Regulation of Pulmonary Vascular Tone in Health and Disease

Special emphasis on exercise and pulmonary hypertension after myocardial infarction

Birgit Houweling

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Regulatie van de pulmonale vaattonus in gezondheid en ziekte

Met de nadruk op inspanning en pulmonale hypertensie na een hartinfarct

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Contents

Chapter 1

General introduction and outline of thesis

Background

In the last decades, ischemic heart disease has become the most prominent cause of heart failure in the western world and is estimated to become world's leading cause of death in the future [3, 4]. Ischemic heart disease occurs after an occlusion of a coronary artery, which deprives the heart muscle behind the occlusion from oxygen. Without reperfusion, this part of the heart muscle will die (myocardial infarction) and loose its ability to contract. Treatment and survival of myocardial infarction has increased due to better treatment options, but secondary problems will occur as a result of the decreased performance of the heart. One of the problems that might occur is pulmonary hypertension, a high blood pressure in the pulmonary circulation. As a result of the increased filling pressures in the left ventricle, pressure in the left atrium and the pulmonary venous system rises and augments the workload for the right ventricle. Since the incidence of ischemic heart failure is growing, incidence of pulmonary hypertension after myocardial infarction also is increasing. Treatment options (as will be described later in this introduction) for pulmonary hypertension are increasing, but there is still a lot unknown about the normal regulation of pulmonary vascular tone and how it is regulated in pulmonary hypertension after myocardial infarction.

During exercise, regulation of the pulmonary vasculature might be different from the normal regulation of the pulmonary vasculature in order to accommodate the increase in cardiac output. An important determinant of the pulmonary vascular bed is pulmonary vascular resistance (PVR). PVR is determined by the pressure-fall over the pulmonary vascular bed (pulmonary artery pressure minus left atrial pressure) divided by the cardiac output. During exercise, pulmonary artery pressure increases concomitant to increases in cardiac output in order to maintain the low PVR. Since patients with pulmonary hypertension already have an increased pulmonary artery pressure, exercise is difficult and sometimes impossible. Therefore it is also important to understand the regulation of pulmonary vascular tone during exercise, in the normal and the hypertensive pulmonary circulation.

Thus the focus of this thesis is to study the regulation of pulmonary vascular tone under normal physiological conditions as well as alterations in regulation of pulmonary vascular tone after myocardial infarction. Regulation of pulmonary vasomotor tone will be studied at resting conditions as well as during exercise.

Circulatory system

The human body is supplied of blood by a double circulatory system, which means that there is a separate pulmonary and systemic circulation. In short, deoxygenated blood (containing little or no oxygen) collects in two major veins: the superior vena cava and the inferior vena cava. The superior and inferior vena cava empty into the right atrium. The blood is then pumped through the tricuspid atrioventricular valve into the right ventricle. From the right ventricle, blood is pumped through the pulmonary semi-lunar valve into the pulmonary trunk. The deoxygenated blood leaves the heart by the pulmonary arteries and travels through the lungs (where it is oxygenated) and into the pulmonary veins. The oxygenated blood then enters the left atrium. The blood then travels through the bicuspid valve, also called mitral valve, into the left ventricle. The left ventricle is thicker and more muscular than the right ventricle because it pumps blood throughout the body against higher pressure. From the left ventricle, blood is pumped through the semi-lunar valve into the aorta. Once the blood goes through the systemic circulation, deoxygenated blood will again collect inside the vena cava and the process will continue.

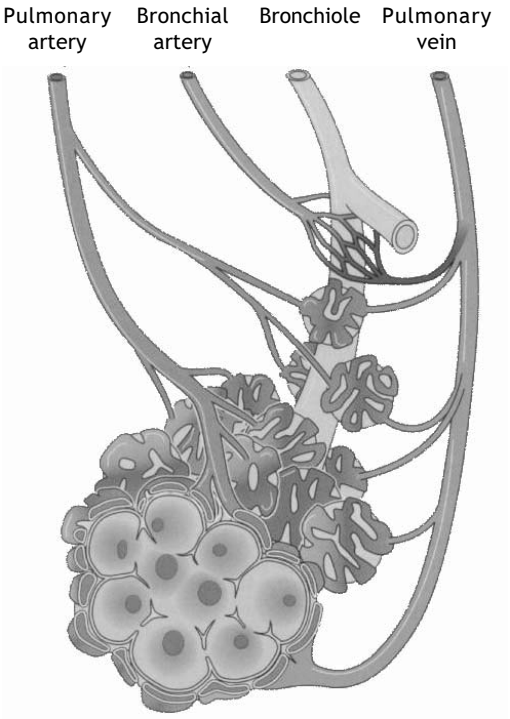

***Figure 1**: Alveoli with pulmonary and bronchial circulation. Pulmonary circulation: deoxygenated blood leaves the heart via the right ventricle and flows through the pulmonary artery to capillaries, which surround the alveoli where oxygen is taken up. Oxygenated blood is directed to the left ventricle by the pulmonary vein. Bronchial circulation: oxygenated blood from the systemic circulation is directed to the bronchiole by a bronchial artery. Deoxygenated blood flows into the pulmonary vein by shunts. Adapted with permission from [6].*

Pulmonary circulation- anatomy

The main function of the lungs is to oxygenate blood by exchanging oxygen and carbon dioxide. Deoxygenated blood is carried to the lungs by the pulmonary arterial bed whereas oxygen is supplied by the bronchi. The pulmonary artery arises from the right ventricle, bifurcates, and carries relatively deoxygenated blood to each lung. The two main branches of the pulmonary artery follow the two mainstem bronchi into the lungs and bifurcate along with the bronchi and bronchioles. A single pulmonary arteriole supplies all the capillaries of a terminal respiratory unit (Fig. 1) [6]. Pulmonary venules collect the oxygenated blood from the capillary network, which then enters the left atrium via the pulmonary veins. The capillaries surrounding the alveoli are part of the pulmonary circulation, whereas lung tissue itself is oxygenated by the bronchial circulation, which is part of the systemic circulation.

Systemic versus pulmonary circulation

The pulmonary circulatory system handles the same cardiac output as the systemic circulation, but there are some distinct differences. The pulmonary circulation is a low pressure system with a low resistance in opposite to the systemic circulation which has a high pressure and resistance. The mean aortic pressure is approximately 90 mmHg in contrast with the approximately 15 mmHg of the mean pulmonary arterial pressure.

Conversely the pulmonary vascular bed has a total resistance that can be ten times lower than that of the systemic circulation. The systemic circulation needs a higher pressure because it has to be able to pump blood to the top of the brain while standing, whereas the pulmonary circulation only needs to pump blood to the top of the lungs. The pulmonary circulation must be a low-pressure system to avoid the consequences of starling forces, which would otherwise flood the lung with edema fluid [6]. The differences in arterial pressures between the two circulations are due to the enormous number of small muscular pulmonary arteries; the large pulmonary capillary bed; and the normally dilated state (low vascular tone) of the pulmonary resistance vessels [1]. Most of the pressure drop in the systemic circulation occurs in the arterioles, while in the pulmonary circulation the arterioles make a much smaller contribution and almost the entire pressure drop occurs rather uniformly between the pulmonary artery and the end of the capillaries. [1, 6] (Fig. 2).

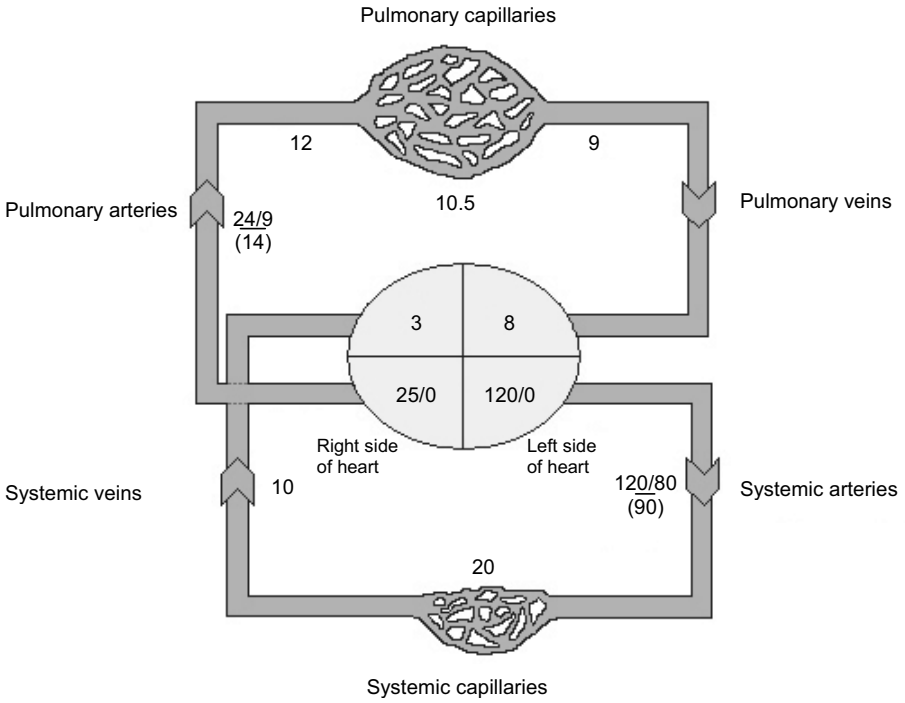

***Figure 2**: Schematic representation of the phasic and mean pressures within the systemic and pulmonary circulation in a normal resting human. Units are mmHg. Adapted with permission from [1]*

Blood vessels are composed of different layers, the inner layer which forms the lumen of the vessel consists of endothelial cells and the layer surrounding these endothelial cells consists of smooth muscle cells. Both endothelial and smooth muscle cells play an important role in regulation of vascular tone. The layer on the outside of the blood vessel is mainly composed of collagen. Pulmonary blood vessels are generally shorter and wider than their counterparts on the systemic side. The walls of pulmonary vessels are also thin. The thinness and paucity of smooth muscle give the pulmonary vessels a high compliance that allows pulmonary vessels to dilate in response to modest increases in pulmonary arterial pressure [6]. In addition, endothelial cells that form the inner layer of the vasculature, differ between the systemic and pulmonary vascular bed. For example, endothelial cells that line in the pulmonary artery of the rat are larger and more rectangular than those lining in the aorta [7].

Pulmonary circulation- regulation of vascular tone

Passive regulation.

In the pulmonary circulation, both passive and active regulation of vascular tone occurs. There are two passive mechanisms at work in the pulmonary vasculature being recruitment and distension of the small vessels (arterioles, capillaries and venules) [1, 6]. Under normal conditions, when pulmonary atrial pressure is low, some pulmonary vessels are open and conducting blood, others are open but not conducting and others are closed. As pressure increases, vessels that previously had been open but not conducting now begin to conduct blood, and some vessels that were completely closed may now open. This recruitment of new vessels will decrease pulmonary vascular resistance and any additional dilation of this vessels (distension) will cause a further reduction in overall pulmonary resistance [6]. This remarkable ability to lower the resistance even further makes it possible to only have a minor increase in mean pulmonary artery pressure during exercise when cardiac output sometimes doubles.

Exercise results in an increase in left atrial pressure that is progressive with exercise intensity and accounts for the majority of the increase in pulmonary arterial pressure that can be observed [8]. For the lungs to perform their gas-exchange function efficiently, the pulmonary circulation must maintain low pulmonary arterial and capillary pressure while accommodating this increased cardiac output. In addition, perfusion must be distributed among the ventilated units so that local flow matches local ventilation. Both pulmonary pressure and vasomotor tone are low in the normal lung under resting conditions, so due to gravity, posture may be an important determinant of local pulmonary perfusion [9].

In humans, in upright posture, the vertical distance from lung base to apex is relatively large resulting in very low perfusion pressures of the top of the lungs while perfusion pressures are larger in the lower lung segments. This "passive" pressure distribution helps to direct the flow to the well-ventilated lower lung areas. During exercise, ventilation increases, thereby recruiting the upper lung segments [10]. Moreover, pulmonary pressure increases which results in improved perfusion of these same regions. Thus, the large decrease in pulmonary vascular resistance from rest to exercise in the upright position mainly reflects recruitment of vessels that were unperfused at rest [11]. In contrast, in the supine posture, the vertical distance is less, such that the whole lung can be perfused rather evenly even at low pulmonary arterial pressures as evidenced by a lower pulmonary vascular resistance as compared to the upright position. Moreover, transition from rest to exercise in the supine position has only little effect on pulmonary vascular resistance [11], reflecting the low levels of pulmonary vascular tone.

In medium-size quadrupeds such as dogs and pigs the vertical distance from the most ventral to the most dorsal part is approximately 15 cm, with the heart lying on the sternum at the "bottom" of the lung. Unlike the human lung with its large base and small apex, quadruped lungs have small ventral volumes but large dorsal volume [12]. Thus, to enable adequate gas-exchange, with most of the lung volume being situated well above the heart, pulmonary artery pressure in these quadrupeds (approximately 15-16 mmHg, [12] needs to be slightly higher than in humans (13-14 mmHg [11, 12]). Since the entire lung is already perfused in prone position in quadrupeds, there is very little effect of posture on the regional pulmonary blood flow [13-16].

During exercise the flow through the pulmonary bed increases, as a result of increasing cardiac output. In quadrupeds, the flows in all lung areas during exercise are proportional to their resting flows, so that the distribution of flow across the pulmonary vasculature does not change [12]. Hence, the fall in pulmonary vascular resistance with exercise is primarily due to decreased pulmonary vascular tone and passive pulmonary arterial distension due to the increase in pulmonary pressure.

Active regulation

There are a lot of vasoconstrictor or vasodilator influences that cause active regulation of the vasculature (Fig. 3). Eventually they all result in vascular smooth muscle cell contraction or relaxation. The vascular smooth muscle cells are lined in the arterial wall just underneath the endothelial cells. They are highly specialized cells whose principal functions are contraction and relaxation [17]. They express a variety of contractile proteins, ion channels, and signalling molecules that regulate contraction. In the intact body, the process of smooth muscle contraction is principally regulated by pharmacomechanic activation (i.e., activation by ligands of cell surface receptors) and electromechanic activation (i.e., stretch, intraluminal pressure) of the contractile proteins myosin and actin [18]. Contraction and relaxation of the smooth muscle cells are caused by an increase or decrease of intracellular calcium respectively, or by deactivation and activation respectively of the myosin light chain phosphatase. Calcium (Ca^{2+}) and myosin light chain phosphatase act synergistically to change vascular tone (Fig. 4).

The cell membrane is permeable to a number of ions, the most important of which are Na^+, K^+, Ca^{2+} and Cl^-. These ions pass across the membrane through specific ion channels that can open (become activated) and close (become inactivated). Their opening and closing can occur in response to 1) voltage changes (voltage gated channels), 2) activation of receptors (receptor gated channels), or specific ions or ligands. Importantly, there are several different types of K^+-channels that play an

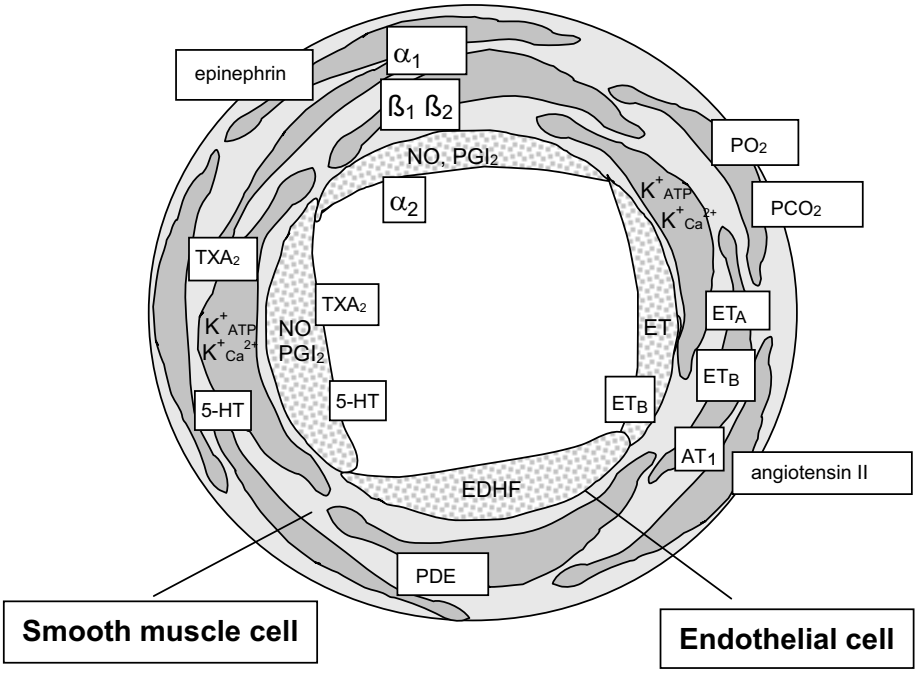

***Figure 3**: Diameter of arteries is influenced by different vasodilators and vasoconstrictors.*
α, alpha adrenoreceptors; β, beta adrenoreceptors; NO, nitric oxide; PGI_2, prostacyclin; PO_2, partial O_2 tension; PCO_2, partial CO_2 tension; K^+_{ATP}, ATP dependent potassium channel; K^+_{Ca2+}. Calcium dependent potassium channel; ET, endothelin; ET_A, endothelin A receptor; ET_B, endothelin B receptor; AT_1, angiotensin 1 receptor; PDE, phosphodiesterase; 5-HT, serotonin; TXA_2, thromboxane. Adapted with permission from [2]

important role in resting membrane potential and in action potentials. Activation of these K^+-channels leads to efflux of K^+-ions, a decrease in membranepotential (hyperpolarization), decrease in intracellular Ca^{2+}, thereby causing smooth muscle relaxation and vasodilation. (Fig. 4) [19, 20].

As described in the paragraphs above, Ca^{2+} is an important signaling molecule in physiology, since it is responsible for the actual constriction of smooth muscle cells. Therefore it is highly regulated with dynamic, localized, and temporal changes in its intracellular concentration. Within the intracellular Ca^{2+} stores of the sarcoplasmic reticulum and in the extracellular space of smooth muscle cells the Ca^{2+} concentration is high (several millimoles per litre). Since basal cytoplasmic Ca^{2+} is in the range of 120 nmol/l [21-23], a large chemical gradient exists for Ca^{2+} to enter from the extracellular space and the sarcoplasmic reticulum lumen into the cytoplasm during smooth muscle cells excitation. The relative contributions of these two major Ca^{2+} sources to the increase in cytoplasmic Ca^{2+}, eliciting contraction of smooth muscle cells, remain in debate [24-28] but may vary depending on smooth

muscle cells type and excitatory stimulus [29].

Ca^{2+} is removed from cells by two basic mechanisms. The first mechanism involves an ATP-dependent Ca^{2+} pump that actively removes calcium from the cell. The second mechanism is the sodium-calcium exchanger. This is a non-ATP-dependent protein that, under steady-state conditions, extrudes Ca^{2+} from the interior of the cell into the extracellular space via facilitated transport [30].

Many vasoactive substances influence vascular resistance by increasing the production of the second messengers cAMP and/or cGMP, which can cause an increase in intracellular Ca^{2+}. These second messengers are short-lived because they are degraded by PDE (Fig. 4) [31]. At least eleven different gene families of PDE are currently known to exist in mammalian tissues but the tissue distribution of the PDE-isoforms as well as their specificity for cAMP and cGMP varies [32]. Inhibition of PDE in vascular smooth muscle can provide a powerful tool to reduce vascular resistance, by prolonging the half-life of cAMP and/or cGMP. The PDE isoforms that are predominantly present in vascular smooth muscle are PDE1, 3,

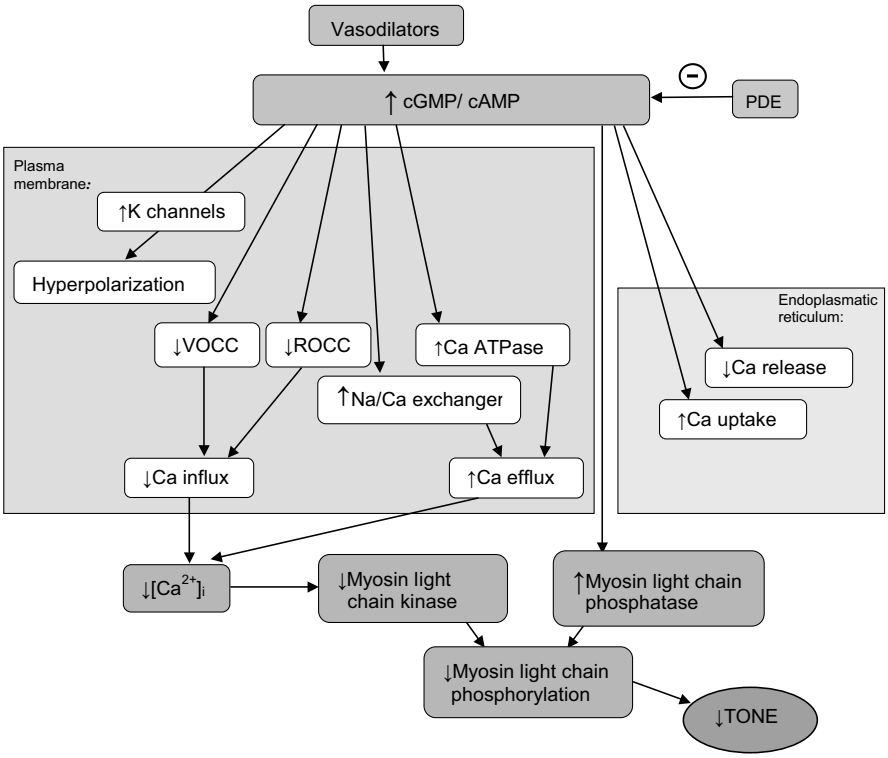

***Figure 4**: Mechanism of vasodilation in smooth muscle cells. cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; PDE, phosphodiesterase; Ca, calcium; K, potassium; VOCC, voltage-operated calcium channels; ROCC, receptor-operated calcium channels; [Ca^{2+}]i, intracellular free calcium ion concentration. Adapted with permission from [5]*

4, 5, 7 and 9 [33, 34]. PDE1, PDE4 and PDE7 are cAMP-specific, while PDE3 has a 4-10 times higher affinity for cAMP than for cGMP. PDE5 and PDE9 are cGMP-specific [32]. However, PDE9 is abundantly expressed throughout the body [32], whereas PDE5 expression is 10 times more abundant in the pulmonary vasculature as compared to the heart [35, 36].

Endothelial cells.

The endothelial cells interact with the smooth muscle cells present in the vascular wall on one hand and with circulating cells on the other hand. Endothelial cells can produce different vasodilators (i.e. nitric oxide (NO), prostacyclin (PGI_2), endothelium derived hyperpolarisation factor (EDHF)) and vasoconstrictors (endothelin (ET)) in response to physiological stimuli such as shear stress. Shear stress is a force acting in the direction of the blood flow on the surface of endothelial cells and increases when flow increases.

Prostaglandins. Lung tissue is particularly active in the synthesis, metabolism and release of a number of prostaglandins, which may play a role in the regulation of pulmonary vascular resistance [37]. The isoenzymes cyclooxygenase (COX)-1 and -2 catalyze the conversion of arachidonic acid into thromboxane A_2 (TXA_2) and prostaglandins (PGs). Prostaglandins I_2 (PGI_2) and E_1 (PGE_1) are active pulmonary vasodilators, whereas $PGF_{2\alpha}$ and PGA_2 are pulmonary vasoconstrictors. PGI_2, prostacyclin, is the principal arachidonic acid metabolite released by the endothelium and possesses both vasodilatory and antiplatelet effects [38]. Physiologically, prostacyclin is a local hormone rather than a circulating one and release of prostacyclin by endothelial cells causes relaxation of the underlying vascular smooth muscle and prevents platelet aggregation within the bloodstream [8]. Prostacyclin exerts its effects through activation of the cyclic adenosine monophosphate (cAMP)-dependent pathways.

Nitric oxide. Nitric oxide (NO) is another vasoactive agent synthesized by the endothelium that plays an important role as a vasodilator [39]. It is synthesized from L-arginine by NO synthase (NOS), which exists in 3 isoforms: inducible NOS (iNOS), an isoform, expressed mainly by macrophages, neuronal NOS (nNOS), an isoform expressed by neurons, and an endothelial form [38]. Endothelial NOS (eNOS) is dependent on calcium and is widely expressed in the pulmonary endothelium of healthy individuals [38]. NO acts through cyclic guanosine monophosphate (cGMP) to regulate potassium channels and has a very localized effect. If any NO diffuses into circulating blood, it immediately and irreversibly binds to the heme iron in hemoglobin. Thus, its action is strictly localized to the vascular bed in which it is produced [1]. Endothelial NO synthase is found in the vascular endothelium of the

normal pulmonary vasculature, where it is responsible for generating NO to govern vascular tone.

Endothelium derived hyperpolarisation factor. Endothelium derived hyperpolarisation factor (EDHF) is a substance synthesized in, and released from, the endothelium which hyperpolarizes vascular smooth muscle cells thus reducing the open probability of voltage-dependent Ca^{2+} channels so that intracellular Ca^{2+} is lowered, and relaxation can take place. The name "EDHF" is confusing because it implies that a single diffusible substance mediates this type of endothelium-dependent relaxation. In fact, numerous endothelium-derived factors, including NO and prostacyclin themselves, can hyperpolarize the underlying smooth muscle. There is still a lot unknown about non-prostanoid–dependent, non-NO–dependent endothelium-dependent hyperpolarization factors, but it is generally assumed that cytochrome p450 plays a role in the production of EDHF. Cytochrome p450 products proposed to be EDHFs are the epoxyeicosatrienoic acids (EETs) Indeed, EETs are generated by endothelial cells and mediate part of the endothelium-dependent dilator effect of arachidonic acid [40]. Other substances that are proposed to be a EDHF include derivates of the lipoxygenase pathway, hydrogen peroxidase and c-type natriuretic peptide [41]

Endothelin. Endothelin is a potent vasoconstrictor peptide that plays an important role in the regulation of pulmonary vascular tone. Three isopeptides of endothelin have been identified, endothelin-1, endothelin-2 and endothelin-3, each containing 21 amino acids [42]. These peptides are synthesised from the 39 amino acid intermediate big ET by ET converting enzyme [43]. ET-1 is the predominant isoform of endothelin in the cardiovascular system and it is produced from big-ET by endothelin-converting-enzym (ECE)-1, while big-ET is produced from preproendothelin by furin-like enzymes [44]. ET-1 is synthesized by a variety of different cell types, including endothelial cells, vascular and airway smooth-muscle cells, leukocytes, macrophages, cardiomyocytes and mesangial cells [45]. In endothelial cells it is released toward the vascular smooth muscle cell, consistent with a paracrine rule. ET-1 is abundantly expressed in the pulmonary vasculature and exerts its major vascular effects through activation of two distinct G-protein-coupled ET_A and ET_B receptors [46].

ET_A receptors are located on the vascular smooth muscle cells were they cause vasoconstriction, while ET_B receptors are localized on endothelial cells and smooth muscle cells [47]. The activation of ET_B receptors on endothelial cells causes the release of NO and PGI_2, thereby causing vasodilation [48]. However, the ET_B receptors that are located on smooth muscle cells induce vasoconstriction [49, 50]. ET_A receptors are the predominant ET vasoconstrictor receptors in arteries

throughout the body. Vasoconstrictor ET_B receptors are present in the veins and pulmonary vessels in larger numbers than in arteries, although ET_A still predominate over ET_B receptors in these vessels [51]. In the large pulmonary arteries, ET-induced constriction is mediated by ET_A receptors, whereas it is mediated by ET_B receptors in the smaller pulmonary resistance vessels [52]. In accordance with these findings, the density of ET_A receptors in the lung decreases with decreasing vessel size, whereas the density of ET_B receptors, both on the endothelium and the smooth muscle increases [53].

The net effect of ET-1 depends mainly on the relative density and activity of ET_A receptors on smooth muscle cells and of ET_B receptors on smooth muscle and endothelial cells [54]. Normally there is a balance between production and clearance. The latter is mediated by the endothelial ET_B receptor such that circulating endothelin is at low level [46].

Neurohumoral control.

The pulmonary vasculature expresses both alpha (α) and beta (β) adrenoreceptors, which help regulate vascular tone by producing vasoconstriction or vasodilation, respectively [55]. These receptors primarily bind norepinephrine that is released from sympathetic adrenergic nerves. Additionally, they bind norepinephrine and epinephrine that circulates in the blood. Vascular smooth muscle has two primary types of alpha-adrenoceptors: $alpha_1$ (α_1) and $alpha_2$ (α_2). The α_1-adrenoceptors are located on the vascular smooth muscle. In contrast, α_2-adrenoceptors are located on the sympathetic nerve terminals as well as on vascular smooth muscle. Smooth muscle α_1 and α_2-adrenoceptors are linked to a G-protein, which activates smooth muscle contraction. Prejunctional α_2-adrenoceptors located on the sympathetic nerve terminals serve as a negative feedback mechanism for norepinephrine release. α_1-Adrenoreceptors in the pulmonary arteries have increased affinity and responsiveness to their agonists when compared with other vessels [56]. The downstream signaling events in α_1-adrenergic stimulation are an increase in calcium levels and activation of protein kinase, which mediate vascular contractile and proliferative responses. The increased sensitivity of α_1-adrenoreceptors to norepinephrine in the pulmonary arteries may greatly facilitate local regulation of vascular tone in response to acute changes in oxygen concentrations, thereby adjusting regional perfusion. Stimulation of α_1-adrenoreceptors increases intracellular free calcium levels by at least two mechanisms: (1) coupling to specific G proteins on the cell membrane and (2) blockade of potassium ion channels [57]. There are two β receptorsubtypes, β_1 and β_2 receptors. Vascular smooth muscle cells have β_2-adrenoceptors that are

normally activated by norepinephrine released by sympathetic adrenergic nerves or by circulating epinephrine. These receptors, are coupled to a Gs-protein, which stimulates the formation of cAMP. Increases in intracellular cAMP caused by β_2-agonists inhibit myosin light chain kinase thereby producing less contractile force (i.e., promoting relaxation).

Angiotensin II. Angiotensin II, the effector peptide of the renin-angiotensin system [58] causes vasoconstriction. It is generated in the lung by means of enzymatic conversion of angiotensin I [46]. It binds to two specific receptor subtypes, AT_1 and AT_2, and activates complex intracellular signalling systems. AT_1 predominates in vascular tissues and contributes to chronic diseases by promoting cell growth, inflammation, and fibrosis, while AT_2 is expressed in pathological settings and is involved in vasodilation and apoptosis [58].

Thromboxane A_2. Thromboxane (TXA_2) is a product of arachidonic acid metabolism and acts as a vasoconstrictor of pulmonary arterial and venous smooth muscle. TXA_2 act or signal by binding to specific receptors, located on the surface of their target cell. In humans, thromboxane signals through two distinct but closely related thromboxane receptors, referred to as the thromboxane receptor α and β. Many cells, particularly macrophages, but also leukocytes, platelets and endothelial cells produce and release TXA_2. The effect is localized mainly in the region where the TXA_2 is released, because the half time of TXA_2 inactivation in blood is only several seconds [1].

Serotonin. Serotonin (5-HT), is an important constituent of platelet-dense granules and is released upon activation. Serotonin can be a vasodilator in healthy vasculature, but is a vasoconstrictor that promotes smooth muscle cell hypertrophy and hyperplasia in damaged vasculature. Normal endothelial cells respond to serotonin by enhancing the release of NO, thereby leading to vascular smooth muscle relaxation and vasodilation. In the setting of endothelial dysfunction, serotonin is unable to stimulate NO release and increases vascular smooth muscle tone, thereby leading to vasoconstriction [59].

Serotonin receptors are classified into 7 types, each type can have subtypes A, B and so on. Vasoconstriction occurs when 5-HT binds to 5-HT receptors. 5-HT can also be transported inside the cells by 5-HT transporters [60]. The 5-HT receptors and 5-HT transporters are abundantly expressed in the lung, where they are predominantly located in the pulmonary arterial smooth muscle cell [61]. A clear distinction between the two classes of molecules, 5-HT transporter and 5-HT receptors, has so far been indefinable. Either or both may play a role in the pulmonary arterial smooth muscle cell response to 5-HT [60].

Oxygen tension.

The effects of changes in PO_2 on the pulmonary circulation are the opposite to those in the systemic circulation [1, 6]. A decrease in PO_2 causes constriction of the pulmonary arteries whereas in the systemic circulation a decrease in PO_2 accounts for relaxation of the resistance vessels. However, in the pulmonary circulation, the PO_2 in the lumen of the arteries appears not to be the most important factor, but rather the PO_2 in the air spaces of the alveoli. Indeed, perfusion of the pulmonary circulation with a hypoxic solution results in less vasoconstriction than ventilating the airways with a low PO_2 air mixture [6]. Oxygen diffuses through the thin alveolar walls into the smooth muscle cells and a reduced alveolair PO_2 results in local vasoconstriction (hypoxic vasoconstriction). Hypoxic vasoconstriction is a unique physiological response in pulmonary arteries and arterioles so that the blood flow is shunted away from hypoxic regions toward better ventilated areas of the lung, improving the ventilation-perfusion matching within the lung [46]. The mechanism behind this hypoxic vasoconstriction remains unknown, but since it also occurs in isolated lung tissue and thus does not rely on the nervous system or systemic hormones, it is believed to act directly on the pulmonary vascular smooth muscle cells [6].

People that live at high altitude have to cope with this hypoxic vasoconstriction due to chronic hypoxia. Altitude decreases the inspired partial pressure of oxygen because of a decrease in barometric pressure [46]. At sea level, PO_2 is on average 150 mm Hg, while at high altitudes, PO_2 decreases to 80 to 100 mm Hg, and at extreme altitudes PO_2 decreases to 40 to 80 mm Hg. Life at altitude is therefore associated with pulmonary hypertension of variable severity [46].

Active regulation during exercise

The regulation of active pulmonary vasomotor tone during exercise is incompletely understood. There are a lot of mechanisms that can play a role. In previous studies we found that β-adrenergic blockade by administration of propanolol increased PVR at rest and during exercise, while α-adrenergic blockade showed the opposite [62]. This was also found in sheep at rest [63]. Since pharmacological β -adrenoceptor stimulation produces vasodilation in pulmonary arteries [64], as well as other vascular beds [65, 66], that is in part mediated by K^+_{ATP} channel activation, the exercise-induced pulmonary vasodilation could also be mediated via K^+_{ATP} channel opening. But blockade of K^+_{ATP} channels showed clearly that the K^+_{ATP} channels are not mandatory for maintaining pulmonary vascular resistance in awake swine at rest and during treadmill exercise [67]. Another well known

vasoactive substance is adenosine, which has been shown to be a vasodilator in the systemic and the coronary vascular bed [68], but its role in the pulmonary circulation is controversial. Saadjian et al [69] found a correlation between PVR and the adenosine plasma concentration in the pulmonary circulation in men, which suggests that adenosine may play a part in the regulation of pulmonary vascular tone. However, a study in swine showed no effect on PVR after blocking adenosine with 8-phenyltheophylline in rest and during exercise [68]. The role of other important vasodilators (NO, prostacyclin, PDE5 inhibition) and vasoconstrictors (ET) in the pulmonary circulation of swine at rest and during exercise will be determined in this thesis.

Pulmonary hypertension

Pulmonary hypertension is characterized by elevated levels of pulmonary arterial pressure and pulmonary vascular resistance. Clinically it is defined as a mean elevation of pulmonary artery pressure > 20 mm Hg at rest or > 30 mm Hg during exercise [70-72]. It is a rare disease with a poor prognosis when untreated [73].

Several years ago there were two classifications for pulmonary hypertension, primary pulmonary hypertension which indicated the patients who were diagnosed with pulmonary hypertension of an unexplained etiology, and secondary pulmonary hypertension, which indicated the patients with pulmonary hypertension as a result of an underlying disease. In 1998, during the Second World Symposium on Pulmonary Hypertension held in Evian, France, a clinical classification of PH was proposed because the term "secondary pulmonary hypertension" was found confusing and without value for diagnosis and treatment [74]. Thus, this term was left out in the new classification, which consist of five groups, the first group to be pulmonary arterial hypertension, which included primary pulmonary hypertension. During the third World Symposium on Pulmonary Hypertension in 2003 some changes were made to the classification and the most important might be the deletion of the name primary pulmonary hypertension. The revised clinical classification of pulmonary hypertension is indicated in table 1 [74].

The first two pulmonary hypertension classifications will shortly be discussed in light of the genetic knowledge of the last few years (idiopathic and familial pulmonary hypertension) and as an outline for the rest of this thesis (pulmonary hypertension with left heart disease)

Idiopathic and familial pulmonary hypertension. Idiopathic and familial pulmonary hypertension are relatively rare with an estimated incidence of 1 or 2

Table 1: Evian Classification of pulmonary hypertension [74]

1. Pulmonary arterial hypertension (PAH)

1.1. Idiopathic (IPAH)
1.2. Familial (FPAH)
1.3. Associated with (APAH):
  1.3.1. Collagen vascular disease
  1.3.2. Congenital systemic-to-pulmonary shunts
  1.3.3. Portal hypertension
  1.3.4. HIV infection
  1.3.5. Drugs and toxins
  1.3.6. Other
  (thyroid disorders, glycogen storage disease, Gaucher disease, hereditary hemorrhagic telangiectasia, hemoglobinopathies, myeloproliferative disorders, splenectomy)
1.4. Associated with significant venous or capillary involvement
  1.4.1. Pulmonary veno-occlusive disease (PVOD)
  1.4.2. Pulmonary capillary hemangiomatosis (PCH)
1.5. Persistent pulmonary hypertension of the newborn

2. Pulmonary hypertension with left heart disease

2.1. Left-sided atrial or ventricular heart disease
2.2. Left-sided valvular heart disease

3. Pulmonary hypertension associated with lung diseases and/or hypoxemia

3.1. Chronic obstructive pulmonary disease
3.2. Interstitial lung disease
3.3. Sleep-disordered breathing
3.4. Alveolar hypoventilation disorders
3.5. Chronic exposure to high altitude
3.6. Developmental abnormalities

4. Pulmonary hypertension due to chronic thrombotic and/or embolic disease

4.1. Thromboembolic obstruction of proximal pulmonary arteries
4.2. Thromboembolic obstruction of distal pulmonary arteries
4.3. Non-thrombotic pulmonary embolism (tumor, parasites, foreign material)

5. Miscellaneous

Sarcoidosis, histiocytosis X, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumor, fibrosing mediastinitis)

per million in the population. In the last few years more understanding towards the genetic basis of pulmonary hypertension has occurred by the suggestion that mutations in the gene encoding the bone morphogenetic protein receptor type II (BMPR2), localized to chromosome 2q33, underlies approximately 50% of cases of familial pulmonary hypertension [75]. Moreover, mutations in BMPR2 have been identified in up to 26% of sporadic cases of PPH [76]. Mutations associated with

pulmonary hypertension also occur in Alk/endoglin, wich is a TGF receptor [77]. However, the chance of a disease gene carrier developing pulmonary hypertension is as low as 20% [74, 78]. It is incompletely understood why this chance is only 20%, but different gene mutations or use of drugs might be the trigger for pulmonary hypertension in patients with the BMPR2 mutation. Moreover, studies are needed to identify other genes, modifiers, and regulatory genes of PH.

Pulmonary hypertension with left heart disease. With the improved treatment and survival of myocardial infarction, the importance of ischemic heart disease increased and has become the most prominent cause of heart failure in the western world [4]. After myocardial infarction a part of the myocardium is dead and has lost its ability to contract. To adequately maintain cardiac output, the remaining 'healthy' part of the myocardium has to work harder and eventually these cells will hypertrophy. When the pumpfunction of the heart is still insufficient, left heart failure will occur. As a result of the decreased ability of the heart to empty the left ventricle, pressure in the left ventricle and left atrium will increase. The increased left atrial pressure is transmitted backwards into the pulmonary circulation wich will lead to reactive increase in PVR and thus pulmonary hypertension and eventually right heart failure [79, 80].

Endothelial dysfunction in pulmonary hypertension.

The precise cause of idiopathic pulmonary hypertension remains unknown, but as with most other forms of pulmonary hypertension, including pulmonary hypertension secondary to an underlying disease, endothelial dysfunction plays an important role. The normal pulmonary vascular endothelial cell maintains the vascular smooth muscle cells in a state of relaxation [37], although in patients with pulmonary hypertension increased pulmonary vascular reactivity and vasoconstriction was found [70]. Indeed, there is growing evidence that the deregulation of pulmonary vascular tone in disease states involves alterations in the counterbalancing systems of the endothelium (such as NO, PGI_2 and ET) [80]. In accordance with that, studies in both experimental models and patients suggest that NO-dependent pulmonary vasodilation is impaired in heart failure [80, 81]. Clinical studies suggest that basal pulmonary artery NO production is relatively deficient in patients with heart failure and secondary pulmonary hypertension, and that the loss of NO-dependent vasodilation may contribute to the development of pulmonary hypertension [82, 83]. However, there are also reports on increased production of NO in early stages of pulmonary hypertension [5]. Hypothetically both the increase and decrease of NO can contribute to the pathogenesis of pulmonary hypertension,

since increased endothelial cell NO could lead to abnormal angiogenesis and VEGF signalling while decreased targeting by NO could contribute to cell proliferation and vasoconstriction [81].

Prostacyclin has very potent pulmonary vasoconstrictor capacities although it seems not to contribute to basal vasomotor tone [84, 85]. However, in patients with severe pulmonary hypertension a decrease in prostacyclin synthase expression is observed [86] and prostacyclin-receptor deficient mice develop severe pulmonary hypertension changes [87] which both indicate a role of prostacyclin in pulmonary hypertension. The feedback mechanism in which NO and prostanoids are capable of inhibition of the release of ET [88-90] will also be decreased with decreased levels of NO and PGI_2, resulting in increased levels of ET. Indeed, increased levels of circulating ET, as well as increased local production [80, 91, 92]. Moreover, there is a correlation between ET plasma levels and the severity of pulmonary hypertension in patients with chronic heart failure [93]. Whether this increased ET influence is the result of the decreased inhibition by NO and PGI_2 or the result of decreased clearance or receptor sensitivity is still incompletely understood.

Morphology and remodelling

Arteries are composed of a tunica intima, which are the endothelial cells that form the inner layer of the vascular wall, a tunica media, the smooth muscle cells and a tunica externa or tunica adventitia which consist of loose fibrous connective fibrous tissue. Pulmonary arteries in pulmonary hypertension are characterized by intimal fibrosis, medial hypertrophy and adventitial proliferation, so thickening of the layers of the vessel wall [94]. This thickening can be the result of hypertrophy and/ or hyperplasia of the cell types within one layer. This alterations in vascular structure are seen in both human and animal models of pulmonary hypertension and take place more rapidly than the remodeling of systemic arteries in systemic hypertension [95].

Pulmonary vascular remodeling occurs in response to a variety of stimuli, both physical (mechanical stretch, shear stress) and chemical (e.g. hypoxia, vasoactive substances, growth factors). The physical factors may involve direct activation of stretch sensitive ion channels [96] or indirect via growth factors [97]. Some of the vasoactive substances that are important in the regulation of vascular tone are also important in remodeling. NO and PGI_2 both have antimitogenic effects in the pulmonary vasculature, meaning they inhibit DNA synthesis and proliferation [95]. Prostacyclin also inhibits the proliferation of vascular smooth muscle cells and decreases platelet aggregation [98]. Furthermore, both NO and PGI_2 can inhibit the production of ET. ET stimulates the production of growth factors such

as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF) and cytokines [54] and strengthens the effects of some growth factors [46]. It has proliferative effect on vascular smooth muscle cells [99, 100] and therefore, ET-1 leads to morphologic abnormalities of small- and medium-sized pulmonary arteries. The consequences of vascular remodeling of the pulmonary vasculature are that due to the wall thickening, vessel inner diameter decreases, and as a result of that pulmonary artery pressure increases. Also the lumen-to-wall ratio is important. Constriction of the smooth muscle cells, induced by a vasoconstrictor, will cause a greater increase in vascular resistance in vessels with a small lumen-to-wall ratio. A thirth important consequence is that due to alterations in the extracellular matrix, compliance of the vessels is reduced and therefore the pulmonary circulation will have less ability to accomodate increases in cardiac output [95], and pulmonary artery pressure will rise more during exercise.

Exercise and pulmonary hypertension

During exercise, pulmonary artery pressure increases concominant to increases in cardiac output in order to maintain the low pulmonary vascular resistance. Since patients with pulmonary hypertension already have an increased pulmonary artery pressure, exercise is difficult and sometimes impossible. These limitations can be explained by findings of Franciosa et al, who found that the right ventricle and the pulmonary arterial pressure limit exercise capacity [101] and Holverda et al, who found that in patients with pulmonary hypertension an exercise-induced rise in pulmonary artery pressure results in further impairment of right ventricular function and underfilling of the left ventricle, which leads to a failing stroke volume response to exercise [102]. However, to date little is known about the regulation of pulmonary vascular tone during exercise and little research is carried out to investigate this regulation even though it is important for patients to be treated with a vasodilator therapy which maintains its vasodilator influence during exercise.

One of the endpoints in many patient studies on therapies for pulmonary hypertension is the six minute walk to investigate the aimed progress in exercise capacity. The change in exercise capacity in patients with pulmonary hypertension appears to parallel other clinical indicators of disease severity, such as survival, hemodynamics, and time to clinical worsening [103]. The distance walked in these 6 minutes is measured and compared with the distance the patient can walk with vasodilator therapy. An average 6 min walk of a patient with pulmonary hypertension is 330 meter, while in healthy subjects this is doubled [104, 105]. In several patient studies the progress in the 6 min walk is between 35 and 75 meter, which is not

even near 'normal' distances [104]. Thus, even though this 6-minute-walk can depict the improvement of exercise capacity in different vasodilator therapies, there is no evidence that the vasoactive substances studied really contribute to the regulation of the hypertensive pulmonary circulation during exercise. In addition, in some studies exogenous vasodilators (e.g. NO and prostacyclin) are studied instead of the endogenous role of these substances.

Current therapies

Therapies have improved over the last years as knowledge of pulmonary hypertension has improved. Since hypoxemia is a pulmonary vasoconstrictor, supplemental oxygen can be used in patients to maintain an oxygen saturation of greater than 90%. Other currently available therapies include inhalation of NO and/ or administration of Ca^{2+}-channel blockers, exogenous prostacyclin and endothelin-receptor blockade. However, these therapies have short-duration, limited efficacy, are expensive and/or associated with significant side effects, indicating that there is a continued need for novel vasodilator therapies of pulmonary hypertension [106, 107]. At this moment a lot of patient studies are being conducted to get more information about effect, dosages, increases in quality of life and long-term effects. In patients who do not respond to the therapies, atrial septostomy (making a hole between atria with a catheter to allow oxygen rich and oxygen poor blood to mix) or lung transplantation are the only options left [78].

Animal models of pulmonary hypertension

There are several animal models to investigate different aspects of pulmonary hypertension. The most frequently used animals in research in all fields are mice. They are small and therefore easy to handle and housing does not require a lot of room. There are some mouse models of pulmonary hypertension, for example there is a BMPR2-deficient mouse that develops pulmonary hypertension [108]. Also wildtype and/ or genetic modified mice exposed to chronic hypoxia develop pulmonary hypertension as a result of hypoxic vasoconstriction [109]. Thus, micemodels have great potential, although hemodynamic measurements are limited. A slightly larger animal, the rat, is also often used in pulmonary hypertension research. Pulmonary hypertension can be induced by hypoxia [109], genetics (spontaneously pulmonary hypertensive rats (Fawn-hooded rat)) [110], myocardial infarction [111] or by monocrotaline, a substance that induces progressive pulmonary hypertension after a single subcutaneous injection. Rats exposed to monocrotaline develop acute pulmonary vascular inflammation resulting in vascular remodeling and pulmonary hypertension [112]. A large animal model is the sheep. Sheeps and lambs are both

often used to perform ligation of the ductus arteriosus, in utero, as a model for persistent pulmonary hypertension of the newborn [113]. Sheep are also used to study hemodynamics in pulmonary hypertension, which can be more extended because of the size of the animal [114].

Also swine are commonly used in cardiovascular research because swine and humans share important anatomic and physiologic characteristics [115, 116]. For example, pulmonary artery pressure of swine is approximately 17 mmHg, while a person at sea level has a pulmonary artery pressure of 12-16 mmHg [117]. The swine model used to study the pulmonary circulation in this thesis is a chronically instrumented swine, which means that hemodynamics of these animals can be monitored while they are awake, providing a lot of data on different vasodilators and vasoconstrictors that can be administered, free from the effect of anesthesia. A subgroup of these animals receives a coronary artery occlusion to induce myocardial infarction and pulmonary hypertension. Hemodynamic and neurohumoral alterations mimic alterations in humans [118]. These animals are habituated to exercise on a treadmill, which provides us with data on the pulmonary circulation during exercise [118, 119].

Our model of pulmonary hypertension is a model with mild pulmonary hypertension early after MI, without severe endothelial dysfunction. Since detection of pulmonary hypertension is improving, more patients are diagnosed before they reach the end stage of the disease. It is therefore important that research is being conducted on early pulmonary hypertension, to map the changes in regulation of pulmonary vascular tone while pulmonary hypertension progresses.

Aim and outline of the thesis

The general aim of this thesis is to study the complex regulation of the pulmonary circulation, in health and disease (pulmonary hypertension after myocardial infarction) in an intact animal model at rest and during exercise.

Knowledge about the regulation of pulmonary vascular tone is necessary to treat diseases of this vascular bed. Although it is common knowledge that there are vast differences between the systemic and pulmonary vascular bed, e.g. high versus low pressure, little is known about the differences in regulation of the pulmonary vascular bed in comparison with the systemic vascular bed. Therefore, in part one of this thesis (*Chapter 2-4*) we studied the contribution of different endothelial derived vasoactive substances on the regulation of vascular tone of the systemic and pulmonary vascular bed under normal physiological circumstances. Specifically we studied the role of the vasodilators NO and prostacyclin (*Chapter 2*) and the role of the potent vasoconstrictor ET (*Chapter 3*). Since ET can increase the production of NO and prostacyclin, which in turn can blunt the release of ET [88-90] or modify the responsiveness of its receptors [120] (Fig. 5), we studied the integrated role of NO, prostacyclin and ET in the pulmonary and systemic circulation at rest and during exercise in *Chapter4*.

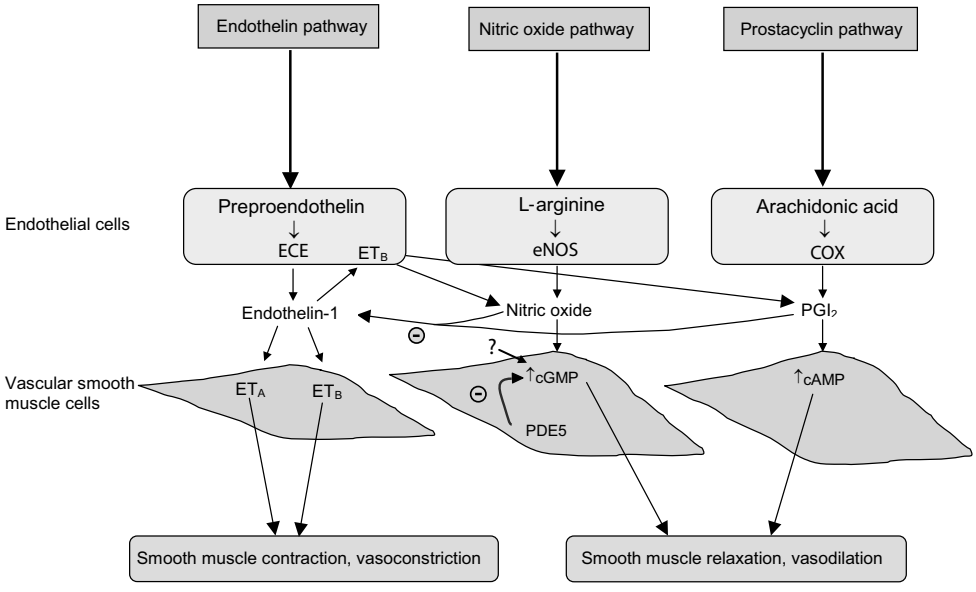

Figure 5: *Three major pathways involved in relaxation and contraction of the smooth-muscle cells of the pulmonary artery are shown. PGI_2, prostacyclin; ET_A, endothelin A receptor; ET_B, endothelin B receptor; cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; PDE, phosphodiesterase.*

Due to increased survival after myocardial infarction, the number of patients with pulmonary hypertension secondary to myocardial infarction is increasing. Yet, there is still a lot unknown about the regulation of pulmonary vascular tone in pulmonary hypertension. Increased levels of circulating ET, as well as increased local production [80, 91, 92] are found in patients with pulmonary hypertension as well as perturbations in vasodilators as a result of endothelial dysfunction [81]. Therefore, in part two of this thesis (*Chapter 5-8*), we studied the alterations in the regulation of pulmonary vascular tone, specifically, we studied the role of ET (*Chapter 5*) and the integrated role of NO, prostcyclin and ET (*Chapter 6*) in swine with MI induced pulmonary hypertension. In addition, since PDE5 inhibition (Fig. 5) is a promising vasodilator therapy for patients with pulmonary hypertension we studied the vasodilator effects of a novel PDE5 inhibitor, EMD360527 in normal (*Chapter 7)* and MI swine (*Chapter 8*). In these chapters we also investigated the importance of NO mediated cGMP production by inhibition of NO.

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Chapter 2

Interaction between prostanoids and nitric oxide in regulation of systemic, pulmonary, and coronary vascular tone in exercising swine

Daphne Merkus, Birgit Houweling, Alisina Zarbanoui and Dirk J Duncker
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Abstract

Prostacyclin and nitric oxide (NO) are produced by the endothelium in response to physical forces such as shear stress. Consequently, both NO and prostacyclin may increase during exercise and contribute to metabolic vasodilation. Conversely, NO has been hypothesized to inhibit prostacyclin production. We therefore investigated the effect of cyclo-oxygenase (COX)-inhibition on exercise-induced vasodilation of the porcine systemic, pulmonary and coronary beds before and after inhibition of NO production. Swine were studied at rest and during treadmill exercise at 1-5 km/h, before and after COX-inhibition with indomethacin (10 mg/kg iv), in the absence and presence of NO synthase inhibition with N^{ω}-nitro-L-arginine (NLA, 20 mg/kg iv). COX-inhibition produced systemic vasoconstriction at rest, that waned during exercise. The systemic vasoconstriction by COX-inhibition was enhanced after NLA, particularly at rest. In the coronary circulation, COX-inhibition also resulted in vasoconstriction at rest and during exercise. However, vasoconstriction was not modified by pretreatment with NLA. In contrast, COX-inhibition had no effect on the pulmonary circulation, either at rest or during exercise. Moreover, a prostanoid influence in the pulmonary circulation could not be detected after NLA. In conclusion, endogenous prostanoids contribute importantly to systemic and coronary tone in awake swine at rest, but are not mandatory for exercise-induced vasodilation in these beds. Endogenous prostanoids are not mandatory for regulation of pulmonary resistance vessel tone. Finally, NO blunts the contribution of prostanoids to vascular tone regulation in the systemic but not in the coronary and pulmonary bed.

Introduction

The endothelium plays an important role in the regulation of vascular tone, by producing both vasodilators (nitric oxide (NO) and prostacyclin) and vasoconstrictors (endothelin). The contribution of these mediators to the regulation of tone varies across vascular beds. Thus, in anesthetized swine, basal pulmonary resistance is strongly influenced by NO and less by prostacyclin whereas the opposite is true for the systemic vascular bed [1]. Prostanoids are produced in skeletal muscle in response to exercise [2-4] and in response to increased levels of adrenaline [5]. Therefore, prostanoids may contribute to metabolic dilation of the coronary [6, 7] and skeletal muscle vasculature [8, 9], although this is not a ubiquitous findings [6, 10-12]. To date no study has investigated the contribution of endogenous prostanoids in the regulation of vasomotor tone in various regional vascular beds during treadmill exercise. Consequently, the first aim of the present study was to determine the contribution of prostanoids to regulation of global and regional systemic, as well as pulmonary and coronary, resistance vessel tone in chronically instrumented swine undergoing treadmill exercise.

Several studies have suggested that an interaction exists between NO and prostanoid production. Thus, not only does NO exert an inhibitory effect on prostacyclin production in vitro [13], but also enhanced prostacyclin production maintains flow-mediated dilation in endothelial NO synthase (eNOS) knockout mice [8]. Furthermore, inhibition of cyclo-oxygenase (COX) affected the duration of reactive hyperemia in dogs treated with L-NAME, but not control dogs [14]. We have previously shown that, whereas endogenous NO exerts a vasodilator influence on the systemic, pulmonary and coronary vasculature of awake swine, the exercise-induced vasodilation was only slightly blunted after inhibition of NO production [15]. Hence, other vasodilators, such as prostacyclin, could have increased contribution to exercise-induced vasodilation and act to compensate when NO synthase activity is blunted. The second aim of the present study was therefore to determine whether the contribution of prostacyclin to regulation of resistance vessel tone is enhanced after inhibition of eNOS in exercising swine.

Methods

Animals

Studies were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH pub. no 86-23, revised 1996), and with approval of the Animal Care Committee of the Erasmus Medical

Center. Fifteen 2 to 3-month old Yorkshire X Landrace swine (23± 1 kg at the time of surgery) of either sex (4 males, 11 females) entered the study. Daily adaptation of animals to laboratory conditions started 1 week before surgery. Studies were started 10 days after surgery.

Surgery

Swine were sedated (ketamine, 20 mg/kg im), anesthetized (thiopental, 10 mg/kg iv), intubated and ventilated with O_2 and N_2O to which 0.2%-1% (v/v) isoflurane was added [15-17]. Anesthesia was maintained with midazolam (2 mg/kg followed by 1 mg/kg per hour iv) and fentanyl (10 μg/kg per hour iv). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid filled polyvinylchloride catheter was inserted into the aortic arch for aortic blood pressure measurement (Combitrans pressure transducers, Braun) and blood sampling for determination of blood gases (Acid-Base Laboratory Model 505, Radiometer), O_2 saturation and hemoglobin concentration (OSM2, Radiometer), and computation of O_2 content, O_2 supply, and O_2 consumption [15-17]. An electromagnetic flow probe (14-15 mm, Skalar) was positioned around the ascending aorta for measurement of cardiac output. A microtipped pressure-transducer ($P_{4.5}$, Konigsberg Instruments) was inserted into the LV via the apex. Polyvinylchloride catheters were inserted into the LV to calibrate the Konigsberg transducer LV pressure signal [15-17], and into the left atrium to measure pressure and inject radioactive microspheres to determine regional blood flows [15].

Catheters were inserted into the pulmonary artery to measure pressure, administer drugs and collect mixed venous samples [15-17]. An angio-catheter was inserted into the anterior interventricular vein for blood sampling [15-17], while a Transonic flow probe (2.5-3.0 mm, Transonic Systems) was placed around the left anterior descending coronary artery. Catheters were tunneled to the back and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine im) for 2 days and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamycin iv) for 5 days.

Experimental Protocols

Dosage and stability of the effect induced by indomethacin. To validate that the employed dose (10 mg/kg dissolved in 20 ml saline pH 9.0, iv) of indomethacin produced complete COX-inhibition, we studied the hemodynamic effects of 1 and 10 mg/kg in eleven resting swine (27±1 kg). After baseline measurements of heart rate, blood pressure and cardiac output had been obtained, animals received a dose of 1 mg/kg iv, and five min later hemodynamic measurements were repeated.

Then, animals received additional indomethacin to achieve a total dose of 10 mg/kg iv and five min after completing administration measurements were again repeated.

To validate the stability of COX-inhibition by indomethacin, i.e. whether the degree of COX-inhibition was maintained during the entire 15-min exercise protocol, we studied the stability of the hemodynamic responses to indomethacin (10 mg/kg iv) in four resting swine over a 20-min period. For this purpose hemodynamic baseline measurements were obtained and animals received indomethacin, administered over a 10-min period. Five min after completion of administration, hemodynamics were measured over a 20-min period.

Role of prostanoids in the regulation of vasomotor tone. Systemic, pulmonary and coronary hemodynamic responses to exercise were studied in 13 swine (27±1 kg). After baseline hemodynamic measurements (lying and standing), blood samples (lying), and temperature (standing) were obtained, a treadmill exercise protocol was begun (1-5 km/h); hemodynamic data and blood samples were collected during the last 30 s of each 3 min exercise stage [15-17]. After completion of the exercise protocol, animals were allowed to rest for 90 min. Subsequently, indomethacin (10 mg/kg) was administered intravenously over a 10 min period and 5 min later the exercise protocol was repeated. We have previously shown excellent reproducibility of the hemodynamic responses to consecutive exercise bouts [16, 17].

On another day, six swine underwent a control exercise trial and after 90 min of rest, animals received the COX inhibitor ibuprofen (60 mg/kg, iv) and underwent a second trial.

Regional blood flows. On a different day, regional blood flows were determined in four swine using the radioactive microsphere technique [15]. Radioactive microspheres were injected at rest (lying) and during exercise at 5 km/h under control conditions as well as 5 min after administration of indomethacin (10 mg/kg iv).

Role of prostanoids in the regulation of vasomotor tone in the presence of NO synthase inhibition. On a different day seven animals (29±2 kg) underwent a control exercise trial and after 90 min of rest, N^{ω}-nitro-L-arginine (NLA, 20 mg/kg dissolved in 100 ml saline) was administered intravenously over a 20 min period, 10 min later followed by the second exercise trial. After another 90 min of rest, animals received indomethacin (10 mg/kg iv) and underwent a third exercise trial.

Data analysis

Digital recording and off-line analysis of hemodynamics and regional blood flow have been described previously [15-17]. Systemic vascular conductance was computed as cardiac output divided by mean aortic blood pressure. Systemic vascular resistance was computed as mean aortic blood pressure divided by cardiac output. Pulmonary vascular resistance is defined as mean pulmonary artery pressure minus mean pulmonary backpressure divided by cardiac output. Pulmonary backpressure is best reflected in the pulmonary capillary wedge pressure, but since the increases in left atrial pressure that we observe in our laboratory in exercising swine ([15-17], present study) agree very well with the reported increases in pulmonary capillary wedge pressure (from 4 mmHg at rest to 11 mmHg during exercise at comparable increases of heart rate [18], we employed mean left atrial pressure as backpressure. Blood O_2 content (mmol/ml) was computed as (Hb·0.621·O_2-saturation) + (0.00131·PO_2). Body O_2 consumption (BVO_2) was calculated as the product of cardiac output and the difference in O_2 content between arterial and mixed venous blood. Myocardial O_2 delivery (MDO_2) was computed as the product of LAD coronary blood flow and arterial blood O_2 content. Myocardial O_2 consumption (MVO_2) in the region of myocardium perfused by the left anterior descending coronary artery was calculated as the product of coronary blood flow and the difference in O_2 content between arterial and coronary venous blood. Myocardial O_2 extraction (MEO_2) was computed as the ratio of MVO_2 and MDO_2.

Statistical analysis

Statistical analysis of hemodynamic data within the separate study protocols (control and indomethacin, control and ibuprofen, and control, NLA and NLA+indomethacin) was performed using two-way (treatment and exercise level) analysis of variance (ANOVA) for repeated measures. When a significant effect was detected, post-hoc testing for exercise and drug effect was performed using Scheffe's test.

To test for the effects of indomethacin and NLA on the relation between VO_2 and hemodynamic and metabolic variables, analysis of co-variance (ANCOVA) was performed in the separate study protocols (control and indomethacin and control, NLA and NLA+indomethacin) using treatment as an independent factor and VO2 as co-variate. Post-hoc testing for drug effect (i.e. control vs NLA and NLA vs NLA+indomethacin) was performed using Scheffe's test.

To test for the interaction of the treatments (indomethacin X NLA), on the relation between VO_2 and hemodynamic and metabolic variables, ANCOVA was performed using indomethacin and NLA as two independent factors and VO_2 as co-

variate. Statistical significance was accepted when P≤ 0.05. Data are presented as mean±SEM.

Results

Dosage and stability of the effect induced by indomethacin. The increase in mean aortic blood pressure and systemic vascular resistance produced by 1 mg/kg indomethacin was identical to the vasoconstrictor and pressure response to 10 mg/kg, although heart rate decreased somewhat less after 1 mg/kg than after 10 mg/kg (Table 1). These findings indicate that the dose of 10 mg/kg indomethacin, which we employed in the exercise studies, produced a maximal effect. In addition, the effect of indomethacin was well maintained over a 20 min period (less than 10 % change, Table 2).

Table 1. Hemodynamic effect of 1 vs 10 mg/kg indomethacin in resting swine

	Baseline	Indomethacin iv	
		1 mg/kg	10 mg/kg
HR (bpm)	121 ± 5	106 ± 4*	87 ± 5*†
MAP (mmHg)	93 ± 2	135 ± 5*	132 ± 6*
CO (l/min)	3.4 ± 0.2	2.8 ± 0.2*	2.6 ± 0.2*
SVR (mmHg/l/min)	28 ± 2	47 ± 3*	51 ± 7*

*Data are Means ± SE, n=11 swine;. HR: Heart rate; MAP: Mean arterial pressure; CO: Cardiac output; SVR: Systemic vascular resistance. * P≤0.05 vs Baseline; † P≤ 0.05 vs Indomethacin 1mg/kg iv*

Table 2. Stability of indomethacin (10 mg/kg iv) induced hemodynamic alterations in resting swine

	Baseline	Time after start of protocol, min		
		t=0	t=10	t=20
HR (bpm)	133 ± 4	92 ± 6*	93 ± 7*	93 ± 7*
MAP (mmHg)	89 ± 2	117 ± 5*	112 ± 5*†	113 ± 5*†
CO (l/min)	3.5 ± 0.4	2.6 ± 0.3*	2.7 ± 0.3*	2.8 ± 0.3
SVR (mmHg/l/min)	27 ± 4	50 ± 10*	47 ± 9*†	45 ± 9*†

*Data are Means ± SE, n=4 swine; * P≤ 0.05 vs baseline; † P≤ 0.05 vs t=0*

Hemodynamic effects of COX-inhibition in exercising swine

Systemic and pulmonary circulation. Exercise up to 5 km/h resulted in more than a doubling of cardiac output which was principally due to the increase in heart rate (up to 85% of maximum heart rate), as stroke volume increased by only 15% (Table 3). Despite the increase in cardiac output, mean aortic blood pressure was maintained, reflecting marked systemic vasodilation, i.e. a doubling of systemic vascular conductance or a 60% decrease in systemic vascular resistance (Fig. 1).

Administration of indomethacin resulted in a 40% increase in aortic blood pressure, due to systemic vasoconstriction (Table 3, Fig. 1). The accompanying decrease in cardiac output was mediated by a (probably baroreflex-mediated) decrease in heart rate (Table 3). The indomethacin-induced systemic vasoconstriction necessitated an increase in O_2-extraction resulting in a decreased mixed venous O_2-saturation (Fig. 1). The pressor and vasoconstrictor responses to indomethacin were progressively blunted during exercise (Table 3, Fig. 1). That the increase in systemic vascular resistance was progressively blunted during exercise was not merely the mathematical result of a smaller absolute effect of indomethacin at lower resistance values, because the relative increase in resistance in response to indomethacin was also greater at rest than during exercise (Fig. 2). Although the absolute indomethacin-induced decrease in systemic conductance was similar at rest and during exercise, the relative decrease in conductance induced by indomethacin was also significantly greater at rest than during exercise (Fig. 2). Furthermore, the effects of indomethacin on body O_2 extraction and mixed venous O_2 saturation (Fig. 1) also waned during exercise. Thus, the slope of the relation between body O_2 consumption and O_2 extraction decreased from 1.13±0.07 under control conditions to 0.76±0.11 in the presence of indomethacin ($P<0.05$), whereas the slope of the relation between body O_2-consumption and mixed venous O_2-saturation decreased from –1.06±0.07 under control conditions to –0.73 ±0.10 in the presence of indomethacin ($P<0.05$).

In six animals, ibuprofen caused systemic vasoconstriction as evidenced by significant increases in blood pressure from (91±4 mmHg to 102±4 mmHg, $P<0.05$) and systemic vascular resistance (from 30±4 mmHg/l/min to 35±4 mmHg/l/min, $P<0.05$), albeit to a lesser extent than indomethacin. Similar to indomethacin, the effect of ibuprofen waned during exercise (at maximal exercise: blood pressure 90±4 mmHg without ibuprofen and 94±4 mmHg with ibuprofen, systemic vascular resistance 15±2 mmHg/l/min without ibuprofen and 17±2 mmHg with ibuprofen).

Pulmonary artery pressure more than doubled during exercise (Table 3), whereas pulmonary vascular resistance decreased by only 25% (Fig. 3). In contrast to the systemic vasculature, indomethacin had no effect on pulmonary vascular

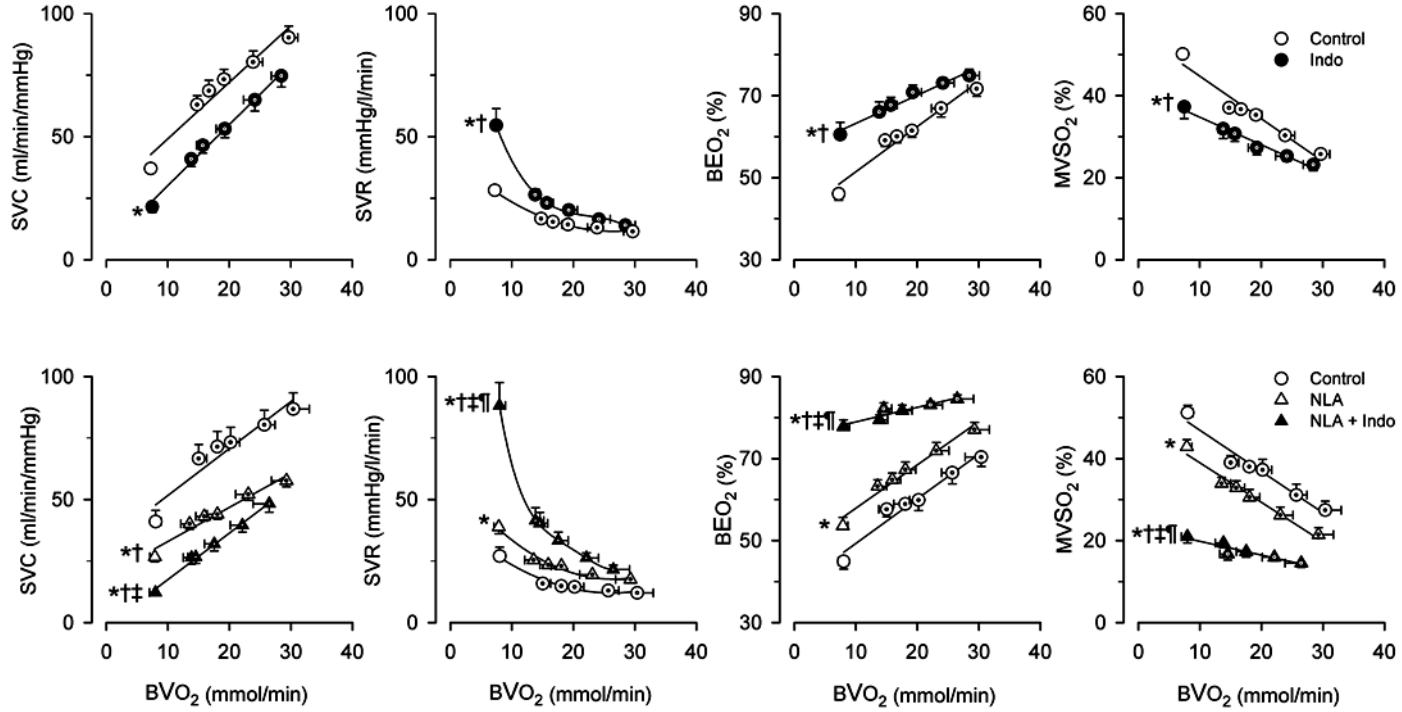


Figure 1. *Effect of inhibition of prostanoid synthesis with indomethacin (10 mg/kg iv) on the relation between body O_2 consumption (BVO_2) and systemic vascular conductance (SVC, left panels), systemic vascular resistance (SVR, second panels from the left), body O_2 extraction (BEO_2, second panels from the right) and mixed venous O_2 saturation ($MVSO_2$, right panels), in the absence (upper panels) and presence (lower panels) of NO synthase inhibition with NLA (20 mg/kg iv). Note that the effects of indomethacin waned during exercise, and that pretreatment with NLA enhanced the vasoconstrictor effect of indomethacin. Dot inside symbol denotes a significant change (P≤ 0.05) from resting (lying) measurement; *P≤ 0.05 vs control relation; †P≤0.05 effect of indomethacin waned during exercise, effect of NLA increased during exercise; ‡P≤ 0.05 NLA + indomethacin vs NLA; ¶P≤ 0.05 effect of indomethacin in the presence of NLA vs effect of indomethacin in the absence of NLA*

Table 3. Hemodynamic parameters before and after administration of indomethacin

		Rest		Exercise level (km/h)				
	Treatment	Lying	Standing	1	2	3	4	5
Systemic hemodynamics								
CO	Control	3.4 ± 0.2	4.3 ± 0.3*	5.3 ± 0.2*	5.8 ± 0.3*	6.3 ± 0.3*	7.1 ± 0.3*	8.1 ± 0.3*
(l/min)	Indomethacin	2.6 ± 0.2†	3.5 ± 0.2*†	4.3 ± 0.2*†	4.8 ± 0.3*†	5.5 ± 0.3*†	6.5 ± 0.4*†	7.4 ± 0.3*†
HR	Control	121 ± 5	139 ± 6*	167 ± 5*	180 ± 5*	201 ± 6*	229 ± 7*	254 ± 4*
(bpm)	Indomethacin	87 ± 5†	103 ± 4*†	119 ± 4*†	128 ± 3*†	148 ± 5*†	174 ± 6*†	203 ± 6*†
SV	Control	28 ± 2	32 ± 2*	32 ± 2*	32 ± 2*	32 ± 2*	31 ± 2*	32 ± 1*
(ml)	Indomethacin	30 ± 2	34 ± 2*	36 ± 2*†	37 ± 2*†	37 ± 2*†	37 ± 1*†	37 ± 1*†
$LVdP/dt_{max}$	Control	2550 ± 150	2910 ± 190*	3490 ± 210*	3710 ± 180*	4280 ± 280*	4770 ± 350*	5460 ± 370*
(mmHg/s)	Indomethacin	2410 ± 210	3060 ± 230*	3050 ± 190*†	3170 ± 240*†	3630 ± 260*†	4240 ± 320*	4870 ± 390*†
$LVdP/dt_{min}$	Control	-2360 ± 100	-2400 ± 120	-2350 ± 90	-2360 ± 90	-2490 ± 100	-2610 ± 130	-2850 ± 160*
(mmHg/s)	Indomethacin	-2930 ± 130†	-3000 ± 170†	-2930 ± 150†	-2860 ± 160†	-2830 ± 150†	-2890 ± 140†	-2980 ± 140
MAP	Control	93 ± 2	89 ± 3	85 ± 3*	86 ± 3*	87 ± 3*	89 ± 3	91 ± 3
(mmHg)	Indomethacin	132 ± 6†	124 ± 7*†	111 ± 5*†	109 ± 5*†	108 ± 5*†	103 ± 5*†	102 ± 4*†
Pulmonary hemodynamics								
PMAP	Control	14 ± 1	14 ± 1	17 ± 2*	19 ± 2*	22 ± 2*	27 ± 2*	31 ± 2*
(mmHg)	Indomethacin	20 ± 1†	18 ± 2	18 ± 2	20 ± 2	22 ± 2	25 ± 2*	27 ± 2*†
LAP	Control	2 ± 1	2 ± 1	0 ± 1	3 ± 1	5 ± 1*	8 ± 1*	10 ± 1*
(mmHg)	Indomethacin	13 ± 1†	8 ± 1*†	6 ± 2*†	8 ± 1*†	8 ± 1*†	9 ± 1*	9 ± 1*
Coronary hemodynamics								
CBF	Control	56 ± 4	73 ± 6*	81 ± 6*	87 ± 7*	99 ± 7*	116 ± 10*	140 ± 11*
(ml/min)	Indomethacin	49 ± 5†	60 ± 5*†	66 ± 5*†	71 ± 6*†	81 ± 8*†	98 ± 11*†	114 ± 11*†

*Data are Means ± SE, n=13 swine; SV: Stroke volume; $LVdP/dt_{max}$: Rate of increase in left ventricular pressure; PMAP: Mean pulmonary artery pressure; LAP: Left atrial pressure; CBF: Coronary blood flow. * P≤ 0.05 vs rest (lying); † P≤ 0.05 vs control*

resistance either at rest or during exercise (Fig. 3).

Regional systemic vascular beds. The exercise-induced increase in cardiac output was principally diverted towards skeletal muscle, and although brain flow increased slightly, flow to most visceral organs decreased (Fig. 4). Vasoconstriction in response to indomethacin occurred in most visceral organs such as kidneys and intestine (with exception of the adrenals and spleen), and various regions of the brain (Fig. 4). In contrast, skeletal muscle flow was not altered by indomethacin either at rest or during exercise.

Coronary Circulation. Exercise resulted in an increase in MVO_2 that was matched by an equivalent increase in coronary blood flow and hence MDO_2, so that MEO_2 and coronary venous PO_2 ($cvPO_2$) were maintained constant over the entire range of MVO_2 (Fig. 5). Indomethacin reduced coronary blood flow at any given level of MVO_2, necessitating an increase in MEO_2 (in order to maintain MVO_2), which resulted in a decrease in $cvPO_2$. The increase in coronary blood flow was blunted in the presence of indomethacin (P<0.05), suggesting that prostanoids may contribute

to metabolic dilation in the coronary vasculature. However, the indomethacin-induced increase in MEO_2 and decrease in $cvPO_2$ were not significantly different during exercise compared to resting conditions (Fig. 5), indicating that the relative contribution of prostanoids to coronary vascular tone was similar at rest and during exercise.

Hemodynamic effects of COX-inhibition in exercising swine with inhibited NO synthase activity

Systemic and pulmonary circulation. NO synthase inhibition with NLA produced vasoconstriction in the systemic circulation, resulting in an increase in aortic blood pressure (Fig. 1, Table 4). Importantly, pretreatment with NLA enhanced the indomethacin-induced vasoconstriction in the systemic circulation, as indicated by the exaggerated increase in systemic vascular resistance and mixed venous O_2-saturation (Fig. 1). Although the absolute indomethacin-induced decreases in systemic vascular conductance were not augmented (Fig. 1), the relative changes in systemic vascular conductance and resistance both showed potentiation of the vasoconstrictor effect of indomethacin by NLA, particularly at rest (Fig. 2). Despite the marked potentiation of the systemic vasoconstrictor response to indomethacin by NLA, the exercise-induced systemic vasodilation was unmitigated.

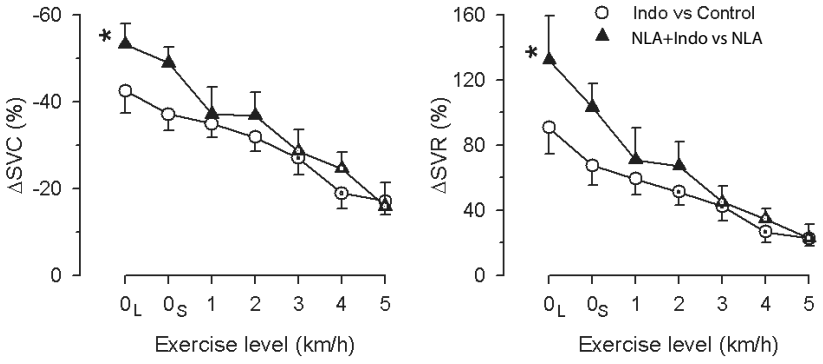


***Figure 2**. Relative changes produced by prostanoid synthesis inhibition with indomethacin (10 mg/kg iv) in systemic vascular conductance (SVC, left panel) and systemic vascular resistance (SVR, right panel) in the absence and presence of NO synthase inhibition with NLA (20 mg/kg iv). Note that both relative changes in conductance and resistance demonstrate that the effects of indomethacin waned during exercise. In contrast, NLA alone produced virtually identical increases in systemic vascular resistance at rest (57±20%) and during exercise (51±11% at 5 km/h) as well as similar decreases in systemic vascular conductance at rest (-30±7%) and during exercise (-31±5%; data not shown). Also note that pretreatment with NLA enhanced the vasoconstrictor effect of indomethacin particularly at rest. O_L: resting (lying), O_S: resting (standing). Dot inside symbol denotes a significant difference (P≤ 0.05) from resting (lying) measurement; *P≤ 0.05 effect of indomethacin in the presence of NLA vs effect of indomethacin in the absence of NLA (ANOVA).*

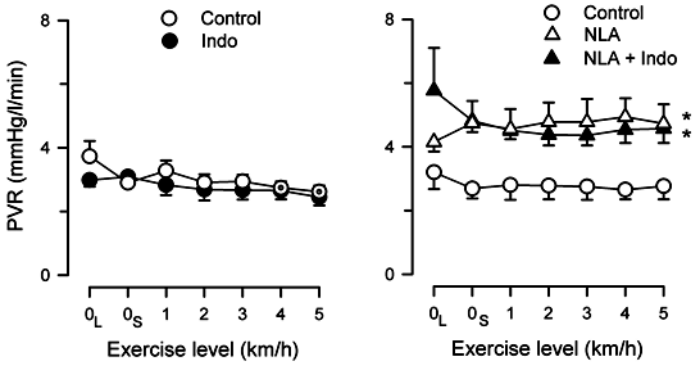


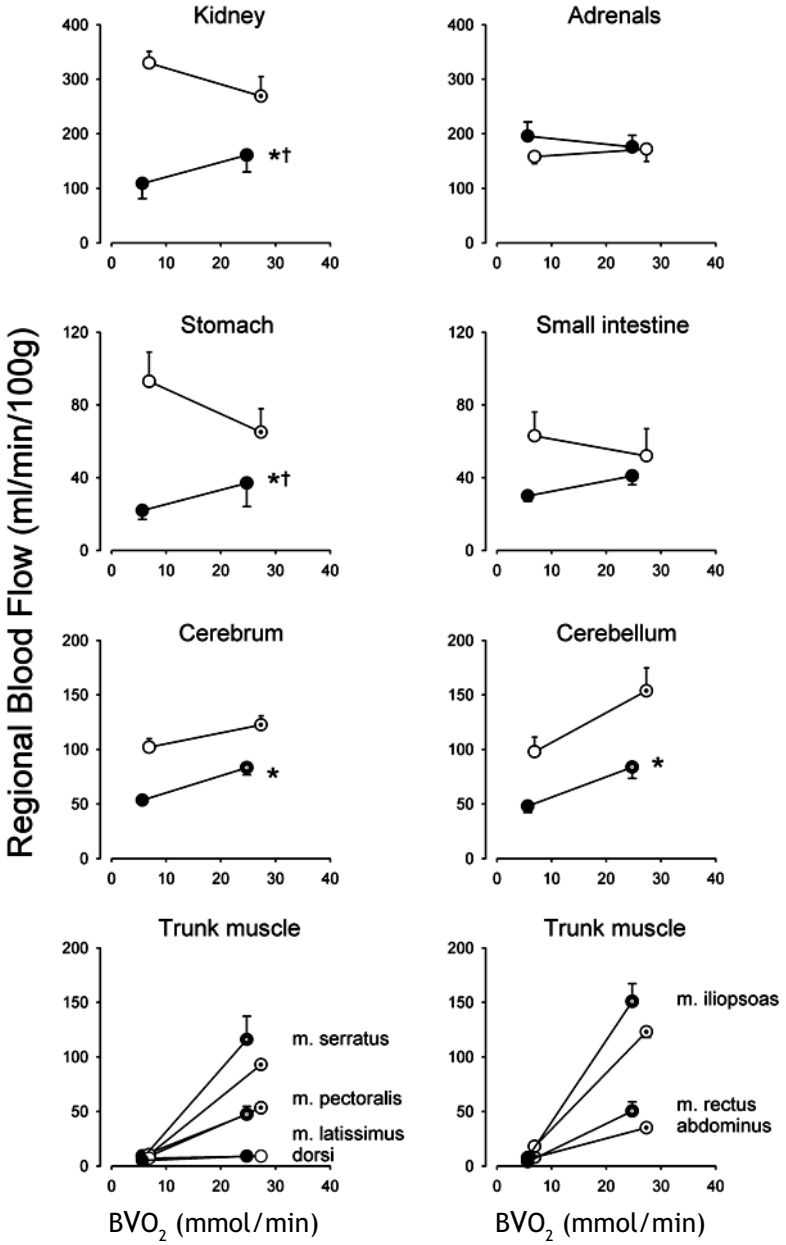
***Figure 3**. Effect of inhibition of prostanoid synthesis with indomethacin (10 mg/kg iv) on the relation between exercise and pulmonary vascular resistance (PVR), in the absence (left panel) and presence (right panel) of NO synthase inhibition with NLA (20 mg/kg iv). Note that indomethacin had no effect on PVR. Dot inside symbol denote changes (P≤0.05) from resting (lying) measurement; *P≤0.05 vs control (ANOVA)*

NO synthase inhibition produced vasoconstriction in the pulmonary circulation (Fig. 3), resulting in an increase in pulmonary artery pressure (Table 4). However, subsequent administration of indomethacin had no additional effect on pulmonary vascular resistance (Fig. 3).

Coronary circulation. NLA reduced coronary blood flow and MDO_2 at any given level of MVO_2, necessating a small increase in MEO_2 (to maintain MVO_2), which resulted in a decrease in $cvPO_2$ (Fig. 5). Although indomethacin resulted in additional vasoconstriction as evidenced by a further decrease in $cvPO_2$, pretreatment with NLA did not enhance the vasoconstrictor effect of indomethacin, indicating that increased prostanoid production does not compensate for the loss of NO in the coronary circulation.

Discussion

The major findings of the present study are that in awake swine free from the effects of anesthesia and acute surgical trauma: (*i*) prostanoids are involved in the regulation of resistance vessel tone in the systemic and coronary circulation, but not in the pulmonary circulation; (*ii*) the vasodilator influence of prostanoids on coronary and cerebral resistance vessels is maintained during exercise, whereas their influence on the total systemic vasculature wanes during exercise; and (*iii*) prostanoids have an increased vasodilator influence in the systemic vascular bed when NO synthase activity is inhibited.


Kidney
Adrenals
Stomach
Small intestine
Cerebrum
Cerebellum
Trunk muscle
Trunk muscle
*†
*†
*
*
m. serratus
m. pectoralis
m. latissimus dorsi
m. iliopsoas
m. rectus abdominus
Regional Blood Flow (ml/min/100g)
BVO2 (mmol/min)
BVO2 (mmol/min)

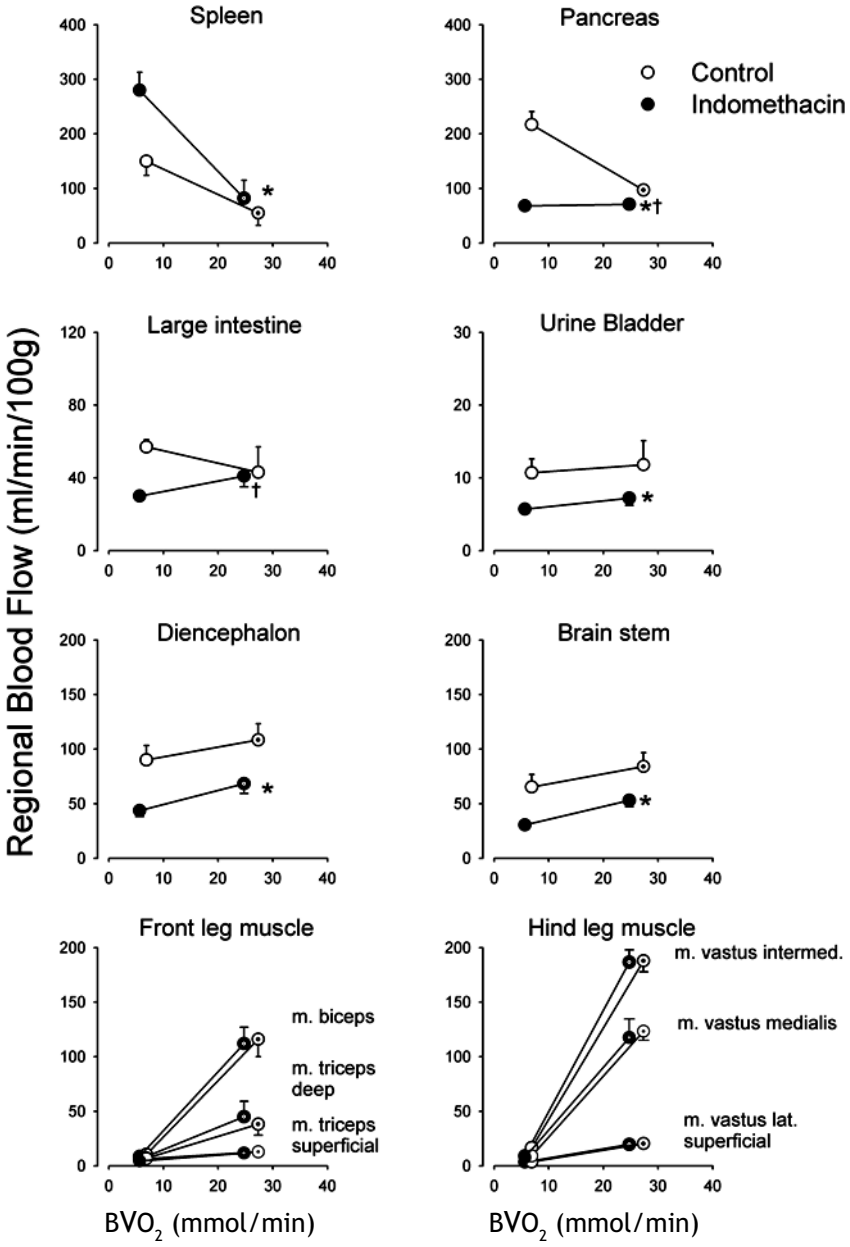


Figure 4 (page 54 & 55). *Effect of inhibition of prostanoid synthesis with indomethacin (10 mg/kg iv) on visceral organ, regional brain and skeletal muscle blood flows at rest (lying) and during exercise at 5 km/h. Dot inside symbol denotes change (P≤0.05) from resting (lying) measurement; *P≤0.05 vs control; †P≤0.05 effect of indomethacin waned during exercise.*

Methodological considerations

Dosage of indomethacin. The dose of indomethacin that we used (10 mg/kg iv) is similar [19, 20] or slightly higher (compared with 3 mg/kg iv [1, 21]) than used by others to inhibit COX in swine. This dose should be sufficient to completely block COX for a prolonged period of time in swine as in our study, administration of indomethacin in a dose of 1 mg/kg yielded similar alterations in hemodynamic variables at rest. To exclude that the decreased effects of indomethacin on the systemic and coronary vasculature during exercise were due to diminished blockade of COX, we established that hemodynamic variables remained stable during a period of 20 min after administration of indomethacin, which encompasses the 15 min duration of the exercise trial. Thus, the decreased effect of indomethacin during exercise is due to either a decreased contribution of endogenous prostanoids to the regulation of vascular tone, or an increased compensation by other vasodilator systems during exercise as will be discussed below.

Indomethacin as a COX inhibitor. Some studies have indicated that COX inhibitors have other effects besides inhibition of COX. Depending on the inhibitor used, transcription factors, MAP kinases, cell cycle proteins and heat shock proteins (HSPs) may be affected [22]. We therefore performed additional experiments with a different COX inhibitor (ibuprofen). Ibuprofen also caused

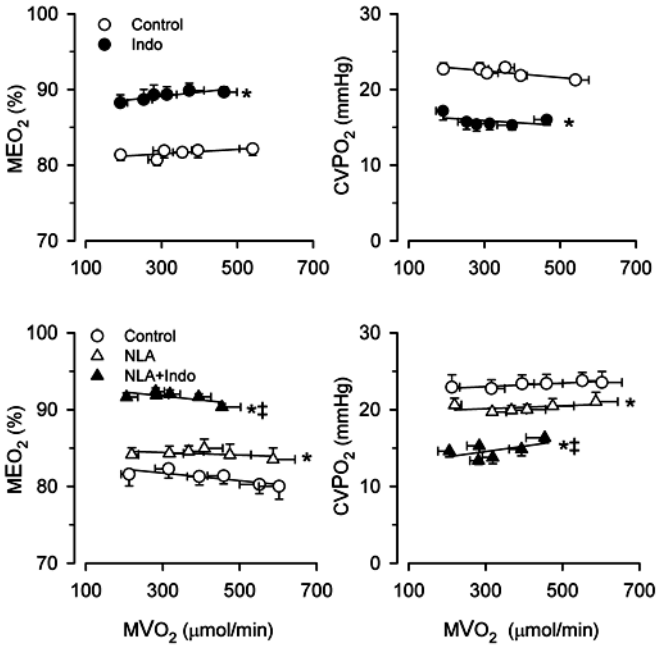


***Figure 5**. Effect of inhibition of prostanoid synthesis with indomethacin (10 mg/kg iv) on the relation between myocardial O_2 consumption (MVO_2) and myocardial O_2 extraction (MEO_2) and between MVO_2 and coronary venous PO_2 ($cvPO_2$), in the absence (upper panels) and presence (lower panels) of NO synthase inhibition with NLA (20 mg/kg iv). Note that NLA did not modify the vasoconstrictor effect of indomethacin. Exercise did not result in changes from resting (lying) measurement. *P≤0.05 vs control; ‡P≤0.05 NLA + indomethacin vs NLA*

Table 4. Hemodynamic parameters before and after administration of NLA and indomethacin

		Rest		Exercise level (km/h)				
	Treatment	Lying	Standing	1	2	3	4	5
Systemic hemodynamics								
CO	Control	3.8 ± 0.4	4.8 ± 0.4*	5.5 ± 0.4*	6.1 ± 0.5*	6.6 ± 0.5*	7.3 ± 0.5*	8.1 ± 0.6*
(l/min)	NLA	3.2 ± 0.3	3.9 ± 0.2*†	4.5 ± 0.3*†	5.0 ± 0.3*†	5.3 ± 0.3*†	6.2 ± 0.3*†	6.9 ± 0.4*†
	NLA+ Indo	1.9 ± 0.2†‡	2.6 ± 0.2*†‡	3.3 ± 0.3*†‡	3.7 ± 0.3*†‡	4.2 ± 0.3*†‡	5.1 ± 0.4*†‡	6.0 ± 0.4*†‡
HR	Control	117 ± 8	137 ± 8*	155 ± 8*	180 ± 8*	200 ± 11*	226 ± 12*	244 ± 9*
(bpm)	NLA	99 ± 4†	117 ± 7*†	129 ± 5*†	141 ± 9*†	155 ± 10*†	188 ± 10*†	220 ± 9*†
	NLA+ Indo	76 ± 10†‡	89 ± 7*†‡	108 ± 10*†‡	111 ± 9*†‡	124 ± 9*†‡	150 ± 10*†‡	176 ± 11*†‡
SV	Control	32 ± 3	35 ± 3	36 ± 3*	34 ± 3	33 ± 2	33 ± 2	33 ± 2
(ml)	NLA	32 ± 3	34 ± 3	35 ± 3*	36 ± 3*	35 ± 3	34 ± 3	31 ± 2
	NLA+ Indo	27 ± 2†‡	31 ± 3*†	33 ± 3*	34 ± 3*	35 ± 3*	35 ± 3*	35 ± 3*
$LVdP/dt_{max}$	Control	2960 ± 200	3220 ± 260	3500 ± 180*	4050 ± 270*	4330 ± 270*	4820 ± 420*	5280 ± 430*
(mmHg/s)	NLA	2790 ± 210†	3310 ± 300*	3610 ± 320*	3880 ± 440*	4140 ± 420*	5080 ± 560*	5880 ± 540*
	NLA+ Indo	2820 ± 370	3360 ± 360	3920 ± 580	3710 ± 290	3920 ± 250*	4500 ± 360*	5340 ± 510*‡
$LVdP/dt_{min}$	Control	-2390 ± 100	-2330 ± 120	-2340 ± 80	-2420 ± 80	-2560 ± 30	-2650 ± 80	-2840 ± 120
(mmHg/s)	NLA	-2790 ± 120†	-2760 ± 100	-2770 ± 90†	-2870 ± 100†	-2860 ± 110	-3010 ± 90	-3170 ± 110
	NLA+ Indo	-3040 ± 220†	-3290 ± 210†‡	-3240 ± 250†	-3300 ± 240†	-3390 ± 240‡	-3380 ± 220†	-3410 ± 180
MAP	Control	93 ± 2	87 ± 5	83 ± 3*	86 ± 3	91 ± 2	92 ± 3	94 ± 3
(mmHg)	NLA	120 ± 4†	109 ± 3*†	111 ± 4*†	114 ± 3†	118 ± 4†	119 ± 3†	119 ± 4†
	NLA+ Indo	159 ± 5†‡	148 ± 5*†‡	134 ± 5*†‡	135 ± 5*†‡	134 ± 5*†‡	130 ± 4*†	124 ± 3*†
Pulmonary hemodynamics								
PMAP	Control	15 ± 1	17 ± 1	18 ± 1*	21 ± 1*	27 ± 1*	30 ± 2*	35 ± 2*
(mmHg)	NLA	22 ± 2†	21 ± 3	25 ± 3*†	28 ± 3*†	33 ± 4*†	39 ± 4*†	42 ± 3*†
	NLA+ Indo	29 ± 2†‡	26 ± 2†	29 ± 2†	31 ± 2†	35 ± 2*†	39 ± 2*†	41 ± 3*†
LAP	Control	3 ± 1	3 ± 2	3 ± 2	3 ± 2	9 ± 2*	11 ± 2*	14 ± 2*
(mmHg)	NLA	10 ± 2	4 ± 3	7 ± 2†	7 ± 2†	11 ± 1	12 ± 1	14 ± 2
	NLA+ Indo	18 ± 4	12 ± 3	14 ± 2	15 ± 2	16 ± 3	17 ± 3	16 ± 2
Coronary hemodynamics								
CBF	Control	60 ± 6	78 ± 7*	84 ± 7*	102 ± 8*	110 ± 8*	133 ± 10*	151 ± 11*
(ml/min)	NLA	60 ± 3	76 ± 5*	84 ± 6*	90 ± 5*	97 ± 6*†	116 ± 8*†	136 ± 10*†
	NLA+ Indo	43 ± 4‡	51 ± 4†‡	64 ± 6*†‡	66 ± 5*†‡	74 ± 6*†‡	90 ± 7*†‡	105 ± 9*†‡

*Data are Means ± SE, n=7; * P≤ 0.05 vs rest (lying); † P≤ 0.05 vs control; ‡ P≤ 0.05 vs NLA.*

systemic vasoconstriction, albeit to a lesser extent than indomethacin. Similar to indomethacin, the effect of ibuprofen waned during exercise. One of the possible differences between ibuprofen and indomethacin is that ibuprofen activates HSP formation while indomethacin is devoid of such action [22]. Increased HSP90 expression may subsequently activate eNOS [23-25], thereby increasing NO production and partially counterbalancing the effect of the loss of the vasodilator prostanoids. However, the observation that indomethacin and ibuprofen induce qualitatively similar effects on systemic hemodynamic parameters, suggests that the effects of indomethacin on cardiovascular function in the present study are indeed due to inhibition of COX. COX catalyzes the conversion of arachidonic acid

into prostaglandin H_2, which is subsequently processed by different enzymes into various prostanoids. The vasodilator prostacyclin and vasoconstrictor thromboxane are the most vasoactive prostanoids, and since COX inhibition resulted in net vasoconstriction it is likely that inhibition of prostacyclin was responsible for the observed vasoconstriction.

Age of the animals. The animals used in the present study were young adults (3 to 4 months), which may have quantitatively influenced the contribution and interaction between NO and prostanoids. However, the contribution of prostanoids in both basal vascular tone as well as vasodilator responses to agonists is independent of age [26] or may decrease slightly from newborn to young adult swine [20, 27]. In contrast, the role of NO in the regulation of tone in the cerebral vasculature does vary with age. However, the largest difference is found between newborn and young adult swine, so that while a contribution of NO is absent in newborn piglets its importance increases in young adult and adult swine [20, 26, 27]. In accordance with these reports, we observed in the young adult swine in the present study that NO contributed importantly to regulation of vascular tone. Taken together, it seems that the swine were of sufficient maturity so that their age did not quantitatively influence the results.

Role of prostanoids in regulation of vascular tone

Systemic circulation. Endogenous prostanoids exerted a strong vasodilator influence on the systemic vasculature at rest as was evidenced by the marked increase in blood pressure and the increase in systemic vascular resistance upon indomethacin administration. Although prostanoids contribute importantly to the regulation of vascular tone at rest, blood pressure and systemic vascular resistance gradually returned to control levels during exercise and mixed venous O_2-saturation approached the values of the control exercise. These findings indicate that either there is no role of prostanoids in metabolic regulation or that their role in the systemic vascular bed is fully compensated by other vasoactive substances such as NO. Little is known about the role of prostanoids in the systemic vasculature during exercise. Because prostacyclin is produced in response to increased levels of adrenaline [5], prostacyclin production will likely increase during exercise, when adrenaline levels increase. Indeed, prostacyclin production increases in the human leg during exercise [2-4]. Furthermore, Sun et al [8] showed that prostacyclin is involved in flow-dependent dilation of isolated murine skeletal muscle arterioles, but did not find a contribution of prostacyclin to basal tone in these vessels. These findings suggest that prostanoids could indeed have increased importance during exercise and thereby contribute to metabolic vasodilation. In apparent support

of this concept, Duffy et al [9] reported that prostanoids contribute to exercise-induced vasodilation in the human forearm. However, in forearm exercise studies, flow is measured immediately after rather than during exercise, which may have influenced the results. For example, inhibition of nitric oxide has been shown to reduce blood flow in the recovery phase following exercise but not during exercise [28]. In line with this view, Beaty et al [10] showed in anesthetized dogs that inhibition of prostanoid synthesis decreased flow to resting skeletal muscle to an equal extent as skeletal muscle flow during electrical stimulation. The present study in awake swine also demonstrates that inhibition of prostanoid production does not affect flow to various skeletal muscle groups with different fiber type composition [15] either at rest or during exercise up to 85% of maximum heart rate.

The present study also shows that the role of prostanoids is strongly dependent on the vascular bed. Inhibition of prostanoid production resulted in a decrease in flow to various regions of the brain of almost 50%, both at rest and during exercise. However, indomethacin did not alter the exercise-induced vasodilation, suggesting that prostanoids are not essential for metabolic vasodilation in the various regions of the brain. Flow to the kidneys and most other visceral organs was also decreased by indomethacin in resting swine, indicating that prostanoids are important for maintaining basal flow to visceral organs. During exercise, however, flow is redistributed away from the visceral organs towards the exercising skeletal muscle. The observation that the effect of indomethacin decreased during exercise suggests that this redistribution may in part be caused by withdrawal of a prostanoid-mediated vasodilator influence in the visceral organs. Conversely, the decreased effect of indomethacin on the visceral organs during exercise is translated into a decreased effect on total systemic vascular resistance, blood pressure and mixed venous O_2 saturation, as flow to skeletal muscle is not affected by indomethacin either at rest or during exercise.

We found in the present study that the effect of inhibition of prostanoid production in the systemic circulation was larger after inhibition of NO synthesis compared to the control experiments, indicating that prostanoids compensate in part for the loss of NO synthesis. There is evidence that NO directly suppresses the activity of COX in vitro [13, 29, 30] as well as in vivo. Prostanoids only contribute to the regulation of coronary tone in dogs after chronic inhibition of nitric oxide synthase [14] as well as in collateral-dependent myocardium [31, 32], whereas NO predominates under normal circumstances. Also, prostacyclin is important in the regulation of flow-induced dilation in eNOS knock-out mice [8]. Functionally, it is important to maintain vasodilator capacity through redundant or back-up

mechanisms. NO and prostacyclin-production are both activated in response to physical stimuli such as shear stress [33, 34], as well as agonists such as acetylcholine and bradykinin [34] and share similar regulatory mechanisms such as activation of COX and NOS by tyrosine phosphorylation [35] and increased intracellular calcium concentrations [36]. Consequently, prostacyclin is a good candidate to act as a back-up system in the systemic circulation when NO bioavailability is reduced.

Pulmonary circulation. We found that prostanoids do not play a role in the regulation of pulmonary vascular tone at rest in either the presence or absence of NO. This is in accordance with a study from Albertini et al [1], who showed that endogenous prostanoids do not contribute to pulmonary vascular tone in anesthetized swine either in the presence or absence of eNOS inhibition. In accordance with a previous study from our laboratory [15] the modest exercise-induced pulmonary vasodilation is principally mediated by NO. However, even when NO-production was inhibited, there was no role for endogenous prostanoids in the regulation of exercise-induced pulmonary vasodilation. Thus, although the porcine pulmonary conductance and resistance vessels are sensitive to the vasodilator actions of exogenous prostanoids [1, 37, 38], endogenous prostanoids do not appear to exert a significant vasodilator influence in the pulmonary circulation.

Coronary circulation. The normal heart is characterized by a high level (80%) of MEO_2 under basal resting conditions [39, 40]. Consequently, the ability of the coronary resistance vessels to dilate in response to increments in myocardial O_2 demand is extremely important to maintain an adequate supply of O_2. A sensitive way to study alterations in coronary vascular tone in relation to myocardial metabolism is the relationship between $cvPO_2$ and MVO_2. For example, an increase in coronary resistance vessel tone will limit coronary blood flow and hence MDO_2, forcing the myocardium to increase its MEO_2 (in order to maintain MVO_2), which results in a lower $cvPO_2$. The $cvPO_2$ thus represents an index of myocardial tissue oxygenation (i.e. the balance between MDO_2 and MVO_2), which is principally determined by coronary resistance vessel tone.

In the coronary vasculature, we found prostanoids to be involved in the regulation of vascular tone both at rest and during exercise, which is in accordance with most [6, 7, 41, 42], but not all [12] data from the human coronary circulation. Prostanoids have been proposed to contribute to metabolic dilation of the coronary resistance vessels in man [7, 42], although this is not a ubiquitous finding [6, 12]. The present study demonstrates that in swine prostanoids do not appear to have increased importance during exercise compared to resting conditions. In contrast to humans and swine, prostanoids do not appear to be important in the regulation of coronary vascular tone at rest or during exercise in the dog [11]. Although

the different observations are difficult to reconcile, they may be attributable to variations in preferential vasodilator systems between species. The contribution of prostanoids to the regulation of coronary vascular tone was not altered by inhibition of NO synthesis. These findings suggest that prostanoids and NO do not act in a compensatory manner when the other pathway is blocked. However, we cannot entirely exclude that the near-maximal MEO_2 (91-93% of the O_2 supplied) that occurred after combined blockade of NO and prostanoid synthesis prevented detection of an interaction between NO and prostanoids.

Metabolic vasodilation after inhibition of NO and prostanoids

Although blockade of production of NO and prostanoids results in severe vasoconstriction at rest and during exercise, exercise-induced vasodilation was principally unperturbed as evidenced by the increase in coronary blood flow and the decrease in systemic vascular resistance during exercise. There are several vasoactive factors that could have contributed to this exercise-induced vasodilation, including adenosine and ATP-sensitive K^+-channels [43] and β-adrenergic vasodilation [44, 45]. In addition, endothelium-derived hyperpolarizing factor may have contributed as it has been shown to be released in response to various agonists [46] as well as pulsatile stress [47], and may thus have increased importance at higher heart rates which occur during exercise.

Conclusion

The present study demonstrates that the contribution of prostanoids to vasomotor control varies markedly between regional vascular beds. Thus, endogenous prostanoids do not play a role in pulmonary resistance vessel control. Conversely, prostanoids contribute significantly to vasomotor and blood flow control in the brain and heart both at rest and during exercise, but are not mandatory for the exercise-induced vasodilation in these organs. In contrast, prostanoids do not appear to play a role in skeletal blood flow regulation either at rest or during exercise. In most visceral organs prostanoids exert a marked vasodilator influence under resting conditions that is withdrawn during exercise. This exercise-induced withdrawal of prostanoid-mediated vasodilation may contribute to the exercise-induced redistribution of blood flow from the visceral organs towards the active skeletal muscle. Finally, prostanoids compensate for an acute loss of NO in the systemic circulation, but not in the coronary and pulmonary circulation.

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Chapter 3

Contribution of endothelin and its receptors to the regulation of vascular tone during exercise is different in the systemic, coronary and pulmonary circulation

Daphne Merkus, Birgit Houweling, Amran Mirza, Frans Boomsma, Anton H. van den Meiracker and Dirk J Duncker
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Abstract

Exercise-induced vasodilation is thought to be mediated through various vasodilator substances, but blunting the influence of vasoconstrictors such as ET may also play a role. However, the role of ET and its receptors in the regulation of systemic, pulmonary and coronary vascular resistance is incompletely understood. The aim of this study was to identify the contribution of endothelin (ET)-1 through the ET_A and ET_B receptors to the regulation of tone in the systemic, coronary and pulmonary beds at rest and during exercise. Ten chronically instrumented swine were studied while running on a treadmill before and after ET_A blockade (EMD122946) or ET_A/ET_B blockade (tezosentan). At rest, EMD122946 resulted in vasodilation in the coronary and systemic circulation, evidenced by a decrease in coronary and systemic vascular resistance and an increase in coronary and mixed venous O_2-saturation. These effects waned during exercise. The effect of tezosentan on the systemic vasculature was similar to that of EMD122946, whereas it was smaller in the coronary circulation. EMD122946 had no effect on the pulmonary vasculature, whereas tezosentan decreased pulmonary resistance but only during exercise. ET exerts a constrictor influence on the coronary and systemic circulation through the ET_A-receptor, which decreases during exercise thereby contributing to metabolic vasodilation. ET exerts a tonic vasodilator influence on coronary resistance vessels through the ET_B-receptor. Finally, ET exerts an ET_B-mediated constrictor influence in the pulmonary vasculature during exercise.

Introduction

Endothelin (ET)-1 is one of the most potent vasoconstrictor agents known. It is produced in endothelial cells by cleavage of its nonvasoactive precursors preproendothelin and big ET [1, 2]. The ET receptors are located both on the endothelium and on vascular smooth muscle. Binding of ET to ET_B receptors on the endothelium leads to production of NO and prostacyclin, which induce vasodilation, whereas binding of ET to the ET_A and ET_B receptors on vascular smooth muscle leads to vasoconstriction [1-3]. Administration of exogenous ET causes ET_B mediated vasodilation at low doses but constriction at high doses, indicating that the ET_B receptor on the endothelium is more sensitive to ET than the receptors on vascular smooth muscle [1-3]. Measurements of ET levels in blood yield concentrations in the picomolar range, while receptor sensitivities are in the nanomolar range [4]. However, reports on the role of endogenous ET have shown that, despite its low plasma concentrations and most likely due to its abluminal secretion, ET contributes to vascular tone in the systemic [5-10], coronary [6, 11] and possibly in the pulmonary circulation [12] under basal physiological conditions.

The role of ET in regulating resistance vessel tone during acute exercise is incompletely understood. Short term exercise does not result in changes in plasma ET-levels [6, 13-15], although small increases have been reported as well [16-18]. Nevertheless, local variations in ET-production may still contribute to redistribution of flow to working muscle. Thus, during one-legged exercise, the ET-levels in the venous blood from the working leg did not change, whereas the ET-levels in the blood from the non-working leg increased [19]. However, because receptor sensitivity to ET can be modulated, for example by exercise training [20] and adenosine [21], changes in ET levels may not accurately reflect its role in exercise-induced changes in flow. Therefore, the aim of the present study was to investigate the role of ET and its receptors in regulation of systemic, pulmonary and coronary resistance vessel tone of swine at rest and during exercise, using a selective ET_A antagonist as well as a mixed ET_A/ET_B antagonist.

Methods

Animals

The study was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and with approval of the Animal Care Committee of the Erasmus MC. Yorkshire X Landrace swine (2-3 months old, n=10,

22±1 kg at the time of surgery) of either sex entered the study. Daily adaptation of animals to laboratory conditions started 1 week before surgery.

Surgery

Swine were sedated (ketamine, 20 mg/kg im), anesthetized (thiopental, 10 mg/kg iv), intubated and ventilated with O_2 and N_2O to which 0.2%-1% (v/v) isoflurane was added [22, 23]. Anesthesia was maintained with midazolam (2 mg/kg followed by 1 mg/kg per hour iv) and fentanyl (10 μg/kg per hour iv). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid filled polyvinylchloride catheter was inserted into the aortic arch for aortic blood pressure measurement (Combitrans pressure transducers, Braun) and blood sampling for determination of blood gases (Acid-Base Laboratory Model 505, Radiometer), O_2-saturation and hemoglobin concentration (OSM2, Radiometer), and computation of O_2-content, O_2-supply, and O_2-consumption (VO_2) [22, 23]. An electromagnetic flow probe (14-15 mm, Skalar) was positioned around the ascending aorta for measurement of cardiac output. A microtipped pressure-transducer ($P_{4.5}$, Konigsberg Instruments) was inserted into the LV via the apex. Polyvinylchloride catheters were inserted into the LV to calibrate the Konigsberg transducer LV pressure signal, into the left atrium to measure pressure and into the pulmonary artery to measure pressure, administer drugs and collect mixed venous blood samples. An angio-catheter was inserted into the anterior interventricular vein for blood sampling, while a Transonic flow probe (2.5-3.0 mm, Transonic Systems) was placed around the left anterior descending coronary artery. Catheters were tunneled to the back and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine im) for 2 days and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamycin iv) for 5 days [22, 23].

Experimental Protocols

Efficacy of ET receptor blockade. In six swine, we tested the efficacy of the ET_A antagonist EMD122946 (a gift from Dr P. Schelling, E Merck Darmstadt, Germany) and the mixed ET_A/ET_B antagonist tezosentan (a gift from Dr Clozel, Actelion Pharmaceuticals Ltd.) in blocking the arterial pressor response to ET. EMD122946 has a pA_2 of 9.5 for ET_A and a pA_2 of 6.0 for ET_B receptors, indicating a 3200-fold selectivity for ET_A compared to ET_B receptors [24]. Tezosentan has a pA_2 of 9.5 for ET_A and a pA_2 of 7.7 for ET_B receptors, indicating only a 63-fold selectivity for ET_A compared to ET_B receptors [6, 25].

While swine were resting quietly, ET-1 (50 ng/kg per ml saline) was infused at rates of 25, 50 and 100 ng/kg/min iv (10 min consecutive infusions) and mean aortic

blood pressure was measured at the end of each infusion. On another day, the same doses of ET-1 were infused after administration of 3 mg/kg iv of EMD122946, dissolved in 40 ml saline (at pH 8), and infused over a 10-min period. On a third day, the same doses of ET-1 were infused after administration of 3 mg/kg iv of tezosentan, dissolved in 15 ml saline, and infused over a 10-min period, followed by 6 mg/kg/h iv infused at a rate of 0.5 ml/min. These three ET-infusion protocols were performed in random order.

Exercise study. Systemic, pulmonary and coronary hemodynamic responses to exercise were studied 1-2 weeks after surgery. After baseline measurements (lying, 0_L, and standing, 0_S) were obtained, a treadmill exercise protocol was started (1-5 km/h). Hemodynamic data and blood samples were collected during the last 30 s of each 3 min exercise stage [22, 23]. After completion of the exercise protocol, swine were allowed to rest for 90 min, during which the hemodynamics returned to baseline resting values that were similar (less than 5% different) to the baseline measurements obtained before the first exercise protocol. Then, either the ET_A antagonist EMD122946 (3 mg/kg iv, n=8) or the mixed ET_A/ET_B antagonist tezosentan (3 mg/kg + 6 mg/kg/h iv, n= 7) was administered as described above, and 10 min later the exercise protocol was repeated. We have previously shown excellent reproducibility of the hemodynamic responses to exercise [22,23,26 27].

Determination of plasma levels of ET

In seven swine, arterial and coronary venous blood samples (5 ml) were collected at rest (Lying) and at 1, 3 and 5 km/h and kept on ice until the end of the exercise trial. Then the blood samples were spun down and plasma was stored at -80°C. Plasma levels of ET-like immuno-reactivity were determined using a radio-immuno assay from Euro-Diagnostica (Malmö, Sweden), which has a cross reactivity of 100% toward ET-1, 48% toward ET-2 and 109% toward ET-3. Since production of ET-2 and ET-3 appears to be absent in the cardiovascular system of the pig [28], the concentrations measured with the radio-immuno assay most likely represents ET-1. Importantly, there was also cross-reactivity of the radio-immuno assay with EMD122946 and tezosentan. To correct for these influences, we dissolved EMD122946 and tezosentan in naïve porcine plasma in the concentrations estimated to be present in vivo (0.04 mg/ml). The ET-level of the plasma before addition of the antagonists was 2.4 pM, while it was 4.3 pM in the presence of EMD122946 and 3.8 pM in the presence of tezosentan. The artificial increases in ET levels produced by EMD122946 (1.9 pM) and by tezosentan (1.4 pM) were subtracted from the ET-values obtained in the in vivo experiments with the respective antagonists to estimate true ET-levels.

Data analysis

Digital recording and off-line analysis of hemodynamics have been described previously [22, 23]. Statistical analysis was performed using two-way (exercise and treatment) analysis of variance (ANOVA) for repeated measures, followed by Dunnett's test (exercise effect) and paired t-test (antagonist effect). Analysis of co-variance (ANCOVA with VO_2 as covariate) was used to detect statistically significant differences of relations between hemodynamic variables and body or myocardial VO_2 in control versus ET_A or ET_A/ET_B blockade. Significance was accepted when $P<0.05$. Data are presented as mean±SE.

Results

Efficacy of blockade

Both ET_A and ET_A/ET_B blockade virtually abolished the ET-1 induced pressor response in the systemic circulation (Fig. 1).

ET levels

Exercise did not influence ET levels (Fig. 2). Furthermore, ET concentrations in arterial and coronary venous blood were not different. ET_A blockade did not alter ET levels at rest, although ET levels were slightly lower during exercise. In contrast, ET_A/ET_B blockade resulted in elevated ET levels both at rest and during exercise.

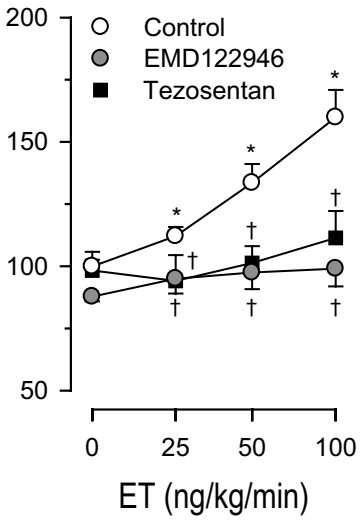

***Figure 1**. Effect of ET_A and mixed ET_A/ET_B blockade on mean aortic blood pressure responses to intravenous infusion of ET-1. EMD122946: ET_A receptor antagonist (3 mg/kg). Tezosentan: ET_A/ET_B receptor antagonist (3 mg/kg+ 6 mg/kg/h iv). MAP: mean arterial pressure. Data are mean±SE; *P<0.05 vs baseline (0 ng/kg/min), †P<0.05 vs corresponding control.*

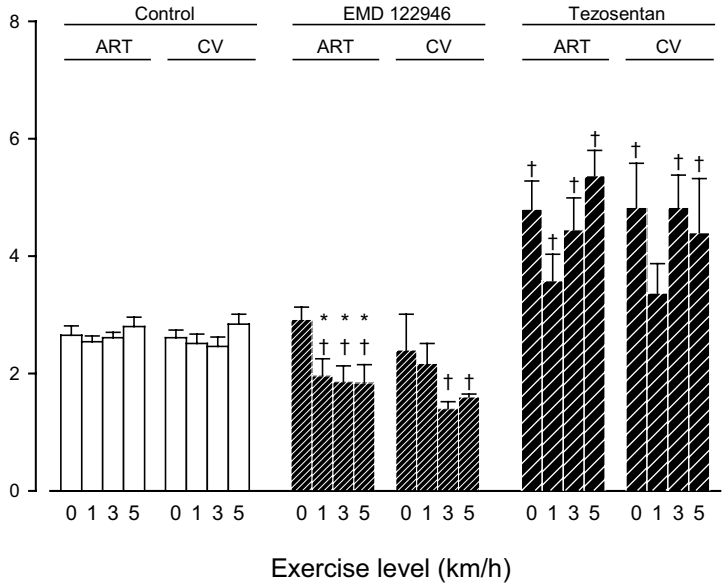

***Figure 2**. Arterial and coronary venous endothelin levels at rest and during exercise. Blood samples are drawn at rest (0 km/h, lying) and at 1, 3 and 5 km/h. ET: endothelin. EMD122946: ET_A receptor antagonist (3 mg/kg). Tezosentan: ET_A/ET_B receptor antagonist (3 mg/kg+ 6 mg/kg/h iv). ART: arterial. CV: coronary venous. Data are mean±SE; *P<0.05 vs 0 km/h, †P<0.05 vs corresponding control.*

Table 1. *Effect of ET_A blockade on hemodynamic responses to graded treadmill exercise*

		Rest		Exercise level (km/h)				
	Treatment	Lying	Standing	1	2	3	4	5
CO	Control	3.6 ± 0.2	4.4 ± 0.3*	5.1 ± 0.4*	5.6 ± 0.3*	6.2 ± 0.4*	7.0 ± 0.4*	7.5 ± 0.3*
(l/min)	EMD122946	3.6 ± 0.3	4.4 ± 0.2*	5.3 ± 0.2*	5.6 ± 0.4*	6.3 ± 0.4*	7.2 ± 0.4*	7.6 ± 0.2*
HR	Control	128 ± 7	144 ± 8*	167 ± 8*	180 ± 7*	199 ± 10*	229 ± 8*	258 ± 8*
(bpm)	EMD122946	132 ± 6	150 ± 7*	173 ± 8*	191 ± 9*†	208 ± 8*	245 ± 9*†	260 ± 7*
SV	Control	29 ± 2	31 ± 2*	31 ± 2*	32 ± 2*	31 ± 2	30 ± 2	29 ± 2
(ml)	EMD122946	28 ± 2	30 ± 2	32 ± 2*	30 ± 2	30 ± 2	29 ± 2	29 ± 2
$LVdP/dt_{max}$	Control	3170 ± 290	3780 ± 470*	3860 ± 330*	3990 ± 290*	4450 ± 430*	5270 ± 410*	5790 ± 410*
(mmHg/s)	EMD122946	3190 ± 310	3810 ± 330*	3990 ± 380*	4260 ± 350*	4580 ± 410*	5190 ± 420*	5460 ± 470*
MAP	Control	89 ± 2	84 ± 3*	79 ± 2*	81 ± 2*	82 ± 2*	86 ± 3	89 ± 3
(mmHg)	EMD122946	82 ± 3†	73 ± 3*†	72 ± 2*†	74 ± 2*†	76 ± 2†	79 ± 2†	80 ± 3†
MPAP	Control	13 ± 1	13 ± 2	16 ± 2	17 ± 1*	21 ± 2*	24 ± 2*	28 ± 2*
(mmHg)	EMD122946	15 ± 2	13 ± 1	15 ± 2	17 ± 2	21 ± 2*	25 ± 2*	27 ± 1*
MLAP	Control	3 ± 1	2 ± 2	3 ± 1	4 ± 1	6 ± 1	7 ± 1	9 ± 2*
(mmHg)	EMD122946	1 ± 2	1 ± 2	1 ± 1	2 ± 2	5 ± 2	7 ± 2*	8 ± 2*
CBF	Control	51 ± 5	63 ± 6*	69 ± 6*	75 ± 6*	87 ± 9*	105 ± 10*	123 ± 6*
(ml/min)	EMD122946	56 ± 5	69 ± 5*	73 ± 6*	83 ± 8*†	96 ± 10*	116 ± 10*†	126 ± 5*

*Data are mean ± SE; n=8. CO, cardiac output; HR, heart rate; SV, stroke volume; LVdPdtmax, maximum rate of rise in left ventricular pressure; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure; CBF, coronary blood flow. *P<0.05 vs rest (Lying), † P<0.05 vs corresponding control measurement*

Systemic circulation

Exercise produced an increase in cardiac output that was principally mediated by the increase in heart rate; LVdP/dtmax almost doubled (Table 1). Mean aortic pressure decreased significantly when animals went from lying to standing, but remained virtually unchanged thereafter despite the increases in cardiac output, reflecting the decrease in systemic vascular resistance (Fig. 3).

Blockade of ET_A or ET_A/ET_B receptors resulted in a decreased mean aortic pressure (Table 1 and 2), which was accompanied by a small, likely baroreceptor reflex mediated, increase in heart rate. Since cardiac output was maintained it follows that the decrease in pressure was the result of the decrease in systemic vascular resistance, which was similar for ET_A and ET_A/ET_B blockade (Fig. 3). Systemic vasodilation was also reflected in the decreased body O_2-extraction and increased mixed venous O_2-saturation (Fig. 3). These effects of ET_A and ET_A/ET_B blockade waned during exercise, indicating that the constrictor influence of ET was blunted during exercise.

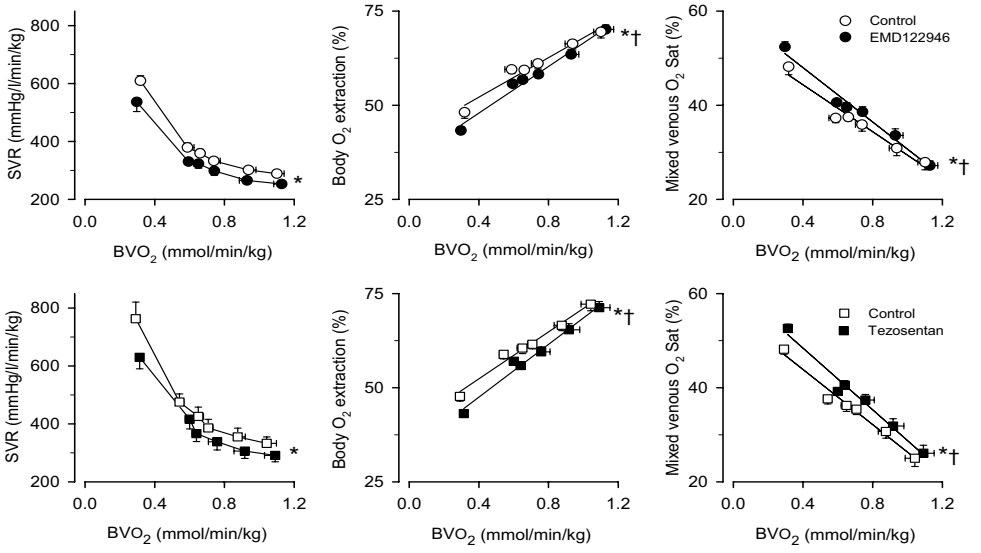

***Figure 3**. Effect of ET_A (EMD122946, upper panels) and ET_A/ET_B (Tezosentan, lower panels) receptor blockade on the relation between body O_2-consumption (BVO_2) and systemic vascular resistance (SVR, left panels), body O_2-extraction (middle panels) and mixed venous O_2 saturation (right panels). ET receptor blockade resulted in systemic vasodilation at rest, that waned during exercise. Data are mean±SE, *P<0.05 vs Control, †P<0.05 effect decreases during exercise*

Coronary circulation

The increase in myocardial O_2-consumption during exercise was accommodated by an increase in coronary blood flow (Table 1), so that myocardial O_2-extraction was maintained constant at approximately 80% (Fig. 4). ET_A blockade resulted in a decreased coronary resistance and, as a result of this vasodilator effect, increased myocardial O_2-supply allowing a decrease in myocardial O_2-extraction and leading to an increased coronary venous O_2-saturation (Fig. 4). The effect of ET_A blockade waned during exercise.

Combined ET_A/ET_B blockade also resulted in a decrease in coronary resistance thereby increasing myocardial O_2-supply and causing a decrease in myocardial O_2-extraction at rest (Fig. 4). The effect of ET_A/ET_B blockade disappeared during exercise. The effects of ET_A/ET_B blockade were smaller than those of ET_A blockade, particularly during exercise.

Pulmonary circulation

Exercise resulted in a doubling of pulmonary artery pressure and a three-fold increase in left atrial pressure. The transpulmonary pressure gradient increased almost in parallel with cardiac output so that pulmonary vascular resistance decreased by less than 10 % (P=NS).

Table 2. Effect of ET_A/ET_B blockade on hemodynamic responses to graded treadmill exercise

		Rest		Exercise level (km/h)				
	Treatment	Lying	Standing	1	2	3	4	5
CO	Control	3.4 ± 0.2	4.4 ± 0.2*	4.9 ± 0.2*	5.7 ± 0.3*	6.1 ± 0.3*	6.9 ± 0.4*	7.5 ± 0.2*
(l/min)	Tezosentan	3.8 ± 0.3	4.4 ± 0.3*	5.2 ± 0.3*	5.7 ± 0.3*	6.5 ± 0.4*	7.3 ± 0.5*	7.9 ± 0.3*
HR	Control	124 ± 5	147 ± 3*	168 ± 6*	185 ± 8*	200 ± 9*	233 ± 12*	263 ± 3*
(bpm)	Tezosentan	149 ± 5†	165 ± 7*†	182 ± 4*†	195 ± 5*	219 ± 8*†	247 ± 12*†	270 ± 7*
SV	Control	28 ± 1	30 ± 1	29 ± 1	31 ± 1*	31 ± 1*	30 ± 1	28 ± 1
(ml)	Tezosentan	26 ± 2	27 ± 1†	28 ± 1*	29 ± 1*	30 ± 1*	29 ± 1	29 ± 1
$LVdP/dt_{max}$	Control	2990 ± 140	3850 ± 260*	4140 ± 270*	4620 ± 390*	4890 ± 440*	5610 ± 480*	6110 ± 330*
(mmHg/s)	Tezosentan	3650 ± 320	4170 ± 380	4240 ± 250*	4590 ± 320*	5250 ± 530	5440 ± 450*	5970 ± 290*
MAP	Control	100 ± 4	91 ± 4	90 ± 3*	93 ± 3*	90 ± 3*	94 ± 3	95 ± 4
(mmHg)	Tezosentan	92 ± 3†	82 ± 3†	81 ± 2*†	80 ± 2*†	83 ± 3†	84 ± 2†	87 ± 3†
PMAP	Control	16 ± 1	14 ± 2	18 ± 1	21 ± 2*	22 ± 2*	27 ± 2*	32 ± 2*
(mmHg)	Tezosentan	15 ± 2	10 ± 2*	15 ± 1	16 ± 2†	20 ± 2*†	22 ± 2*†	26 ± 2*†
LAP	Control	5 ± 0	1 ± 3	2 ± 1*	5 ± 1	5 ± 1	7 ± 1	10 ± 3*
(mmHg)	Tezosentan	3 ± 2†	-3 ± 1*	2 ± 0	2 ± 1†	5 ± 1	6 ± 1*	9 ± 1*
CBF	Control	51 ± 3	66 ± 5*	77 ± 6*	88 ± 7*	93 ± 7*	112 ± 9*	134 ± 5*
(ml/min)	Tezosentan	59 ± 6	67 ± 3	75 ± 6*	83 ± 7*	100 ± 8*	120 ± 11*	139 ± 3*

*Data are mean ± SE; n=7. *P<0.05 vs rest (Lying), † P<0.05 vs corresponding control measurement*

ET_A blockade had no effect on pulmonary artery pressure, left atrial pressure or cardiac output (Table 1), so that pulmonary vascular resistance was unchanged (Fig. 5). In contrast, ET_A/ET_B blockade reduced pulmonary artery pressure during exercise, whereas left atrial pressure and cardiac output remained unaffected (Table 2), reflecting a decrease in pulmonary vascular resistance (Fig. 5).

Discussion

The major findings in this study are that: (*i*) endogenous ET contributes to vascular tone in the systemic, coronary and pulmonary vasculature; (*ii*) the vasoconstrictor effect of ET on the systemic and coronary vasculature is mediated through the ET_A receptor, whereas it is mediated through the ET_B receptor in the pulmonary vasculature; (*iii*) in contrast, the ET_B receptor exerts a vasodilator influence on the coronary circulation; (*iv*) although arterial ET levels do not change during exercise, its vasoconstrictor influence on the systemic and particularly the coronary resistance vessels decreases, whereas its vasoconstrictor influence on the pulmonary resistance vessels increases.

Methodological considerations

Efficacy and selectivity of ET receptor antagonists. Both EMD122946 and tezosentan virtually abolished the ET-1 induced pressor response in resting swine. These observations demonstrate that receptor blockade was effective during a 30 minute period, which encompasses the duration of the exercise protocol and that the pressor response to exogenous ET-1 was principally mediated via ET_A stimulation.

The selectivity of EMD122946 and tezosentan for the ET_A and the ET_B receptor has been studied in vitro. EMD122946 has a 3200-fold selectivity for ET_A compared to ET_B receptors [24], whereas Tezosentan has only a 63-fold selectivity for ET_A compared to ET_B receptors [6, 25]. In accordance with these in vitro studies, we found that ET levels rose after tezosentan, but not after EMD122946. The increase in ET levels with tezosentan is due to the blockade of ET_B receptors in the lungs, which are responsible for clearance of ET [29]. The absence of an increase in ET-levels with EMD122946, indicates that this compound has no ET_B receptor blocking properties in the dose employed in the present study in vivo.

Rationale for using ET_A and ET_A/ET_B receptor antagonists to assess the functional role of ET_B receptors. ET is preferentially released on the abluminal side of the vasculature. Thus, plasma ET levels may not adequately reflect the

contribution of ET in the regulation of vascular tone. Hence, the most adequate way to measure the contribution of ET to the regulation of vascular tone is the use of ET receptor antagonists. Since the ET_B receptor is responsible for clearance of ET in the lungs [29], we chose to use a selective ET_A and a mixed ET_A/ET_B antagonist to determine the role of the ET_A and ET_B receptor, rather than a selective ET_B antagonist. Selective ET_B blockade would increase ET levels, which could then cause vasoconstriction through the ET_A receptor, thereby confounding interpretation of the findings. When ET levels rise in the presence of ET_A/ET_B blockade, this will not affect vascular tone. Thus, the role of the ET_B receptor must be derived from the difference in response between selective ET_A blockade and combined ET_A/ET_B blockade.

Role of ET in the regulation of tone at rest and during exercise

Coronary circulation. The normal heart is characterized by a high level (80%) of myocardial O_2-extraction under basal resting conditions [30, 31]. Consequently, the ability of the coronary resistance vessels to dilate in response to increments

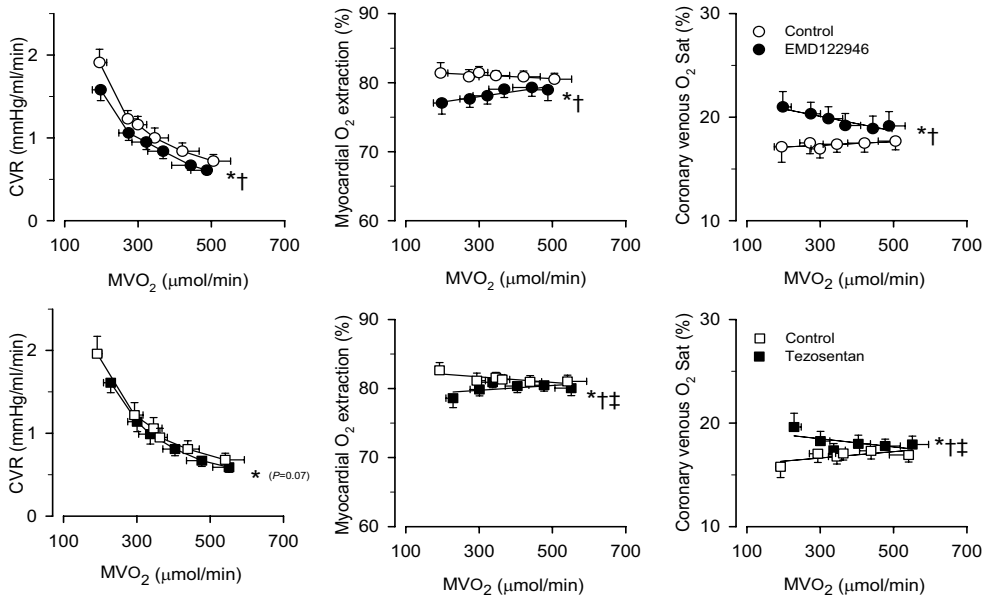

***Figure 4**. Effect of ET_A (EMD122946, upper panels) and ET_A/ET_B (Tezosentan, lower panels) receptor blockade on the relation between myocardial O_2-consumption (MVO_2) and coronary vascular resistance (CVR, left panels), myocardial O_2-extraction (middle panels) and coronary venous O_2-saturation (right panels). ET receptor blockade resulted in coronary vasodilation at rest, that waned during exercise. Data are mean±SE, *P<0.05 vs Control, †P<0.05 effect of receptor blockade decreases during exercise, ‡P<0.05 vs EMD122946-induced response.*

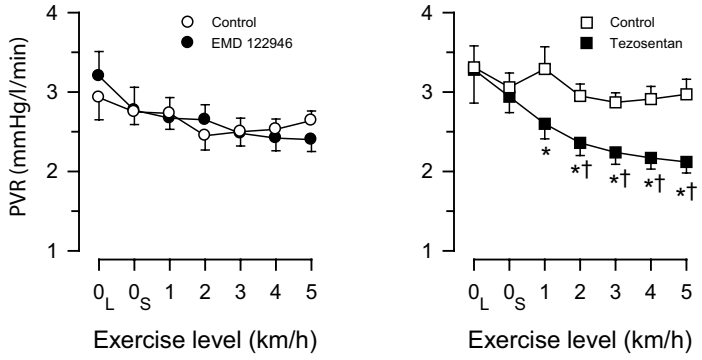

***Figure 5**. Effect of ET_A (EMD122946, left panel) and ET_A/ET_B (Tezosentan, right panel) blockade on pulmonary vascular resistance (PVR). ET_B blockade resulted in pulmonary vasodilation during exercise.Data are mean±SE, 0_L:lying; 0_S: standing; *P<0.05 vs Control, †P<0.05 vs lying*

in myocardial O_2-demand is extremely important to maintain an adequate O_2-supply. A sensitive way to study alterations in coronary vascular tone in relation to myocardial metabolism is the relationship between coronary venous O_2-content and myocardial O_2-demand. Thus, an increase in coronary resistance vessel tone will limit CBF and hence myocardial O_2-supply at a given level of myocardial O_2 consumption, forcing the myocardium to increase its myocardial O_2-extraction (in order to meet myocardial O_2-demand), which results in a lower coronary venous O_2-content. Conversely, a decrease in resistance vessel tone increases myocardial O_2-supply at a given level of myocardial O_2-consumption resulting in an increased coronary venous O_2-content. The coronary venous O_2-content thus represents an index of myocardial tissue oxygenation (i.e. the balance between myocardial O_2-supply and O_2-demand) which is determined by the coronary resistance vessel tone. Using this approach, several laboratories have indicated roles for myriad vasodilator systems such as NO, adenosine, K^+_{ATP} channels and β-adrenoceptors in metabolic vasodilation [22, 23, 26, 27, 30-33].

In the present study, we extended the investigation of metabolic vasodilation to the contribution of withdrawal of the endothelin-induced vasoconstriction. Using the myocardial O_2 balance, we demonstrated ET_A-mediated coronary constriction and simultaneous ET_B-mediated coronary vasodilation in swine. This is in accordance with studies that have shown that the constrictor responses of coronary conductance and resistance arteries are predominantly ET_A-mediated [34-37], whereas ET_B-mediated vasodilation has been found in the large coronary arteries [38, 39] and coronary arterioles [40], although an ET_B-mediated constrictive component also exists in large coronary arteries [36, 41]. Overall, dose response curves to ET in isolated coronary arteries [20] and arterioles [42] indicate that the sensitivity for ET increases with decreasing vessel size.

In dogs, the effect of ET_A/ET_B blockade on coronary vascular tone tended to decrease during incremental levels of exercise [6]. This led us to hypothesize that there is a role for withdrawal of the vasoconstrictor effect of ET in metabolic regulation of coronary vascular tone. Indeed we recently showed that the effect of ET_A blockade on the coronary circulation decreased during exercise and that the effects of ET were modified by the cardiomyocytes according to their metabolic status [11]. However, in the present study we found that only the ET_A-mediated vasoconstriction waned during exercise, whereas an ET_B-mediated vasodilation was tonically present. Although a decrease in local ET release, for example due to an increased NO production during exercise [2, 43] could theoretically have contributed to metabolic coronary vasodilation, this is unlikely. Thus, neither coronary arterial nor coronary venous ET-levels changed during exercise, which is in agreement with the study of Takamura et al [6]. Moreover, the vasodilator influence of the ET_B receptor was constant despite a decrease in the vasoconstrictor influence of the ET_A receptor. There are several other mechanisms that may account for exercise-induced modulation of the effects of ET. First, interstitial adenosine levels increase during exercise, which can decrease sensitivity of the vasculature to ET [21]. Second, NO production increases during exercise, which can directly modulate the binding of ET to the ET_A receptor [44, 45]. NO has been shown to induce depalmitoylation of the β-adrenoceptor thereby affecting its signaling [46] and may also result in depalmitoylation of the ET_A receptor, thereby decreasing its signal transduction [45, 47, 48]. Thus, ET_A receptor sensitivity may be decreased during exercise through an increase in NO, adenosine or both, thereby facilitating metabolic vasodilation.

Systemic circulation. In the systemic circulation, effects of endogenous ET were predominantly exerted through the ET_A receptor with no net contribution of the ET_B receptor. In addition, the vasoconstriction caused by exogenously administered ET was predominantly ET_A-mediated since the increase in aortic blood pressure was abolished by both the ET_A and the ET_A/ET_B antagonists. Hence, in the systemic vascular bed the effects of both endogenous and exogenous ET appear to be mediated exclusively through the ET_A receptor. These findings are in line with other studies demonstrating that the constrictor response of systemic conductance and resistance arteries is principally ET_A-mediated [34-36]. Although there was no net contribution of endogenous ET through the ET_B receptor in our study, we cannot exclude that some regional vascular beds exhibit ET_B mediated constriction whereas others vasodilate, as suggested by studies showing an ET_B-mediated constrictive component in the renal arteries [36, 41] as well as ET_B-mediated vasodilation in the systemic circulation [49].

In the present study, we found that the contribution of ET to overall systemic vascular tone decreased during exercise. From our experiments, it is impossible to elucidate the mechanism behind the blunted effect of ET during exercise. The ET levels in arterial blood did not change, which is in accordance with data obtained in humans [13, 15, 18], dogs [6, 50], and swine [14]. However, in view of the preferentially abluminal release of ET, this observation does not exclude alterations in the contribution of ET to regulation of resistance vessel tone. Thus, plasma concentrations of ET may not adequately reflect local ET levels. Furthermore, ET binds tightly to its receptors and the rate of dissociation is low [1-3], so that locally secreted ET binds to its receptors and acts, with minimal accumulation in the blood [51]. Further support for a discrepancy between local and arterial ET-levels is provided by the fact that although the ET-levels in blood were below the vasoactive threshold [20, 42], blockade of ET receptors resulted in vasodilation at rest, an observation that is corroborated by studies in dogs [6] and humans [8-10]. However, spill-over into the blood may occur. For example, Maeda et al reported that during one-leg exercise, ET-levels increased in the venous effluent from the non-working leg, while venous ET-levels were unchanged in the exercising leg [19]. Hence, ET production may vary locally according to the metabolic status of the tissue [18, 52, 53]. Alternatively, local increases in NO and adenosine during exercise may have decreased ET_A receptor signal transduction, and thereby modulated the constriction to ET during exercise [21, 44, 46-48]. Thus, local modulation of ET release and/or receptor sensitivity at sites of increased metabolism provides an additional way to increase flow to areas that need it most, while tone remains intact in other areas, thereby ensuring adequate (re)distribution of flow commensurate with metabolic needs.

Pulmonary Circulation. ET-induced constriction is mediated by ET_A receptors in the large pulmonary arteries, whereas it is mediated by ET_B receptors in the smaller pulmonary resistance vessels [54]. In accordance with these findings, the density of ET_A receptors in the lung decreases with decreasing vessel size, whereas the density of ET_B receptors, both on the endothelium and the smooth muscle increases [55]. In swine, exogenously administered ET induces transient pulmonary hypotension (endothelial ET_B receptor), followed by sustained pulmonary hypertension (ET_B receptor on vascular smooth muscle) [49]. The role of endogenous ET in the regulation of pulmonary resistance is less well understood. In some studies in the porcine and human circulation, ET receptor blockade had no effect [7, 56], while a small vasodilator response was reported in other studies [12, 57]. In our study, blockade of neither ET_A nor ET_B receptors affected pulmonary vascular resistance at rest, indicating that endogenous ET does not contribute to

resting tone in the pulmonary resistance vessels. During exercise however, an ET_B-mediated vasoconstriction became apparent, which contrasts with the blunted ET-mediated constriction in the systemic and coronary beds. Since hypoxia-induced pulmonary vasoconstriction is in part ET-mediated [56, 57], it could be speculated that the decrease in pulmonary arterial PO_2 which occurred during exercise may have contributed to the ET_B mediated vasoconstriction.

Conclusions and implications

In awake swine, free from the effects of anesthesia, endothelin contributes to basal resting tone in resistance vessels of the systemic and coronary, but not the pulmonary circulation. During treadmill exercise, the ET_A-mediated constriction of both the systemic and coronary bed wanes, thereby contributing to metabolic vasodilation in these beds. A tonic ET_B-mediated vasodilator influence is present in the coronary circulation. In contrast, an ET_B-mediated constriction becomes apparent in the pulmonary bed during exercise, which blunts the exercise-induced decrease in pulmonary vascular resistance.

The present study provides evidence for a novel concept of metabolic dilation in the systemic and coronary circulation during exercise, which involves not only increased vasodilator influences [22, 23, 26, 27, 30, 31], but also inhibition of vasoconstrictor influences. Importantly, our study predicts that loss of the capacity to inhibit ET mediated vasoconstrictor influence (e.g. in situations of endothelial dysfunction) may result in impaired vasodilation during increased O_2-demand. In support of this concept, McEniery et al [58] recently showed that ET receptor blockade normalized the impaired exercise-induced vasodilation in the fore-arm of hypertensive humans, whereas ET blockade had no effect on exercise responses in normotensive subjects.

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Chapter 4

Nitric oxide blunts the endothelin-mediated pulmonary vasoconstriction in exercising swine

Birgit Houweling, Daphne Merkus, Marjolein M D Dekker and Dirk J Duncker
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Abstract

We have previously shown that vasodilators and vasoconstrictors that are produced by the vascular endothelium, including nitric oxide (NO), prostanoids and endothelin (ET), contribute to the regulation of systemic and pulmonary vascular tone in swine, in particular during treadmill exercise. Since NO and prostanoids can modulate the release of ET, and vice versa, we investigated the integrated endothelial control of pulmonary vascular resistance in exercising swine. Specifically, we tested the hypothesis that increased NO and prostanoid production during exercise limits the vasoconstrictor influence of ET, so that loss of these vasodilators results in exaggerated ET-mediated vasoconstriction during exercise. Fifteen instrumented swine were exercised on a treadmill at 0-5 $km \cdot h^{-1}$ before and during ET_A/ET_B receptor blockade (tezosentan, 3$mg \cdot kg^{-1}$ iv) in the presence and absence of inhibition of NO synthase (N^{ω}-nitro-L-arginine, 20 $mg \cdot kg^{-1}$ iv) and/or cyclo-oxygenase (indomethacin, 10 $mg \cdot kg^{-1}$ iv). In the systemic circulation, ET receptor blockade decreased vascular resistance at rest, which waned gradually with increasing exercise intensity. Prior inhibition of either NO or prostanoid production augmented the vasodilator effect of ET receptor blockade, and these effects were additive. In contrast, in the pulmonary bed, ET-receptor blockade had no effect under resting conditions, but decreased pulmonary vascular resistance during exercise. Prior inhibition of NO synthase, enhanced the pulmonary vasodilator effect of ET receptor blockade both at rest and during exercise, whereas inhibition of prostanoids had no effect, even after prior NO synthase inhibition. In conclusion, endogenous endothelin limits pulmonary vasodilation in response to treadmill exercise. This vasoconstrictor influence is blunted by NO but not by prostanoids.

Introduction

The pulmonary vascular bed is a low-resistance system that is capable of accommodating large amounts of blood flow at low levels of pulmonary artery pressure [1]. Consequently, under basal resting conditions the normal arterio-venous pressure difference across the pulmonary vascular bed is approximately 10 mmHg, which contrasts sharply with the 90-100 mmHg arterio-venous pressure gradient across the systemic bed. Despite the low pulmonary vascular resistance under resting conditions, a small further decrease in pulmonary resistance occurs during exercise, albeit significantly less (10-30%) than the 60-80% decrease in systemic vascular resistance [1]. The exercise-induced decrease in pulmonary vascular resistance involves both passive pulmonary vasodilation, including vascular recruitment and passive distension due to the exercise-induced increase in pulmonary artery pressure, as well as an active reduction in pulmonary vasomotor tone [1]. The mechanism of vasomotor tone regulation in pulmonary resistance vessels during exercise is still incompletely understood, but differs from that in the systemic vascular bed. For example, while endogenous adenosine and K^{+}_{ATP} channel activity have been shown to exert a vasodilator influence in the systemic bed during treadmill exercise, they are not mandatory for the regulation of tone in pulmonary resistance vessels [2, 3].

The vascular endothelium releases a variety of vasoactive substances, including nitric oxide (NO), prostanoids and endothelin (ET), that contribute to vasomotor control. However, the endothelial lining is not a homogeneous compartment as it is characterized by significant structural and functional heterogeneity. For example, the endothelium in the pulmonary bed differs markedly in ultrastructure and function from the systemic endothelium [4, 5]. In support of this concept, recent studies in swine indicate that while both NO and prostanoids exert a vasodilator influence on the systemic vascular bed during exercise, only NO, but not prostanoids, contributes to the exercise-induced pulmonary vasodilation [6, 7]. Furthermore, we recently observed in exercising swine that in the systemic circulation, the vasoconstrictor influence of ET wanes with increasing exercise intensities, whereas in the pulmonary circulation an ET vasoconstrictor influence emerges during exercise [8]. Since ET can increase the production of NO and prostanoids, which in turn can blunt the release of ET [9-11] or modify the responsiveness of its receptors [12], the present study was undertaken to investigate the integrated vasomotor control of pulmonary vascular resistance by NO, prostanoids and ET, in chronically instrumented swine under resting conditions and during graded treadmill exercise.

Methods

Animals

Studies were performed in accordance with the Council of Europe Convention (ETS123)/ Directive (86/609/EEC) for the protection of vertebrate animals used for experimental and other scientific purposes, and with approval of the Animal Care Committee of the Erasmus Medical Center. Fifteen 2-3-month-old Yorkshire X Landrace swine (22±1 kg at the time of surgery) of either sex entered the study.

Surgery

Swine were sedated with ketamine (30 $mg \cdot kg^{-1}$ im), anaesthetized with thiopental (10 $mg \cdot kg^{-1}$ iv), intubated and ventilated with a mixture of O_2 and N_2O (1:2) to which 0.2-1% (v/v) isoflurane was added [3, 13]. Anaesthesia was maintained with midazolam (2 $mg \cdot kg^{-1}$ + 1 $mg \cdot kg^{-1}$ per hour iv) and fentanyl (10 $\mu g \cdot kg^{-1}$ per hour iv). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid-filled polyvinylchloride catheter was inserted into the aortic arch for aortic blood pressure measurement (Combitrans pressure transducers, Braun) and blood sampling. An electromagnetic flow probe (14-15 mm, Skalar) was positioned around the ascending aorta for measurement of cardiac output. Polyvinylchloride catheters were inserted into the left atrium to measure pressure, and into the pulmonary artery to measure pressure, administer drugs and collect mixed venous blood samples. Catheters were tunnelled to the back and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine im) for 2 days and antibiotic prophylaxis (25 $mg \cdot kg^{-1}$ amoxicillin and 5 $mg \cdot kg^{-1}$ gentamycin iv) for 5 days.

Experimental protocols

Studies were performed 1-3 weeks after surgery with animals exercising on a motor driven treadmill. The excellent reproducibility of consecutive exercise trials has been reported previously [2, 3, 7, 13]. In the present study, four exercise protocols were performed on different days and in random order.

Endothelin. With swine (n=11) lying quietly on the treadmill, resting hemodynamic measurements, consisting of heart rate, cardiac output, mean aortic pressure (MAP), mean pulmonary artery pressure (MPAP), and mean left atrial pressure (MLAP) were obtained and blood samples collected. Hemodynamic measurements were repeated and rectal temperature was measured with animals standing on the treadmill. Subsequently, a five-stage (1-5 $km \cdot h^{-1}$) treadmill

exercise protocol was started; each exercise stage lasted 2-3 min. Hemodynamic variables were continuously recorded and blood samples collected during the last 45 s of each stage. After completing the exercise protocol animals were allowed to rest on the treadmill for 90 min after which the mixed ET_A and ET_B receptor (ET_A/ET_B) antagonist tezosentan (a gift from Dr Clozel, Actelion Pharmaceuticals Ltd.) was intravenously administered over 10 min in a dose of 3 mg·kg^{-1}, followed by a continuous infusion of 6 mg·kg^{-1}·h^{-1} iv [8], and the exercise protocol was repeated.

Prostanoids and endothelin. Ninety min after 9 swine had undergone a control exercise trial (as described above), animals received the cyclo-oxygenase inhibitor indomethacin ((Sigma) 10 mg·kg^{-1} iv over 10 min [6]) and 5 min later underwent a second exercise trial. Ninety min later, animals received indomethacin in a dose of 5 mg·kg^{-1} iv, which resulted in hemodynamic conditions that were identical to those following administration of 10 mg·kg^{-1} prior to the second exercise trial. Subsequently, animals received tezosentan (3 mg·kg^{-1} iv + 6 mg·kg^{-1}·h^{-1} iv) and underwent a third exercise trial.

NO and endothelin. Ninety min after 7 swine had undergone a control exercise trial, animals received the NO-synthase inhibitor N$^{\omega}$-nitro-L-arginine (NLA (Sigma),

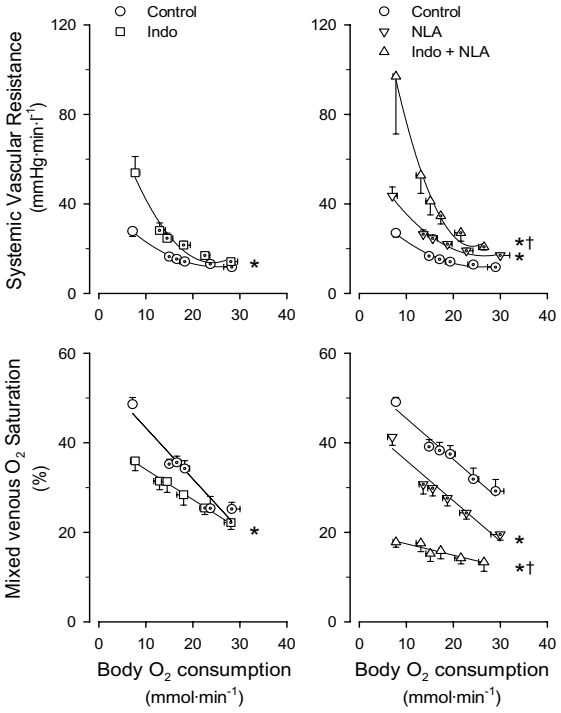

***Figure 1**. Effect of cyclo-oxygenase inhibition (indomethacin (Indo), left panels) and NO synthase inhibition (NLA, right panels) and combined inhibition of cyclo-oxygenase and NO synthase (Indo + NLA, right panels) on the relation between body O_2 consumption and systemic vascular resistance (upper panels) and the mixed venous O_2 saturation (lower panels). Dot inside symbol denotes P<0.05 vs corresponding value at rest, *P<0.05 vs control, †P<0.05 vs NLA*

20 mg·kg^{-1} iv; [7]), and underwent a second exercise trial. Ninety min later, animals received tezosentan (3 mg·kg^{-1} iv + 6 mg·kg^{-1}·h^{-1} iv) and underwent a third exercise trial.

NO, prostanoids and endothelin. Ninety min after 5 swine underwent an exercise trial in the presence of NLA (20 mg·kg^{-1} iv), animals received indomethacin (10 mg·kg^{-1} iv) and underwent a second exercise trial. Ninety min later, animals received indomethacin (5 mg·kg^{-1} iv) and tezosentan (3 mg·kg^{-1} iv and 6 mg·kg^{-1}·h^{-1} iv) and underwent a third exercise trial.

Blood gas measurements

Blood samples were kept in iced syringes until the conclusion of each exercise trial. Measurements of PO_2 (mmHg), PCO_2 (mmHg) and pH were then immediately performed with a blood gas analyser (Acid-Base Laboratory Model 505, Radiometer, Copenhagen, Denmark). Oxygen saturation (%) and hemoglobin (grams per 100 ml) were measured with a hemoximeter (OSM3, Radiometer). Blood O_2 content (mmol·ml^{-1}) was computed as (Hb·0.621·O_2-saturation) + (0.00131·PO_2). Body O_2 consumption (BVO_2) was calculated as the product of cardiac output and the difference in O_2 content between arterial and mixed venous blood [3, 13].

Data analysis

Digital recording and off-line analysis of hemodynamics have been described previously [2, 13]. Systemic vascular resistance was computed as mean aortic blood pressure divided by cardiac output. Pulmonary vascular resistance was computed as mean pulmonary artery pressure minus mean left atrial pressure divided by cardiac output [6].

Statistical analysis

Analysis of variance (ANOVA) for repeated measures or analysis of co-variance (ANCOVA) were used as appropriate. Post-hoc testing for exercise and drug effect was performed using Scheffe's test. Statistical significance was accepted when $P<0.05$. Data are presented as mean±SEM.

Results

The role of prostanoids and NO in the regulation of vascular tone

Systemic circulation. Table 1 shows that exercise up to 5 km·h-1 resulted in more than a doubling of cardiac output which was principally due to an increase in heart rate (up to 85% of maximum heart rate), as stroke volume increased by

Table 1: *Hemodynamics of swine at rest and during exercise*

		Rest		Exercise level (km·h^{-1})				
	Treatment	lying	standing	1	2	3	4	5
HR	Control	126 ± 5	147 ± 5*	169 ± 6*	186 ± 5*	202 ± 6*	232 ± 8*	265 ± 5*
(bpm)	Tezo	150 ± 5‡	163 ± 5*‡	184 ± 6*	198 ± 8*	214 ± 7*‡	243 ± 9*‡	267 ± 6*
	Indo	86 ± 7†	97 ± 5*†	121 ± 6*†	134 ± 6*†	150 ± 6*†	174 ± 6*†	204 ± 7*†
	Indo+Tezo	97 ± 7†‡	112 ± 5*†‡	128 ± 6*†	141 ± 6*†	157 ± 7*†	178 ± 8*†	212 ± 9*†
	NLA	97 ± 5†	115 ± 3*†	129 ± 6*†	139 ± 6*†	156 ± 6*†	190 ± 9*†	225 ± 9*†
	NLA+Tezo	112 ± 6†	136 ± 7*†‡	146 ± 8*†‡	154 ± 7*†‡	176 ± 8*†‡	201 ± 8*†‡	234 ± 10*†
	NLA+Indo	82 ± 7†	93 ± 8*†	110 ± 8*†	122 ± 12*†	134 ± 10*†	164 ± 13*†	193 ± 12*†
	NLA+Indo+Tezo	100 ± 10†	116 ± 8*†‡	127 ± 6*†‡	138 ± 7*†	153 ± 13*†‡	171 ± 11*†	194 ± 10*†
CO	Control	3.6 ± 0.2	4.5 ± 0.2*	5.3 ± 0.3*	5.9 ± 0.3*	6.4 ± 0.3*	7.1 ± 0.3*	7.8 ± 0.4*
(l·min^{-1})	Tezo	4.0 ± 0.2‡	4.7 ± 0.3*	5.5 ± 0.3*	6.1 ± 0.4*	6.8 ± 0.4*‡	7.6 ± 0.4*‡	8.2 ± 0.4*
	Indo	2.6 ± 0.3†	3.2 ± 0.3*†	4.1 ± 0.3*†	4.5 ± 0.3*†	5.1 ± 0.3*†	6.2 ± 0.3*†	7.2 ± 0.3*
	Indo+Tezo	3.0 ± 0.2†	3.8 ± 0.3*†‡	4.4 ± 0.3*†	5.2 ± 0.4*‡	5.6 ± 0.4*‡	6.4 ± 0.4*	7.5 ± 0.5*
	NLA	3.1 ± 0.4†	3.8 ± 0.2*†	4.5 ± 0.3*†	4.9 ± 0.3*†	5.4 ± 0.3*†	6.4 ± 0.4*†	7.1 ± 0.4*†
	NLA+Tezo	3.8 ± 0.4‡	4.7 ± 0.4*‡	5.2 ± 0.4*†‡	5.6 ± 0.4*†‡	6.4 ± 0.3*†‡	7.3 ± 0.4*†‡	8.2 ± 0.5*‡
	NLA+Indo	2.0 ± 0.3†	2.3 ± 0.3†	3.1 ± 0.3*†	3.7 ± 0.4*†	4.1 ± 0.3*†	5.1 ± 0.5*†	6.0 ± 0.3*
	NLA+Indo+Tezo	3.1 ± 0.3	3.8 ± 0.4‡	4.2 ± 0.3*‡	4.9 ± 0.3*	5.2 ± 0.3*‡	5.8 ± 0.3*	6.6 ± 0.4*‡
MAP	Control	97 ± 3	90 ± 3*	89 ± 2*	90 ± 3*	89 ± 2*	92 ± 2*	93 ± 2
(mmHg)	Tezo	91 ± 2	82 ± 3*‡	81 ± 2*‡	79 ± 2*‡	82 ± 2*‡	83 ± 2*‡	85 ± 2*‡
	Indo	124 ± 7†	120 ± 7†	107 ± 6*†	106 ± 6*†	107 ± 5*†	104 ± 5*	101 ± 4*
	Indo+Tezo	111 ± 3†	93 ± 3*†‡	89 ± 2*†‡	89 ± 3*†‡	89 ± 3*‡	88 ± 3*‡	89 ± 3*‡
	NLA	124 ± 3†	118 ± 3†	115 ± 3†	115 ± 2*†	118 ± 2†	119 ± 2†	119 ± 3†
	NLA+Tezo	112 ± 5†	103 ± 3†‡	99 ± 4*†‡	99 ± 3*†‡	100 ± 3*†‡	98 ± 3*†‡	98 ± 2*†‡
	NLA+Indo	166 ± 8†	163 ± 8†	151 ± 6*†	145 ± 5†	140 ± 3*†	134 ± 4*†	128 ± 5*†
	NLA+Indo+Tezo	128 ± 5†‡	118 ± 7*†‡	113 ± 6*†‡	114 ± 6*†‡	115 ± 7*†‡	113 ± 6*†‡	113 ± 8*†‡
MPAP	Control	15 ± 1	15 ± 2	19 ± 1*	21 ± 1*	23 ± 1*	28 ± 2*	33 ± 1*
(mmHg)	Tezo	16 ± 1	13 ± 2	17 ± 2	18 ± 2	21 ± 2*‡	25 ± 2*‡	28 ± 2*‡
	Indo	19 ± 1	18 ± 2	18 ± 2	20 ± 2	22 ± 2*	26 ± 2*	29 ± 2*
	Indo+Tezo	17 ± 2	14 ± 2*‡	15 ± 2	17 ± 2	20 ± 2	23 ± 2*	26 ± 2*
	NLA	25 ± 2†	22 ± 2	25 ± 2†	27 ± 2†	31 ± 2*†	38 ± 2*†	44 ± 1*†
	NLA+Tezo	18 ± 2	19 ± 2	18 ± 2†‡	21 ± 1‡	25 ± 2*†‡	29 ± 3*‡	33 ± 3*‡
	NLA+Indo	24 ± 2†	26 ± 3†	27 ± 3	29 ± 3	31 ± 3*	35 ± 2*†	37 ± 3*
	NLA+Indo+Tezo	19 ± 2	17 ± 3	20 ± 2	23 ± 3	25 ± 3	28 ± 4*‡	31 ± 3*‡
MLAP	Control	4 ± 1	2 ± 2	3 ± 1	4 ± 1	6 ± 1	7 ± 1*	11 ± 1*
(mmHg)	Tezo	4 ± 1	0 ± 1*	3 ± 1	3 ± 1	6 ± 1	8 ± 1*	10 ± 1*
	Indo	10 ± 2†	7 ± 2	3 ± 2*	6 ± 1*	6 ± 1*	7 ± 1	8 ± 2
	Indo+Tezo	8 ± 1	2 ± 1*‡	3 ± 2*	4 ± 1*	6 ± 1*	7 ± 2	8 ± 2
	NLA	11 ± 1†	5 ± 2*	7 ± 1*	8 ± 1*	9 ± 1*	10 ± 1*	12 ± 1*
	NLA+Tezo	3 ± 3	5 ± 1*	4 ± 2*	7 ± 1*	9 ± 1*	11 ± 2*	13 ± 2*
	NLA+Indo	16 ± 3†	15 ± 4†	13 ± 4	14 ± 3†	14 ± 3†	12 ± 3	10 ± 3
	NLA+Indo+Tezo	7 ± 3‡	4 ± 4	5 ± 2	8 ± 2	9 ± 2	11 ± 3	12 ± 2

*HR: Heart Rate, CO: Cardiac Output, MAP: Mean Arterial Pressure, MPAP: Mean Pulmonary Arterial Pressure, MLAP: Mean Left Atrial Pressure. Data are mean ± SEM; * P<0.05 vs rest (lying); † P<0.05 vs corresponding control, ‡ P<0.05 effect of Tezosentan*

only 15% (not shown). The increase in cardiac output, was balanced by a similar decrease in systemic vascular resistance (Fig. 1), so that mean aortic blood pressure was minimally affected (Table 1).

Administration of the NO synthase inhibitor NLA or the cyclo-oxygenase inhibitor indomethacin resulted in a marked increase in aortic blood pressure, which was due to systemic vasoconstriction (Table 1, Fig. 1). The accompanying decrease in cardiac output resulted from a (probably baroreflex-mediated) decrease in heart rate. The systemic vasoconstriction necessitated an increase in O_2-extraction resulting in a decreased mixed venous O_2-saturation. During exercise, the pressor and vasoconstrictor responses to cyclo-oxygenase inhibition were progressively blunted, whereas the responses to NO synthase inhibition were maintained (Table 1, Fig. 1).

Pretreatment with NLA enhanced the indomethacin-induced vasoconstriction in the systemic circulation, as indicated by the exaggerated increase in systemic vascular resistance and exaggerated decrease in mixed venous O_2-saturation. Importantly, despite the marked potentiation of the systemic vasoconstrictor response to cyclo-oxygenase inhibition by NO synthase inhibition under resting conditions, the exercise-induced vasodilation was unmitigated in the presence of indomethacin and NLA (Fig. 1).

Pulmonary circulation. Pulmonary artery pressure doubled during exercise (Table 1). However, the transpulmonary pressure gradient (MPAP-MLAP) increased slightly less than cardiac output, reflecting a 20% decrease in pulmonary vascular resistance (Fig. 2). In contrast to the systemic bed, inhibition of cyclo-oxygenase

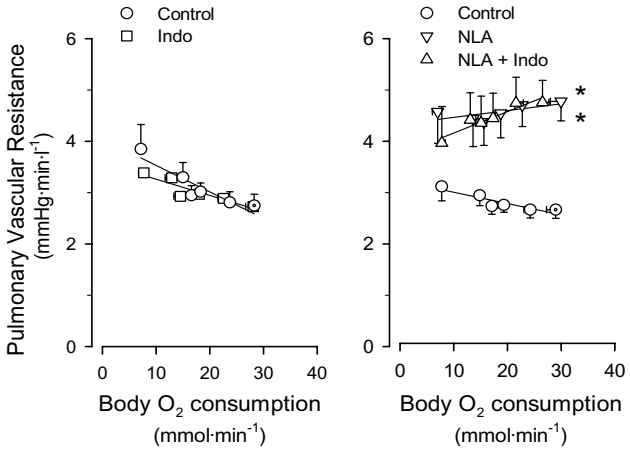

Figure 2: *Effect of cyclo-oxygenase inhibition (Indo, left panels) and NO synthase inhibition (NLA, right panels) and combined inhibition of cyclo-oxygenase and NO synthase (Indo + NLA, right panels) on the relation between body O_2 consumption and pulmonary vascular resistance. Indomethacin had no effect on pulmonary vascular resistance either under control conditions or in the presence of NLA. Dot inside symbol denotes P<0.05 vs rest, *P<0.05 vs control*

had no effect on pulmonary vascular resistance either at rest or during exercise. NO synthase inhibition produced vasoconstriction in the pulmonary circulation, resulting in an increase in pulmonary artery pressure. Subsequent inhibition of cyclo-oxygenase had no additional effect on pulmonary vascular resistance (Fig. 2).

The role of endothelin in the regulation of vascular tone

Systemic circulation. Administration of the mixed ET_A/ET_B antagonist tezosentan resulted in a small decrease in aortic blood pressure under resting conditions (Table 1), which was caused by systemic vasodilation as demonstrated by a decrease of systemic vascular resistance and an increase in mixed venous O_2 saturation (Fig. 3). The vasodilator response to tezosentan waned progressively with incremental exercise intensity. Pretreatment with indomethacin enhanced the systemic vasodilation by tezosentan, particularly at rest. In contrast, pretreatment with the NO synthase inhibitor NLA enhanced the vasodilator response to tezosentan both at rest and during exercise. Finally, combined pretreatment with indomethacin and NLA caused a further increase in the vasodilator responses to tezosentan (Fig. 3). These observations indicate that both prostanoids and NO blunt the endothelin vasoconstrictor influence in the systemic circulation at rest and during exercise.

Pulmonary circulation. Tezosentan had no effect on the pulmonary circulation under resting conditions (Table 1, Fig. 4). In contrast during exercise, tezosentan reduced pulmonary artery pressure, with no effect on left atrial pressure and cardiac output (Table 1), reflecting a decrease in pulmonary vascular resistance (Fig. 4). Pre-treatment with indomethacin did not change the vasodilator response to tezosentan. In contrast, in the presence of NLA, the pulmonary vasodilator response to tezosentan was markedly enhanced. Additional pretreatment with indomethacin did not further enhance pulmonary vasodilation by tezosentan, as compared to NLA alone. These findings indicate that NO, but not prostanoids, blunts the vasoconstrictor response to ET in the pulmonary circulation during exercise.

Discussion

The main findings in the present study in awake swine, free from the effects of anaesthesia and acute surgical trauma are that: (*i*) prostanoids blunt the vasoconstrictor influence of ET in the systemic but not the pulmonary circulation, (*ii*) NO blunts the vasoconstrictor influence of ET in both the systemic and pulmonary circulation, particularly during exercise, (*iii*) prostanoids and NO blunt the vasoconstrictor influence of ET on the systemic bed in an additive manner, but

(*iv*) loss of NO does not unmask a role of prostanoids in blunting the vasoconstrictor influence of ET in the pulmonary circulation.

Pulmonary vascular resistance during exercise

Exercise produced a small decrease in pulmonary vascular resistance, which was likely due to a decrease in vasomotor tone in the pulmonary resistance vessels. Pulmonary vasomotor tone is the resultant of an interplay between vasodilator and vasoconstrictor influences, as is also illustrated by previous findings from our laboratory that α-adrenoceptor blockade [13] and ET-receptor blockade [8] induce pulmonary vasodilation, while β-adrenoceptor blockade [13] as well as NO synthase inhibition [6, 7] result in pulmonary vasoconstriction in exercising pigs. Importantly, the increase in pulmonary vascular resistance produced by blockade of vasodilator pathways was accompanied by an increase in pulmonary arterial pressure. Since an increase in pulmonary arterial pressure would act to cause a passive decrease in resistance, these studies indicate that the increase in pulmonary vascular resistance must have been the result of an increase in vasomotor tone, and further support the concept that the exercise-induced decrease in pulmonary vascular resistance is principally due to a decrease in pulmonary resistance vessel tone [14].

Role of endothelium in regulation of pulmonary vascular resistance

Nitric oxide. The role of endogenous NO in maintaining the low basal pulmonary vascular resistance is species dependent. Thus, while the majority of studies suggest a role for NO in the regulation of basal pulmonary vascular resistance in swine, sheep, horses and humans, most studies do not support such a role of NO in dogs (see [15] for references). The role of NO in the pulmonary vasodilation during exercise is similarly species dependent. Thus, despite causing a significant pulmonary vasoconstriction under resting conditions, NLA had no effect on the exercise-induced pulmonary vasodilation in sheep [16] or horses [17]. In contrast, we have consistently observed that NO synthase inhibition blunts the exercise-induced pulmonary vasodilation in swine [6, 7]. The results of the present study confirm our previous observations and indicate that significant interspecies differences exist with respect to vasomotor control mechanisms within the pulmonary resistance vessels [15].

Prostanoids. Inhibition of endogenous prostanoid production does not alter basal pulmonary vascular resistance in sheep [18] and swine [6], but causes vasoconstriction in dogs [19, 20]. In most species, endogenous prostanoids do also not appear to contribute to the exercise-induced pulmonary vasodilation [6, 18,

Figure 3: *From left to right: effect of endothelin receptor blockade (tezosentan (Tezo)), cyclo-oxygenase inhibition and endothelin receptor blockade (Indo + Tezo), NO synthase inhibition and endothelin receptor blockade (NLA + Tezo) and combined inhibition of cyclo-oxygenase and NO synthase and endothelin receptor blockade (NLA+Indo+Tezo) on the relation between body O_2 consumption and systemic vascular resistance (upper panels) and the mixed venous O_2 saturation (lower panels). *P<0.05 effect of Tezo vs corresponding control, †P<0.05 effect of Tezo in presence of Indo, NLA or NLA+Indo vs Tezo alone, ‡P<0.05 effect of Tezo in presence of NLA or NLA+Indo vs effect of Tezo in the presence of Indo, P<0.05 effect of Tezo in presence of NLA+Indo vs effect of Tezo in the presence of NLA.*

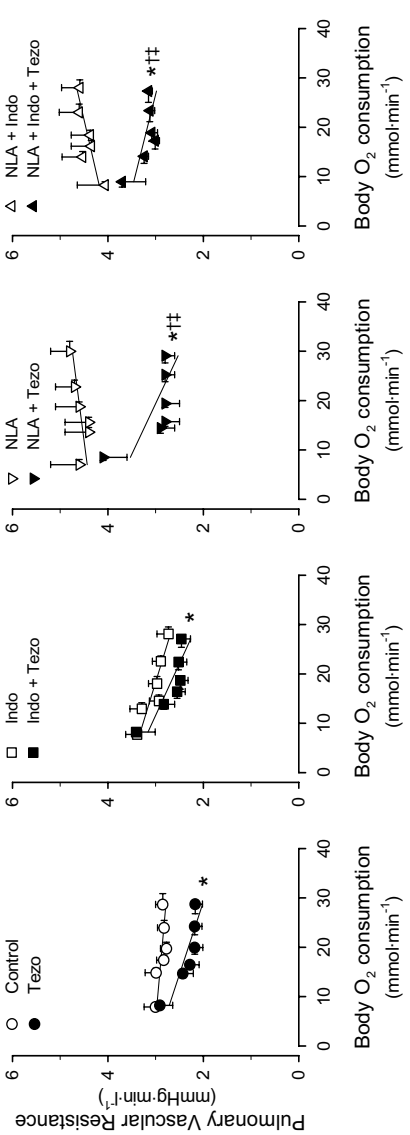

***Figure 4**: From left to right: effect of endothelin receptor blockade (tezosentan (Tezo)), cyclo-oxygenase inhibition and endothelin receptor blockade (Indo + Tezo), NO synthase inhibition and endothelin receptor blockade (NLA + Tezo) and combined inhibition of cyclo-oxygenase and NO synthase and endothelin receptor blockade (NLA + Indo + Tezo) on the relation between body O_2 consumption and pulmonary vascular resistance. Indomethacin had no effect on the response of pulmonary vascular resistance to tezosentan either under control conditions or in the presence of NLA. *P<0.05 effect of Tezo vs corresponding control, †P<0.05 effect of Tezo in presence of Indo, NLA or NLA+Indo vs Tezo alone, ‡P<0.05 effect of Tezo in presence of NLA or NLA+Indo vs effect of Tezo in the presence of Indo.*

19]. It could be argued that the dose of indomethacin used in the present study was not sufficient to inhibit cyclo-oxygenase in the pulmonary circulation. This is unlikely, however, in view of our observation that indomethacin in a dose of 10 $mg \cdot kg^{-1}$ does not produce a greater vasoconstrictor response in the systemic bed than 1 $mg \cdot kg^{-1}$ [6], suggesting a maximal effect. On the other hand, it cannot be excluded that cyclo-oxygenase inhibition may have gone without an apparent effect on pulmonary vascular resistance due to the opposing effects of simultaneously blocking vasodilator and vasoconstrictor prostanoids. Interestingly, the vasoconstrictor response to indomethacin in the systemic circulation was enhanced following NO synthase inhibition, which is likely due to the inhibition of prostacyclin synthase activity by NO and peroxynitrite [21]. In contrast, cyclo-oxygenase inhibition in the presence of NLA still had no effect on pulmonary vascular resistance [22],[6] suggesting that prostacyclin activity was not increased following NO synthase inhibition. Future studies, using thromboxane A_2 receptor antagonists or thromboxane A_2 synthase inhibitors in conjunction with cyclo-oxygenase inhibitors, are required to evaluate in detail the integrated control of pulmonary vascular resistance by vasodilator and vasocontrictor prostanoids during exercise.

Endothelin. ET-induced constriction is mediated by ET_A receptors in the large pulmonary arteries, whereas it is mediated by ET_B receptors in the smaller pulmonary resistance vessels [23]. In accordance with these findings, the density of ET_A receptors in the lung decreases with decreasing vessel size, whereas the density of ET_B receptors, both on the endothelium and the smooth muscle increases [24]. In our study, blockade of both ET_A and ET_B receptors did not affect pulmonary vascular resistance at rest, indicating that endogenous ET does not contribute to resting tone in the pulmonary resistance vessels. During exercise however, an ET-mediated vasoconstriction became apparent, which contrasts with the blunted ET-mediated constriction in the systemic bed [8].

Interactions between NO, prostanoids and endothelin in the regulation of pulmonary vascular resistance

The present study shows that there is an interaction between NO and ET in the pulmonary and the systemic circulation. This interaction was demonstrated by the larger effect on systemic and pulmonary vascular resistance of combined ET_A and ET_B receptor blockade in the presence of NO synthase inhibition, as compared to the effect of combined ET_A and ET_B receptor blockade under control conditions. Possible mechanisms behind this interaction include modification by NO of ET production or ET receptor binding affinity [25, 26]. Indeed Kelly et al [27] showed

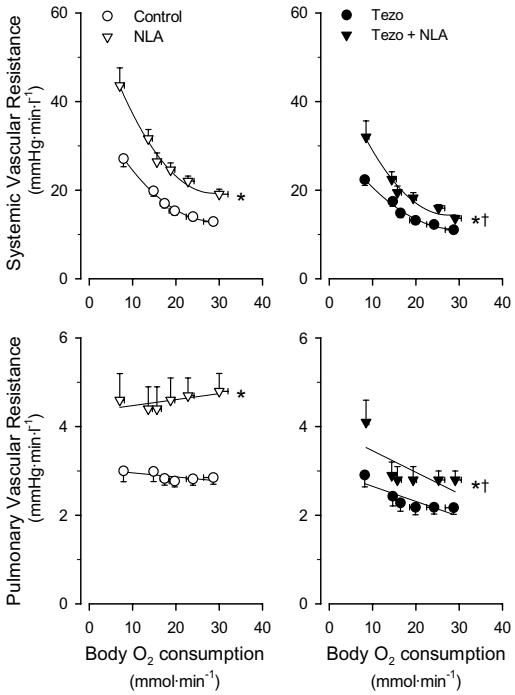

Figure 5: *Effect of NO synthase inhibition (NLA) in the absence (left) and presence (right) of endothelin receptor blockade (Tezo) on the relation between body O_2 consumption and systemic vascular resistance (upper panels) and on the relation between body O_2 consumption and pulmonary vascular resistance (lower panels). *P<0.05 effect of NLA vs corresponding control, †P<0.05 effect of NLA in presence of Tezo vs NLA alone.*

in vitro, that in pulmonary arterial endothelial cells, NO decreases endothelin-1 secretion through the activation of soluble guanylyl cyclase. Moreover, Wiley and Davenport [12] showed that NO can modulate the binding of ET to the ET receptor. It has been suggested that the principal vasodilator effect of NO occurs through the inhibition of ET-induced constriction [25]. If this were the case, the level of vasodilation reached by ET-receptor blockade alone would be identical to the level of vasodilation obtained by the combined effect of ET-receptor blockade and NO synthase inhibition. At rest, pulmonary vascular resistance was significantly higher after combined administration of NLA and tezosentan compared to tezosentan alone, which indicates that NO acts predominantly in a direct manner (Fig. 5). However during exercise, pulmonary vascular resistance was only slightly higher ($P<0.05$ by ANCOVA) after combined administration of NLA and tezosentan as compared to administration of tezosentan alone. This suggests that, although a significant part of the vasodilator effect of NO on the pulmonary vasculature during exercise occurs via inhibition of ET, NO also has a direct vasodilator effect on the pulmonary circulation. In the systemic vasculature, the effect of NLA in control is approximately twice as large as the effect of NLA after tezosentan, indicating that the direct vasodilator effect of NO is approximately equal to the vasodilator effect of the NO-mediated inhibition of ET (Fig. 5).

In the systemic circulation, prostanoids limit ET-induced vasoconstriction, particularly after NO synthase inhibition. The mechanism behind the interaction between prostanoids and ET is not fully understood, but may involve inhibition of transcription, translation, secretion and/or action of ET by prostacyclin [28]. In contrast with the findings in the systemic circulation, our data do not support an interaction between prostanoids and ET in the pulmonary circulation under physiological conditions. Exogenous prostanoids can induce pulmonary vasodilation [22, 29, 30], indicating that prostacyclin receptors are present in the pulmonary circulation of swine, as. However, we previously found that inhibition of endogenous prostanoid production did not affect the low basal pulmonary vascular resistance either under normal conditions or in the presence of NO synthase inhibition [6]. Although we cannot exclude that cyclo-oxygenase inhibition may have gone without an apparent effect due to the opposing effects of simultaneously blocking vasodilator and vasoconstrictor prostanoids, it could also be argued that endogenous prostanoid production in the pulmonary circulation is too small under physiological conditions. This concept is supported by in vitro data by Wort et al [31], who found no cyclo-oxygenase protein in human pulmonary arterial smooth muscle cells under normal conditions. However, under stimulation with cytokines, which mimics pathological conditions, cyclo-oxygenase-2 protein expression could be detected, and inhibited the production of ET. Also the prostacyclin-mimetic cicaprost inhibited ET production [31]. Taken together, these data suggest that prostanoids can exert an inhibitory influence on endothelin production in the pulmonary circulation, similar to what we found in the systemic vasculature. However, under physiological conditions, prostanoid levels may be insufficient to modulate pulmonary vasomotor tone and blunt the ET-induced pulmonary vasoconstriction during exercise.

Clinical relevance

Pulmonary arterial hypertension can arise from a multitude of etiologies. However, in the later stages of the disease, endothelial dysfunction becomes a central feature [5, 15]. Endothelial dysfunction is associated with a loss of endothelial vasodilator substances, including NO and prostanoids. The present study shows that under conditions of endothelial dysfunction, in particular a reduced NO bioavailability, exercise-induced pulmonary vasodilation is blunted and pulmonary hypertension during exercise is exacerbated, which is due to "unopposed" ET-mediated pulmonary vasoconstriction. These observations support clinical studies that have shown the efficacy of ET-receptor antagonism and/or exogenous NO in the treatment of chronic pulmonary arterial hypertension [32-35].

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Chapter 5

Role of endothelin receptor activation in secondary pulmonary hypertension in awake swine after myocardial infarction

Birgit Houweling, Daphne Merkus, Oana Sorop,
Frans Boomsma and Dirk J Duncker
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Abstract

We previously observed that pulmonary hypertension secondary to myocardial infarction (MI) in swine is characterized by elevated plasma endothelin (ET) levels and pulmonary vascular resistance (PVR). Consequently, we tested the hypothesis that an increased ET-mediated vasoconstrictor influence contributes to secondary pulmonary hypertension after MI and investigated the involvement of ET_A and ET_B receptor subtypes. Chronically instrumented swine with (n=25) or without (n=19) MI were studied at rest and during treadmill exercise (up to 4 km h^{-1}), in the absence and presence of the ET_A antagonist EMD 122946 or the mixed ET_A/ET_B antagonist tezosentan. In normal swine, exercise caused a small decrease in PVR. ET_A blockade had no effect on PVR at rest or during exercise. Conversely, ET_A/ ET_B blockade decreased PVR but only during exercise (from 3.0±0.1 mmHg min l^{-1} to 2.3±0.1 mmHg min l^{-1} at 4 km h^{-1}; P≤0.05). MI increased pulmonary artery pressure and vascular resistance both at rest and during exercise (both P≤0.05). The increased pulmonary artery pressure correlated with the increased plasma ET levels in resting MI swine (r=0.71, P≤0.01). Furthermore, the pulmonary vasoconstrictor response to ET-1 infusion was enhanced after MI (P≤0.05). ET_A/ ET_B blockade decreased pulmonary resistance in MI swine from 3.6±0.3 to 3.1±0.5 mmHg min l^{-1} at rest and from 3.4±0.3 to 2.4±0.2 mmHg min l^{-1} during exercise at 4 km h^{-1} (both P≤0.05). This increased response to mixed ET_A/ET_B blockade in MI compared to normal swine appeared to be the result of an increased ET_A mediated vasoconstriction, as ET_A blockade decreased pulmonary resistance in MI swine from 3.4±0.4 to 2.8±0.2 mmHg min l^{-1} at rest and from 3.1±0.3 to 2.6±0.2 mmHg min l^{-1} at 4 km h^{-1} (both P≤0.05). In conclusion, increased plasma ET levels together with increased pulmonary resistance vessel responsiveness to ET result in an exaggerated pulmonary vasoconstrictor influence of ET in swine with a recent MI. This vasoconstrictor influence is the result of an emergent tonic ET_A vasoconstriction in addition to the exercise-induced ET_B vasoconstriction that is already present in normal swine.

Introduction

Congestive heart failure is the only major cardiovascular disorder of which the incidence has increased over the past decade, which is principally due to a reduction in mortality associated with acute myocardial infarction (MI) [1]. The loss of viable myocardial tissue after MI causes left ventricular dysfunction leading to an increase in left ventricular filling pressure and neurohumoral activation particularly during exercise [2-4]. The increase in left ventricular filling pressure is transmitted backwards into the pulmonary circulation, resulting in pulmonary congestion and an increase in pulmonary arterial blood pressure, thereby elevating right ventricular afterload [2, 5]. The resultant right ventricular hypertrophy is a risk factor for the development of right-sided heart failure [6].

In addition to the backward transmission of left atrial pressure into the pulmonary circulation, an elevated pulmonary vascular resistance, associated with activation of the endothelin system, also contributes to pulmonary hypertension secondary to MI [2, 7, 8]. Endothelin-1 (ET-1) is one of the most potent vasoconstrictors known to date and is produced in endothelial cells. Its role in pulmonary hypertension is supported by increased levels of circulating endothelin (ET), as well as increased local production [9-11]. Moreover, there is a correlation between ET plasma levels and severity of pulmonary hypertension in patients with heart failure [12].

Both ET_A and ET_B receptors have been identified in the pulmonary vasculature [13, 14]. ET_A and ET_B receptors on vascular smooth muscle induce vasoconstriction, whereas ET_B receptors on the endothelium induce vasodilation through production of NO and prostacyclin. We have previously shown, that endogenous ET-mediated pulmonary vasoconstriction in normal swine occurs predominantly through the ET_B receptors [15], indicating that the vasoconstrictor effect of ET_B receptors on vascular smooth muscle is larger than the vasodilator effect of ET_B receptors on the endothelium, while ET_A receptors have virtually no influence on the pulmonary circulation. The endothelial ET_B receptors are also responsible for the plasma clearance of ET in the lungs [16], which is reduced in relation with the severity of pulmonary hypertension in patients with chronic heart failure [17]. The reduced pulmonary clearance of ET and the increased plasma ET levels after MI suggest that ET can contribute to the aggravation of pulmonary hypertension. Indeed, studies in patients with pulmonary hypertension secondary to MI suggest that ET_A as well as ET_A/ET_B receptor blockade are capable of improving clinical conditions [18]. However, to date no study has addressed the effect of ET receptor antagonists on pulmonary vascular resistance after MI.

The aim of the present study was therefore to test the hypothesis that an

increased ET vasoconstrictor influence in the pulmonary circulation contributes to secondary pulmonary hypertension in swine with a recent MI. For this purpose, we first investigated in awake resting swine, whether plasma ET levels and pulmonary vascular responsiveness to exogenous ET are increased after MI. Subsequently, we investigated whether the vasoconstrictor influence of endogenous ET was increased in the pulmonary circulation and assessed the role of ET_A and ET_B receptor subtypes in this process. Since in normal swine a pulmonary ET vasoconstrictor influence emerges during exercise, but is negligible under resting conditions [15], the vasoconstrictor influence of endogenous ET in MI swine was studied both at rest and during treadmill exercise.

Methods

Animals

Studies were performed in accordance with the Council of Europe Convention (ETS123)/ Directive (86/609/EEC) for the protection of vertebrate animals used for experimental and other scientific purposes, and with approval of the Animal Care Committee at Erasmus Medical Center. A total of 47 Yorkshire X Landrace swine (2-3-month-old, 22±1 kg at the time of surgery) of either sex entered the study.

Surgery

Swine were sedated with ketamine (30 mg kg^{-1} im), anaesthetized with thiopental (10 mg kg^{-1} iv), intubated and ventilated with a mixture of O_2 and N_2O (1:2) to which 0.2-1% (v/v) isoflurane was added [19, 20]. Anaesthesia was maintained with midazolam (2 mg/kg + 1 mg kg^{-1} per hour iv) and fentanyl (10 µg kg^{-1} per hour iv). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid-filled polyvinylchloride catheter was inserted into the aortic arch and pulmonary artery for blood pressure measurement (Combitrans pressure transducers, Braun, Melsungen, Germany) and blood sampling. An electromagnetic flow probe (14-15 mm, Skalar) was positioned around the ascending aorta for measurement of cardiac output. A polyvinylchloride catheter was also inserted into the left atrium to measure pressure. In all 47 swine the proximal part of the left coronary circumflex artery (LCx) was exposed, but only in 22 animals the LCx was permanently occluded with a silk suture to produce an MI [2, 4]. Three MI swine died during surgery due to recurrent fibrillation. Catheters were tunnelled to the back and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine im) for 2 days and antibiotic prophylaxis (25 mg kg^{-1} amoxicillin and 5 mg kg^{-1} gentamycin iv) for 5 days.

Resting protocols

Experimental Design. Resting studies were performed 17±1 days after surgery with animals resting quietly on the treadmill. A total number of 19 normal and 18 MI swine were used in the resting protocols. In the first resting protocol we evaluated the effects of MI on resting hemodynamics and plasma ET levels. In the second resting protocol we evaluated the pulmonary resistance vessel responses to ET by intravenously infusing exogenous ET-1.

Pulmonary arterial pressure and plasma levels of ET. Plasma levels of ET were determined in 15 normal and 18 MI swine. Since we have recently shown that exercise does not affect plasma levels of ET in either normal swine or swine with MI [3], we elected to determine plasma ET levels only under resting conditions. Arterial blood samples (5 ml) in all swine were collected in tubes containing EDTA. Samples were centrifuged (3000g; 10 min; 4°C) and plasma was stored at -80°C. Plasma levels of ET-like immuno-reactivity were determined using a radio-immuno assay from Euro-Diagnostica (Malmö, Sweden), which has a cross reactivity of 100% toward ET-1, 48% toward ET-2 and 109% toward ET-3 [15]. Since production of ET-2 and ET-3 appears to be absent in the cardiovascular system of the swine [21], the concentrations measured with the radio-immuno assay most likely represent ET-1. Resting hemodynamic measurements consisting of aortic, left atrial, and pulmonary artery blood pressures and cardiac output were obtained.

Exogenous ET-1 infusions. With swine (6 normal and 3 MI) resting quietly, resting hemodynamic measurements were obtained. Subsequently, ET-1 was infused iv via the pulmonary artery catheter at rates of 20 and 40 pmol kg^{-1} min^{-1} and resting hemodynamic measurements were again obtained at the end of each 10 min infusion period.

Exercise protocols

Experimental Design. Studies were performed 11±1 days after surgery with animals exercising on a motor-driven treadmill. The two exercise protocols were performed on different days and in random order. A total number of 14 normal and 9 MI swine were used in the exercise protocols, of which 8 normal and 8 MI swine were also used in the resting protocols. In the first exercise protocol we evaluated the vasoconstrictor influence of endogenous ET, using a mixed ET_A/ET_B receptor antagonist. In the second protocol we assessed the role of the ET_A receptor subtype in the vasoconstrictor influence of endogenous ET in MI swine, using a selective ET_A receptor antagonist. We refrained from studying the effects of a selective ET_B receptor antagonist, because the ET_B receptor is responsible for clearance of ET in the lungs [16]. Hence, ET_B blockade increases ET levels, which can then act through

the ET_A receptor to cause vasoconstriction, thereby confounding interpretation of the results. Consequently, the contribution of the ET_B receptor must be derived from the difference in response to the ET_A and the ET_A/ET_B receptor antagonist.

Effects of combined ET_A and ET_B receptor blockade. With swine (13 normal and 9 MI) lying quietly on the treadmill, resting hemodynamic measurements consisting of left atrial, aortic and pulmonary artery blood pressures and cardiac output were obtained and arterial and mixed venous blood samples were collected. Hemodynamic measurements were repeated and rectal temperature measured with animals standing on the treadmill. Subsequently, a four stage exercise protocol (1, 2, 3, and 4 km h^{-1}) was begun with each stage lasting 2-3 min. Hemodynamic variables were measured and blood samples collected during the last 30 seconds of each exercise stage, when hemodynamics had reached a steady state. At the conclusion of the exercise protocol animals were allowed to rest for ninety min. Then, animals received the mixed ET_A and ET_B receptor (ET_A/ET_B) antagonist tezosentan (a gift from Dr Clozel, Actelion Pharmaceuticals Ltd., Allschwill, Switzerland) intravenously over 10 min in a dose of 3 mg kg^{-1}, followed by a continuous infusion of 6 mg kg^{-1} h^{-1} iv [15] and the exercise protocol was repeated. Tezosentan has a pA_2 of 9.5 for ET_A and a pA_2 of 7.7 for ET_B receptors, indicating only a 63-fold selectivity for ET_A compared to ET_B receptors [22, 23]. Moreover, we have previously shown that the employed dose of tezosentan blocks the pressor response to intravenous ET infusion and results in an increase in plasma ET levels, indicating that both ET_A and ET_B receptors are blocked [15].

Effects of ET_A receptor blockade. Ninety min after swine (9 normal and 8 MI) underwent the exercise protocol under control conditions, swine received the ET_A receptor antagonist EMD 122946 (a gift from Prof. Schelling, E. Merck Darmstadt, Darmstadt, Germany) in a dose of 3 mg kg^{-1} intravenously [15]. EMD122946 has a pA_2 of 9.5 for ET_A and a pA_2 of 6.0 for ET_B receptors, indicating a 3200-fold selectivity for ET_A compared to ET_B receptors [24]. EMD122946 in a dose of 3 mg kg^{-1} blocks the pressor response to intravenously infused ET. In contrast, this dose of EMD122946 does not block ET_B receptors as ET_B-mediated clearance of ET is not affected by administration of EMD122946 as evidence by unaltered ET-plasma levels [15]. Five minutes after completion of the infusion, resting measurements were obtained and the exercise protocol repeated.

Blood gas measurements

Blood samples obtained in the exercise protocols, were kept in iced syringes until the conclusion of each exercise trial. Measurements of PO_2 (mmHg), PCO_2

(mmHg) and pH were then immediately performed with a blood gas analyser (Acid-Base Laboratory Model 505, Radiometer, Copenhagen, Denmark). Oxygen saturation (%) and haemoglobin (grams dl^{-1}) were measured with a haemoximeter (OSM3, Radiometer). Blood O_2 content (mmol ml^{-1}) was computed as ($Hb \cdot 0.621 \cdot O_2$-saturation) + ($0.00131 \cdot PO_2$). Body O_2 consumption (BVO_2) was calculated as the product of cardiac output and the difference in O_2 content between arterial and mixed venous blood [19, 20].

Data analysis

Digital recording and off-line analysis of hemodynamics have been described in detail elsewhere [19, 20]. Systemic vascular resistance was computed as mean aortic blood pressure divided by cardiac output. Pulmonary vascular resistance (PVR) was computed as mean pulmonary artery pressure minus mean left atrial pressure divided by cardiac output.

Statistical analysis

The effect of MI on pulmonary arterial pressure and plasma ET levels was assessed using an unpaired t-test. The relation between endogenous ET and pulmonary arterial pressure was assessed using linear regression while statistical analysis of the effect of exogenous ET on systemic vascular resistance and PVR in normal versus MI swine was performed using analysis of variances (ANOVA). To test for the effects of MI and drug treatment (EMD 122946 or tezosentan) on the

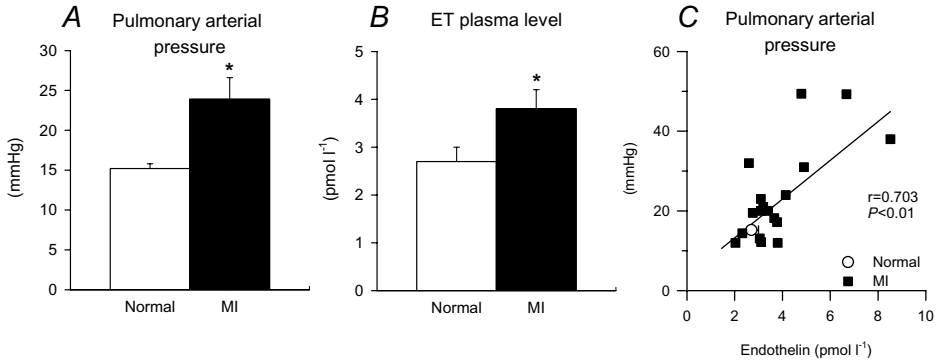

Figure 1: *Pulmonary artery pressure (A) and ET plasma level (B) in 18 MI and 19 normal swine. C, shows the correlation between pulmonary artery pressure and ET plasma level in swine with MI (squares), while the data of normal swine are shown as mean±SEM (open circle). Inclusion of both MI and normal swine resulted in a highly similar correlation between pulmonary artery pressure (PAP) and ET plasma levels (PAP = 4.9ET + 3.5, r=0.703), as compared to inclusion of MI swine alone (PAP = 5.2ET + 3.6; r=0.708).*P≤0.05 MI versus normal swine*

relation between BVO_2 and systemic vascular resistance or PVR, regression analysis was performed using MI, drug treatment and BVO_2 as well as their interaction as independent variables and assigning a dummy variable to each animal. Statistical significance was accepted when P≤0.05. Data are presented as mean±S.E.M.

Results

Endothelin and myocardial infarction at rest

After MI, mean resting pulmonary artery pressure increased from 15±1 mmHg to 24±3 mmHg (Fig. 1A), which was in part the result of the increased mean left atrial pressure (4±1 mmHg in normal swine and 12±2 mmHg in MI swine). Resting ET plasma levels were elevated in MI (3.8±0.4 pmol l^{-1}) compared to normal swine (2.7±0.3 pmol l^{-1}, P≤0.05) (Fig. 1B). Furthermore, a significant correlation existed between pulmonary artery pressure and ET levels in MI swine (r=0.708; P≤0.01) (Fig. 1C), supporting a role for ET in pulmonary hypertension after MI.

To investigate whether the responsiveness to ET is changed after MI, different dosages of ET-1 were infused. In the systemic circulation, ET-1 infusion resulted in a progressive increase in the vascular resistance that was similar in both normal and MI swine (Fig. 2). In contrast, whereas ET-1 in a dose of 20 pmol kg^{-1} min^{-1} induced no significant change in PVR in either group of swine, ET-1 in a dose of 40 pmol kg^{-1} min^{-1} produced a significantly greater increase in PVR in MI compared to normal swine (Fig. 2).

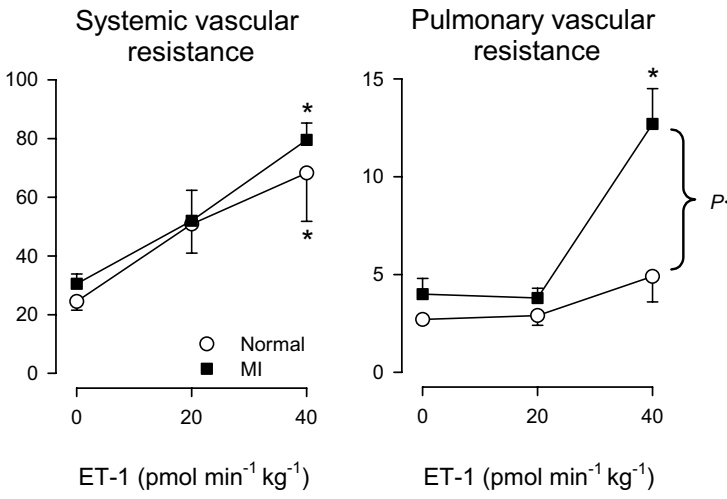

Figure 2: *The effects of ET infusions on systemic vascular resistance (left panel) and pulmonary vascular resistance (right panel) of 6 normal swine (open circles) and 3 MI swine (solid squares).*
**P≤0.05 vs ET-1 in a dose of 0 pmol min^{-1} kg^{-1}*

Changes in hemodynamics after a myocardial infarction at rest and exercise

Exercise up to 4 km h^{-1} in normal swine resulted in a tripling of BVO_2 which was met by a doubling of cardiac output and a ~40% increase in O_2 extraction (Fig. 3). The increase in cardiac output was principally due to an increase in heart rate, as stroke volume increased by only 7%. Mean aortic blood pressure was minimally affected (Table 1), implying that the increase in cardiac output was balanced by a similar decrease in systemic vascular resistance. Mean pulmonary artery pressure almost doubled in normal swine during exercise (Fig. 3). However, the transpulmonary pressure gradient (pulmonary artery pressure minus left atrial pressure) increased slightly less than cardiac output, reflecting a small decrease in PVR during exercise.

Despite a 17% lower stroke volume in MI compared to normal swine, cardiac output was virtually maintained both at rest and during treadmill exercise, which was the result of a higher heart rate (Fig. 3). The slightly lower cardiac output during exercise necessitated a small increase in O_2-extraction, resulting in a decrease in mixed venous O_2 saturation. Pulmonary artery pressure was elevated, which was due to a marked increase in left atrial pressure but also due to a ~15% increase in PVR (Fig. 3)

The role of endothelin in the regulation of vascular tone

In normal swine, ET_A/ET_B receptor blockade with tezosentan had no effect on the pulmonary circulation under resting conditions (Table 1, Fig. 4). During exercise, however, tezosentan reduced pulmonary arterial pressure with no effect on left atrial pressure and caused a small increase in cardiac output (Table 1), implying a decrease in PVR. The tezosentan-induced decrease in PVR increased progressively with incremental exercise intensity (Fig. 4). In contrast, the ET_A receptor antagonist EMD 122946 had no effect on pulmonary artery or left atrial pressure in normal animals either at rest or during exercise (Table 2), so that PVR remained unchanged (Fig. 4). Hence in normal swine, the vasoconstrictor influence of endothelin on the pulmonary circulation during exercise is mediated by ET_B receptors.

In MI swine ET_A/ET_B receptor blockade caused a significant decrease in PVR at rest and during exercise (Fig. 4). ET_A receptor blockade also resulted in vasodilation, demonstrated by a modest decrease in PVR at rest, which was maintained during exercise. The difference between the effect of ET_A/ET_B blockade and of ET_A blockade alone (representing the ET_B component) was unchanged in MI compared to normal swine (Fig. 4). Thus, the increased pulmonary vasoconstrictor influence by endogenous ET in MI swine is principally mediated by ET_A receptors

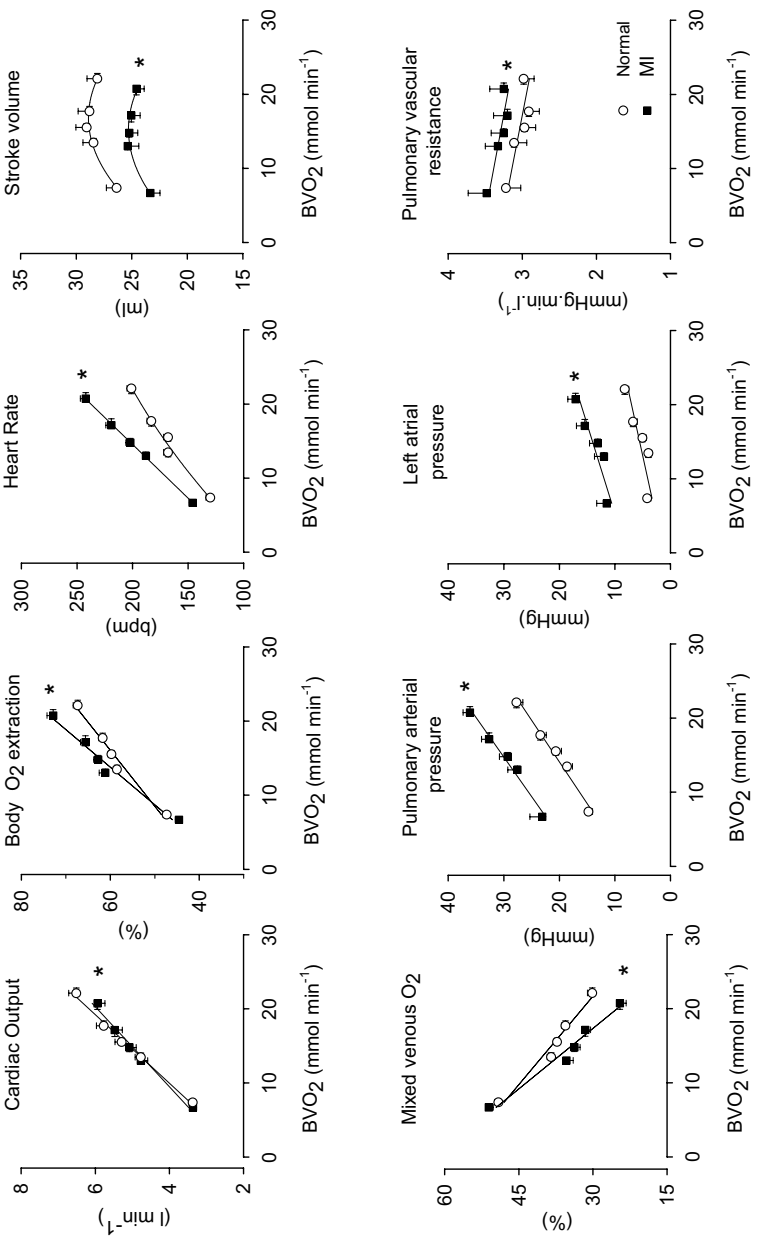

***Figure 3**: The responses to exercise of various parameters in relation to the body oxygen consumption (BVO_2) under control conditions in 14 normal swine (open circles) and 9 MI swine (solid squares). *P≤0.05 MI versus normal swine*

Table 1: *Haemodynamics of swine at rest and during exercise before and after administration of tezosentan*

			Rest		Exercise level (km h^{-1})			
	Treatment	Infarct	lying	standing	1	2	3	4
HR	Control	-	128 ± 4	147 ± 4*	168 ± 5*	183 ± 5*	200 ± 5*	232 ± 7*
(bpm)	Tezosentan	-	149 ± 4†	161 ± 5*†	183 ± 6*†	196 ± 7*†	212 ± 6*†	243 ± 7*†
	Control	+	147 ± 4‡	158 ± 5*	187 ± 2*‡	203 ± 4*‡	219 ± 6*‡	244 ± 8*
	Tezosentan	+	157 ± 4†	169 ± 4*†	190 ± 4*	205 ± 4*	222 ± 7*	243 ± 6*
MAP	Control	-	95 ± 3	88 ± 3*	88 ± 2*	88 ± 2*	87 ± 2*	89 ± 2*
(mmHg)	Tezosentan	-	88 ± 2†	79 ± 2*†	78 ± 2*†	77 ± 2*†	80 ± 2*†	81 ± 2*†
	Control	+	90 ± 2	83 ± 2*	81 ± 2*‡	81 ± 2*	82 ± 3*	83 ± 3
	Tezosentan	+	80 ± 2†‡	76 ± 2†	75 ± 2†	76 ± 2†	76 ± 2†	77 ± 2†
PMAP	Control	-	15 ± 1	15 ± 1	19 ± 1*	21 ± 1*	23 ± 1*	28 ± 1*
(mmHg)	Tezosentan	-	15 ± 1	13 ± 1*	17 ± 1†	18 ± 2*†	21 ± 1*†	25 ± 2*†
	Control	+	25 ± 3‡	24 ± 2‡	29 ± 2*‡	31 ± 2*‡	34 ± 2*‡	38 ± 2*‡
	Tezosentan	+	21 ± 2‡	21 ± 2†‡	25 ± 2†‡	28 ± 2*†‡	31 ± 2*‡	33 ± 2*†‡
LAP	Control	-	4 ± 1	2 ± 1	4 ± 1	5 ± 1	6 ± 1*	8 ± 1*
(mmHg)	Tezosentan	-	5 ± 1	0 ± 1*	3 ± 1	4 ± 1	6 ± 1*	9 ± 1*
	Control	+	12 ± 2‡	10 ± 2‡	13 ± 2‡	14 ± 2‡	16 ± 2*‡	18 ± 2*‡
	Tezosentan	+	11 ± 2‡	10 ± 2‡	12 ± 2‡	15 ± 2*‡	17 ± 2*‡	18 ± 2*‡
CO	Control	-	3.3 ± 0.1	4.2 ± 0.2*	4.8 ± 0.2*	5.4 ± 0.2*	5.9 ± 0.2*	6.6 ± 0.3*
($l\ min^{-1}$)	Tezosentan	-	3.7 ± 0.2†	4.4 ± 0.2*†	5.1 ± 0.3*	5.6 ± 0.3*	6.2 ± 0.3*†	7.0 ± 0.3*†
	Control	+	3.4 ± 0.1	3.9 ± 0.2*	4.7 ± 0.2*	5.0 ± 0.2*	5.5 ± 0.3*	5.9 ± 0.3*
	Tezosentan	+	3.5 ± 0.2	4.5 ± 0.3*†	5.1 ± 0.3*	5.5 ± 0.3*†	5.9 ± 0.3*†	6.3 ± 0.3*†
SVR	Control	-	29 ± 1	22 ± 1*	19 ± 1*	17 ± 1*	15 ± 1*	14 ± 1*
($mmHg\ min\ l^{-1}$)	Tezosentan	-	24 ± 1†	19 ± 1*†	16 ± 1*†	14 ± 1*†	13 ± 1*†	12 ± 1*†
	Control	+	27 ± 1	22 ± 1*	17 ± 1*	16 ± 1*	15 ± 1*	14 ± 0*
	Tezosentan	+	23 ± 1†	17 ± 1*†	15 ± 1*	14 ± 1*†	13 ± 1*†	12 ± 1*†
Hb art	Control	-	8.0 ± 0.2	-	8.3 ± 0.2*	8.4 ± 0.2*	8.6 ± 0.2*	8.7 ± 0.2*
($g\ dl^{-1}$)	Tezosentan	-	8.5 ± 0.3†	-	8.5 ± 0.2	8.6 ± 0.2	8.6 ± 0.2	8.6 ± 0.2
	Control	+	7.7 ± 0.3	-	8.0 ± 0.4	8.2 ± 0.4	8.5 ± 0.4*	8.6 ± 0.4
	Tezosentan	+	8.0 ± 0.3	-	8.4 ± 0.4*	8.2 ± 0.4	8.2 ± 0.3	8.3 ± 0.4
O_2Sat art	Control	-	93 ± 1	-	92 ± 1	92 ± 1	93 ± 1	92 ± 1
(%)	Tezosentan	-	93 ± 1	-	92 ± 1*	93 ± 1	92 ± 1	92 ± 1
	Control	+	91 ± 1	-	89 ± 1	89 ± 1	90 ± 1‡	90 ± 1‡
	Tezosentan	+	91 ± 1	-	89 ± 1	90 ± 1‡	89 ± 1‡	89 ± 1‡
O_2Sat mv	Control	-	49 ± 1	-	39 ± 1*	38 ± 1*	36 ± 1*	30 ± 1*
(%)	Tezosentan	-	54 ± 1†	-	42 ± 1*†	41 ± 1*†	37 ± 1*	31 ± 2*
	Control	+	52 ± 2	-	34 ± 2*	33 ± 2*‡	30 ± 2*‡	23 ± 2*‡
	Tezosentan	+	55 ± 1†	-	38 ± 1*‡	35 ± 1*‡	31 ± 2*‡	23 ± 2*‡
BVO_2	Control	-	7.3 ± 0.3	-	13.1 ± 0.6*	15.3 ± 0.7*	17.6 ± 0.7*	21.8 ± 0.7*
($mmol\ min^{-1}$)	Tezosentan	-	7.7 ± 0.3	-	13.5 ± 0.7*	15.3 ± 0.6*	18.3 ± 0.8*	22.5 ± 0.9*
	Control	+	6.8 ± 0.5	-	13.0 ± 0.8*	14.6 ± 0.9*	17.5 ± 1.3*	21.2 ± 1.2*
	Tezosentan	+	6.4 ± 0.4‡	-	13.6 ± 0.7*	15.5 ± 0.8*	17.9 ± 1.1*	21.8 ± 1.0*

*HR: Heart Rate, MAP: Mean Arterial Pressure, PMAP: Mean Pulmonary Arterial Pressure, LAP: Mean Left Atrial Pressure, CO: Cardiac Output. SVR: Systemic Vascular Resistance, Hb art: Arterial Hemoglobin, O_2sat art: Arterial Oxygen Saturation, O_2Sat mv: Mixed Venous Oxygen Saturation, BVO_2: Body Oxygen Consumption. Data are mean± S.E.M. from 13 normal and 9 MI swine; * P≤0.05 vs rest (lying); † P≤0.05 vs corresponding control, ‡ P≤0.05 vs corresponding normal swine*

Table 2: *Haemodynamics of swine at rest and during exercise before and after administration of EMD 122946*

			Rest		Exercise level (km h^{-1})			
	Treatment	Infarct	lying	standing	1	2	3	4
HR	Control	-	132 ± 6	146 ± 7*	168 ± 6*	182 ± 6*	201 ± 8*	232 ± 8*
(bpm)	EMD 122946	-	133 ± 5	150 ± 5*	173 ± 7*	190 ± 8*	211 ± 8*	247 ± 8*†
	Control	+	144 ± 5	159 ± 5*	190 ± 6*‡	200 ± 7*	218 ± 8*	239 ± 6*
	EMD 122946	+	152 ± 5‡	163 ± 5*	177 ± 6*	199 ± 5*	215 ± 4*	241 ± 5*
MAP	Control	-	90 ± 2	86 ± 3	82 ± 2*	83 ± 2*	84 ± 2*	87 ± 2
(mmHg)	EMD 122946	-	82 ± 2†	73 ± 2*†	72 ± 1*†	74 ± 2*†	76 ± 2*†	79 ± 1†
	Control	+	95 ± 2	84 ± 2*	81 ± 2*	81 ± 2*	83 ± 2*	83 ± 3*
	EMD 122946	+	85 ± 2†	75 ± 2*†	74 ± 2*†	73 ± 2*†	75 ± 2*†	77 ± 2*†
PMAP	Control	-	14 ± 1	15 ± 1	18 ± 1*	20 ± 2*	24 ± 2*	27 ± 2*
(mmHg)	EMD 122946	-	15 ± 1	14 ± 1	17 ± 1	20 ± 2*	23 ± 2*	27 ± 2*
	Control	+	21 ± 3	22 ± 2‡	26 ± 2*‡	28 ± 2*‡	31 ± 2*‡	34 ± 1*‡
	EMD 122946	+	20 ± 2‡	19 ± 2	22 ± 2	26 ± 2*‡	29 ± 2*‡	33 ± 1*†‡
LAP	Control	-	4 ± 1	3 ± 2	4 ± 1	5 ± 1	8 ± 1*	9 ± 1*
(mmHg)	EMD 122946	-	2 ± 2	2 ± 2	2 ± 1	4 ± 2	6 ± 2*	9 ± 2*
	Control	+	10 ± 3‡	9 ± 2‡	11 ± 3‡	12 ± 2‡	15 ± 2‡	17 ± 2*‡
	EMD 122946	+	10 ± 2‡	7 ± 2*	9 ± 3‡	12 ± 2‡	14 ± 2*‡	17 ± 2*‡
CO	Control	-	3.5 ± 0.2	4.3 ± 0.3*	4.9 ± 0.3*	5.4 ± 0.3*	5.9 ± 0.4*	6.7 ± 0.4*
(l min^{-1})	EMD 122946	-	3.6 ± 0.2	4.4 ± 0.2*	5.2 ± 0.2*	5.6 ± 0.3*	6.2 ± 0.4*	7.0 ± 0.4*†
	Control	+	3.3 ± 0.2	4.1 ± 0.3*	4.8 ± 0.3*	5.1 ± 0.3*	5.5 ± 0.3*	6.0 ± 0.3*
	EMD 122946	+	3.6 ± 0.2	4.4 ± 0.2*	4.7 ± 0.2*	5.3 ± 0.3*	5.7 ± 0.3*†	6.3 ± 0.3*†
SVR	Control	-	27 ± 2	21 ± 1*	17 ± 1*	16 ± 1*	15 ± 1*	14 ± 1*
(mmHg min l^{-1})	EMD 122946	-	23 ± 2†	17 ± 1*†	14 ± 1*†	14 ± 1*†	13 ± 1*†	12 ± 1*†
	Control	+	29 ± 2	21 ± 1*	17 ± 1*	16 ± 1*	15 ± 1*	14 ± 1*
	EMD 122946	+	24 ± 1†	17 ± 1*†	16 ± 1*	14 ± 1*†	13 ± 1*†	12 ± 1*†
Hb art	Control	-	7.9 ± 0.3	-	8.2 ± 0.3*	8.5 ± 0.3*	8.5 ± 0.3*	8.7 ± 0.3*
(g dl^{-1})	EMD 122946	-	8.0 ± 0.2	-	8.3 ± 0.2	8.4 ± 0.2*	8.5 ± 0.2*	8.4 ± 0.3
	Control	+	7.8 ± 0.5	-	7.9 ± 0.4	8.3 ± 0.4*	8.2 ± 0.5	8.4 ± 0.5
	EMD 122946	+	8.0 ± 0.4	-	8.3 ± 0.3	8.3 ± 0.3	8.2 ± 0.3	8.4 ± 0.3
O_2Sat art	Control	-	93 ± 1	-	93 ± 1	92 ± 1	93 ± 1	92 ± 1
(%)	EMD 122946	-	92 ± 1	-	92 ± 0	92 ± 1	93 ± 1	92 ± 1
	Control	+	91 ± 1	-	91 ± 1‡	91 ± 1	92 ± 1*	92 ± 1
	EMD 122946	+	91 ± 1	-	90 ± 1	91 ± 1	91 ± 1†	91 ± 1†
O_2Sat mv	Control	-	49 ± 2	-	38 ± 2*	36 ± 1*	35 ± 2*	30 ± 2*
(%)	EMD 122946	-	53 ± 1†	-	41 ± 1*	39 ± 1*†	38 ± 1*†	32 ± 1*†
	Control	+	51 ± 1	-	36 ± 2*	35 ± 1	33 ± 1	26 ± 2*
	EMD 122946	+	53 ± 2	-	40 ± 1*	37 ± 2*	33 ± 2*	25 ± 2*
BVO_2	Control	-	7.6 ± 0.4	-	14.1 ± 1.2*	16.0 ± 0.7*	18.0 ± 1.3*	22.7 ± 1.5*
(mmol min^{-1})	EMD 122946	-	7.2 ± 0.5	-	13.9 ± 0.8*	15.6 ± 1.0*	18.1 ± 1.1*	22.4 ± 1.6*
	Control	+	6.6 ± 0.3	-	13.0 ± 0.7*	14.9 ± 0.8*	16.8 ± 1.3*	20.7 ± 1.2*
	EMD 122946	+	7.1 ± 0.4	-	12.6 ± 0.5*	15.0 ± 0.6*	16.9 ± 0.8	21.8 ± 0.7*

*HR: Heart Rate, MAP: Mean Arterial Pressure, PMAP: Mean Pulmonary Arterial Pressure, LAP: Mean Left Atrial Pressure, CO: Cardiac Output. SVR: Systemic Vascular Resistance, Hb art: Arterial Hemoglobin, O_2sat art: Arterial Oxygen Saturation, O_2Sat mv: Mixed Venous Oxygen Saturation, BVO_2: Body Oxygen Consumption. Data are mean ± S.E.M. from 9 normal and 8 MI swine; * P≤0.05 vs rest (lying); † P≤0.05 vs corresponding control, ‡ P≤0.05 vs corresponding normal swine*

Administration of the ET_A/ET_B receptor antagonist tezosentan to normal swine resulted in a decrease in systemic vascular resistance, reflecting vasodilation in the systemic vasculature at rest and during exercise. There were no differences between the response to tezosentan and EMD 122946 in the systemic circulation of normal swine (Tables 1 and 2). In MI swine the ET_A/ET_B receptor blockade and ET_A receptor blockade resulted in similar decreases of systemic vascular resistance compared to normal swine. These findings indicate that the ET vasoconstrictor influence in the systemic circulation is principally ET_A mediated and is not increased after MI.

Discussion

The main findings in the present study in awake swine with pulmonary hypertension secondary to MI are that (*i*) circulating plasma levels of ET as well as the pulmonary vascular responsiveness to exogenous ET-1 are increased; (*ii*) the increase in ET-mediated vasoconstrictor influence after MI is principally mediated by the ET_A receptor; (*iii*) the pulmonary vasoconstrictor influence of ET that is mediated through the ET_B receptor is not altered as compared to normal swine; (*iv*) the vasoconstrictor influence of endogenous ET in the systemic circulation did not change after MI. Since the role of ET in the systemic circulation, which has been discussed extensively in one of our previous papers [15], was not altered after MI, we will focus on the pulmonary circulation.

In agreement with previous studies from our laboratory [2, 25]), MI swine with a 2 wk old MI have LV dysfunction, characterized by a lower stroke volume and increased left atrial pressure. The accompanying increase in heart rate resulted in an essentially maintained cardiac output, indicating that overt congestive heart failure is absent [2, 25]. In agreement with clinical observations [12], and with previous observations from our own laboratory [2, 25], MI swine were characterized by increases in pulmonary arterial pressure and PVR. Furthermore, similar to clinical observations [12], we also found that the severity of pulmonary hypertension and the increase in plasma levels of ET were highly correlated. Moreover, the range of plasma ET levels after MI, which demonstrate increases of up to four-fold as compared to normal swine, is similar to that found in patients with pulmonary hypertension secondary to MI [12]. These observations indicate that our porcine model recapitulates several aspects of secondary pulmonary hypertension observed in patients after MI.

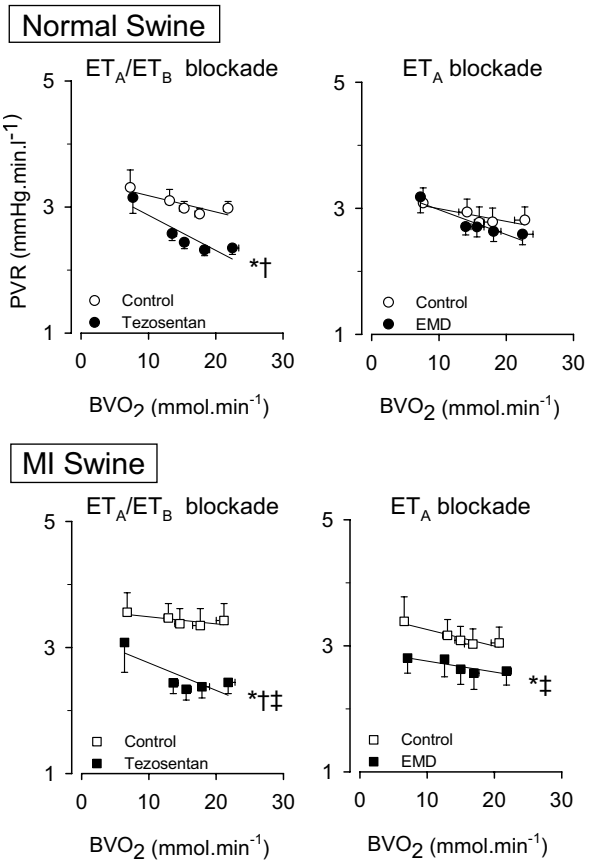

Figure 4: *The effects of the ET_A/ET_B receptor antagonist tezosentan (13 normal and 9 MI swine) and the ET_A receptor antagonist EMD122946 (9 normal and 8 MI swine) on pulmonary vascular resistance (PVR) plotted as a function of body O_2 consumption (BVO_2). Normal swine are represented by circles and MI swine are represented by squares. Control exercise trials are shown as open symbols, exercise trials in the presence of antagonists are shown as solid symbols. *P≤0.05 effect of EMD 122946 or tezosentan vs corresponding Control, †P≤0.05 effect of EMD 122946 vs effect of tezosentan, ‡P≤0.05 effect of EMD 122946 or tezosentan in MI versus normal swine.*

Elevation of plasma ET levels in pulmonary hypertension

In agreement with previous studies from our laboratory [2, 25], circulating levels of ET were increased after MI. These elevated ET levels could have been the result from decreased ET clearance, increased ET production, or both.

ET clearance. The pulmonary circulation, together with the kidneys and the liver, is the predominant site in the body for clearance of ET, with single pass-clearance reaching values of up to 30-50% [11, 26]. This clearance is mediated through binding of ET to the ET_B receptor on the pulmonary endothelium [16]. In the pulmonary circulation of healthy individuals the ET-production and clearance are balanced, such that there is no net difference between pulmonary arterial and venous ET plasma levels [11, 26]. In secondary pulmonary hypertension, increased plasma ET levels can be the result of decreased clearance of ET in the lungs. Indeed, in both experimental models and humans with heart failure, clearance of ET was shown to be reduced [17, 27, 28] although this was not a unanimous finding [29].

ET production. ET is produced from big-ET by endothelin-converting-enzym (ECE)-1, while big-ET is produced from preproendothelin by furin-like enzymes [30]. Hence, increases in either ECE activity or in preproendothelin levels could have contributed to an increase in ET production. The rate-limiting step in the ET production is thought to be the conversion from big-ET to ET by ECE-1. Increased ET-production in pigs with pacing-induced heart failure was attributed to an increased ECE-1 activity [29]. The ECE-1-isoform that is involved is still incompletely understood, but is most likely the ECE-1a isoform, as ECE-1a mRNA is upregulated, while ECE-1c mRNA appears to remain unaltered [31]. However, an upregulation of ECE-1 mRNA could not be confirmed in rats with either mild LV dysfunction or overt heart failure [32]. Conversely, the latter authors [32] as well as others [33] reported that increased preproendothelin mRNA levels in the lungs contribute to increased ET production in rats with MI-induced heart failure. However, in animals with mild LV dysfunction (resembling the porcine MI model in the present study) preproendothelin mRNA levels were still normal [32], suggesting that increased preproendothelin expression might be a feature of more advanced stages of heart failure. It should be noticed that in all these studies, involving protein and/or mRNA expression, total lung tissue is used. Since ECE-1 as well as preproendothelin are also present in bronchial epithelial cells [34-37] , interpretation of these studies using total lung tissue is difficult. Future studies using only pulmonary resistance vessels, are required to investigate the molecular alterations underlying an increased production of ET in more detail.

The mechanisms underlying the reported increases in ECE activity and/or preproendothelin levels in pulmonary hypertension secondary to LV failure are incompletely understood, but could be caused, at least in part, by endothelial dysfunction. Thus, in patients with secondary pulmonary hypertension due to heart failure, basal pulmonary NO production is reduced [10]. NO is capable of limiting ET-production [38-40], and in support of this concept, we recently found that loss of NO-synthesis, resulted in an enhanced pulmonary vasoconstrictor influence of ET in normal swine [41]. However, we previously failed to observe a loss of the vasodilator influence of NO in the pulmonary circulation after MI [25], suggesting that at this stage of LV dysfunction, the increased plasma ET levels are not the result of loss of NO bioavailability. Future studies, investigating the effects of ET receptor antagonists following NO synthase inhibition in swine with pulmonary hypertension after MI, are required to test this hypothesis more rigorously.

ET-receptor subtypes involved in pulmonary hypertension

ET-induced constriction of large pulmonary arteries is principally mediated by

ET_A receptors [14, 42], whereas constriction in the smaller pulmonary resistance vessels is mediated by ET_B receptors [14]. These findings are supported by observations that the density of ET_A receptors in the lung decreases with decreasing vessel size, whereas the density of ET_B receptors, both on the endothelium and the smooth muscle increases [13].

In normal swine, blockade of either ET_A receptors alone or combined blockade of ET_A and ET_B receptors had no effect on PVR at rest, indicating that endogenous ET does not contribute to resting tone in the pulmonary resistance vessels [15]. During exercise, however, an ET-mediated vasoconstriction became apparent that was ET_B receptor-mediated with no contribution of ET_A receptors [15, 41]. This ET_B-mediated vasoconstriction that limits the pulmonary vasodilation in response to exercise [15], is consistent with the localization of the ET_B receptors on the pulmonary resistance vessels [13].

In swine with secondary pulmonary hypertension, the increased pulmonary vasodilator response to mixed ET_A/ET_B receptor blockade was increased in MI compared to normal swine. Both plasma ET levels and sensitivity of the pulmonary circulation to exogenous ET (via intravenous infusion) were increased, and likely acted in concert to increase the pulmonary vasoconstrictor influence of ET in MI swine. Although we cannot entirely exclude that other neurohormones, including noradrenaline and angiotensin II, contributed to the increased pulmonary resistance vessel tone after MI, it is important to note that mixed ET_A/ET_B receptor blockade abolished the differences in pulmonary vascular resistance between normal and MI swine (see Fig. 4). This finding is consistent with the concept that an increased ET vasoconstrictor influence was principally responsible for the increase in pulmonary resistance vessel tone after MI.

Although the ET_A receptor does not contribute to the regulation of pulmonary vasomotor tone in normal swine at rest or during exercise, an ET_A-mediated vasoconstriction emerged after MI. This ET_A-mediated vasoconstriction appeared tonic in nature, so that it did not increase further during exercise. This increased vasoconstrictor influence of the ET_A receptor may also have contributed to the increased pulmonary sensitivity to exogenous ET. This is in accordance with a study in pigs with pacing-induced heart failure, that showed that ET_A receptor mRNA and ET_A binding are increased [31]. Similarly, data in dogs show that, while pulmonary vasoconstriction in response to exogenous ET is predominantly mediated through the ET_B receptor in normal dogs, there is an ET_A-mediated component in dogs with pacing-induced heart failure [43]. Conversely, Docherty and MacLean [44] investigated the effect of ET on isolated pulmonary arterioles in animals with MI using ET receptor agonists and antagonists, and found no contribution of the ET_A

receptor to pulmonary vasoconstriction in either normal rabbits or rabbits with MI. Similarly, several studies in rats show no alterations in ET_A mRNA and/or protein expression in the lung after myocardial infarction [32, 45]. However, in all studies, involving protein and/or mRNA expression, total lung tissue is used. Since ET_A as well as ET_B receptors are also present in bronchial airway smooth muscle and lung alveoli [46], interpretation of these studies using total lung tissue is difficult.

Comparison of the vasodilator effect of ET_A and ET_A/ET_B receptor blockade in normal and MI swine suggests that the contribution of the ET_B receptor to the regulation of pulmonary vasomotor tone remained unaltered after MI. These data are in apparent contrast with the majority of studies on ET_B receptor expression, both at the level of mRNA [32, 47] and the level of protein binding [31, 45], that indicate that ET_B receptor expression as well as ET_B mediated clearance are reduced in lungs of animals with heart failure. However, in these studies, ET_B receptors on the endothelial cells and on the vascular smooth muscle cells cannot be distinguished. It is therefore possible that ET_B receptors on the endothelium, which are responsible for vasodilation and clearance, are reduced to the same extent as ET_B receptors on vascular smooth muscle, which induce vasoconstriction. The net effect would then be that the ET_B-mediated vasoconstriction to endogenous ET would remain more or less constant, while clearance is reduced.

Clinical relevance

In the treatment of pulmonary hypertension, ET antagonism is a promising therapy. There are several clinical trials that demonstrate improvement in hemodynamics and exercise capacity and reduction in the number of clinical events after administration of an ET_A receptor antagonist or a mixed ET_A/ET_B receptor antagonist [48-51]. It has been proposed that ET_A receptor blockade would be favourable because it leaves the ET_B-mediated clearance of ET as well as the endothelium ET_B mediated pulmonary vasodilation unaffected. However, comparison of clinical trials using either ET_A or combined ET_A/ET_B receptor antagonists [18], suggests that exercise capacity of patients with pulmonary hypertension improves more with combined ET_A/ET_B than with ET_A receptor antagonists. The observations in the present study in MI swine provide further rationale for the use of combined ET_A/ET_B receptor antagonism in patients with secondary pulmonary hypertension. This important issue is currently being investigated in the prospective STRIDE-2 clinical trial, comparing treatment with ET_A receptor antagonism versus combined ET_A/ET_B receptor antagonism [52, 53].

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Chapter 6

Alterations in endothelial control of the pulmonary circulation in exercising swine with secondary pulmonary hypertension after myocardial infarction

Daphne Merkus, Birgit Houweling, Vincent J de Beer
Zaida Everon and Dirk J Duncker
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Abstract

Secondary pulmonary hypertension after myocardial infarction (MI) has been associated with endothelial dysfunction and activation of the endothelin (ET) system. Here, we investigated whether an increased ET-mediated pulmonary vasoconstrictor influence contributes to pulmonary hypertension after MI, and whether this increased ET vasoconstriction is caused by impaired NO and prostanoid production. For this purpose, chronically instrumented swine with and without MI ran on a treadmill at 0-4 km h^{-1}. Mixed ET_A /ET_B receptor blockade (tezosentan) was performed in the absence and presence of single or combined endothelial NO synthase (eNOS, with N^{ω}-Nitro-L-arginine) and cyclo-oxygenase (COX, with indomethacin) inhibition. In normal swine, mixed ET_A /ET_B blockade decreased pulmonary vascular resistance, but only during exercise. In MI swine, an increased ET-mediated vasoconstrictor influence was observed in the pulmonary circulation both at rest and during exercise. Inhibition of COX resulted in pulmonary vasoconstriction at rest in MI, but not in normal swine; this vasoconstriction in MI swine was normalized by ET_A /ET_B receptor blockade. Inhibition of eNOS enhanced the vasodilator response to ET_A /ET_B blockade, indicating that NO blunts the pulmonary vasoconstrictor influence of ET. However, this vasodilator response was enhanced to a similar degree in MI and normal swine. In summary, swine with a recent MI are characterized by an exaggerated pulmonary vasoconstrictor influence of ET. This increased ET- mediated pulmonary vasoconstrictor influence is not caused by a loss of NO bioavailability, and is blunted by an increased prostanoid-mediated vasodilation. In conclusion, an increased ET-mediated vasoconstriction, which does not appear to be the result of loss of endothelial vasodilators, contributes to pulmonary hypertension after MI.

Introduction

Myocardial infarction (MI) results in decreased left ventricular (LV) function, thereby increasing LV filling pressures. These increased LV filling pressures are transmitted backwards into the pulmonary circulation, leading to an increase in pulmonary arterial pressure (PAP). This so called "secondary pulmonary hypertension" is frequently associated with a 'reactive' increase in pulmonary vascular resistance (PVR), resulting in a further increase in PAP [1]. Thus, the pulmonary circulation after MI is characterized by elevated PAP and PVR, which increase the afterload of the right ventricle (RV) and may contribute to RV dysfunction and eventually RV failure [2].

Pulmonary hypertension is exacerbated during exercise, as the vasodilator capacity of the pulmonary vasculature is reduced [3-5]. Moreover, in patients with heart failure, exercise capacity is inversely correlated with PVR and the failure to decrease PVR during exercise [6]. The mechanism underlying the pulmonary vasoconstriction after MI is still incompletely understood, but may involve alterations in serotonin, thromboxane A_2, angiotensin-II [7] as well as endothelial dysfunction [1, 8]. Under normal, physiological conditions, the endothelium plays a key role in maintaining vascular homeostasis by carefully balancing the production of vasodilators such as nitric oxide (NO) and prostacyclin, and vasoconstrictors such as endothelin (ET). Moreover, NO [9-12] and prostacyclin [13, 14] can limit the production and/or reduce receptor sensitivity of ET, while ET, by binding to the ET_B receptor on the endothelium, stimulates the production of NO and prostanoids [9, 15-18]. Indeed, we have recently shown that in the pulmonary circulation of normal swine endogenous NO blunts the vasoconstrictor influence of ET, thereby maintaining a low PAP and PVR particularly during exercise [19].

In general, an increase in shear stress results in an increased production of NO and prostanoids [20], while an increase in intravascular pressure may increase the production of ET [21, 22]. Hence, following MI, a lower cardiac output may contribute to a reduced production of NO and prostanoids [23-25] while the increased PAP may contribute to an increased production of ET [26], by the pulmonary endothelial cells. This imbalance between production of vasodilators and vasoconstrictors likely contributes to the sustained state of pulmonary vasoconstriction after MI. In support of this concept, we recently observed an increased ET-mediated vasoconstrictor influence in the pulmonary circulation after MI [27]. Interestingly, the vasodilator influence of endogenous NO appeared to be maintained [28], while perturbations in the vasomotor influence of prostanoids have not been investigated to date.

In light of these considerations, the aim of the present study was to investigate alterations in integrated endothelial control of pulmonary vascular tone in swine with secondary pulmonary hypertension following a recent MI. Specifically, we investigated whether the increased pulmonary vasoconstrictor influence of endogenous ET after MI is due to a decreased influence of NO and/or prostanoids, or whether the increased ET vasoconstriction occurs independent of these endothelial vasodilators. For this purpose we investigated the effects of ET receptor blockade in the absence and presence of single or combined NO synthase (NOS) and cyclo-oxygenase (COX) inhibition. In view of the increased contribution of ET to pulmonary vasomotor tone during exercise [27], and the close correlation between plasma ET levels and exercise capacity in patients with heart failure [29] we investigated the interaction between NO, prostanoids and ET at rest and during graded treadmill exercise.

Methods

Animals

Studies were performed in accordance with the Council of Europe Convention (ETS123)/ Directive (86/609/EEC) for the protection of vertebrate animals used for experimental and other scientific purposes, and with approval of the Animal Care Committee of the Erasmus Medical Center. Thirty-four 2-3-month-old Yorkshire X Landrace swine (22±1 kg at the time of surgery) of either sex entered the study. After completing all experimental protocols, animals were killed by an intravenous overdose of pentobarbitone sodium.

Surgery

Swine were sedated with ketamine (30 mg·kg^{-1} im), anaesthetized with thiopental (10 mg·kg^{-1} iv), intubated and ventilated with a mixture of O_2 and N_2O (1:2) to which 0.2-1% (v/v) isoflurane was added [30]. Anaesthesia was maintained with midazolam (2 mg kg^{-1} + 1 mg kg^{-1} h^{-1} iv) and fentanyl (10 μg kg^{-1} h^{-1} iv); depth of anaesthesia was checked regularly using a pain stimulus (toe-pinch). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid-filled polyvinylchloride catheter was inserted into the aortic arch for aortic blood pressure measurement (Combitrans pressure transducers, Braun) and blood sampling. A calibrated electromagnetic flow probe (14-15 mm, Skalar) was positioned around the ascending aorta for measurement of cardiac output. Polyvinylchloride catheters were inserted into the left atrium to measure pressure, and into the pulmonary artery to measure pressure, administer drugs and collect

mixed venous blood samples. In 14 swine the circumflex artery was permanently occluded with a suture to induce MI of the lateral left ventricular wall (infarct size ~20% of the left ventricle [28, 31]). Catheters were tunnelled to the back and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine im) for 2 days and antibiotic prophylaxis (25 mg kg^{-1} amoxicillin and 5 mg kg^{-1} gentamycin iv) for 5 days. Three MI swine died in the first week after surgery.

Experimental protocols

Studies were performed ~2 weeks after surgery with animals exercising on a motor driven treadmill. We previously demonstrated excellent reproducibility of the cardiovascular response to consecutive exercise trials with 90 minutes of rest in between in both normal swine [30] and MI swine [28, 32]. In the present study, four exercise protocols were performed on different days and in random order. Most swine participated in several protocols. Overlap of animals between protocols is shown in Table 1. We have previously published part of our data on endothelial control of pulmonary vasomotor tone in normal swine [19] and on the role of endothelin in control of pulmonary vasomotor tone in MI swine [27].

Endothelin. With swine (Normal, n=17 and MI, n=9) lying quietly on the treadmill, resting hemodynamic measurements, consisting of heart rate, cardiac output, mean aortic pressure (MAP), mean pulmonary arterial pressure (PAP), and mean left atrial pressure (LAP) were obtained and blood samples collected. Hemodynamic measurements were repeated and rectal temperature was measured with animals standing on the treadmill. Subsequently, a four-stage (1-4 km h^{-1}) treadmill exercise protocol was started; each exercise stage lasted 2-3 min. This exercise protocol results in heart rates of 230-240 beats per minute, which equals approximately 85% of the estimated maximal heart rate of these animals [31, 32]. Hemodynamic variables were continuously recorded and blood samples collected during the last 45 s of each stage. The signal of the electromagnetic flow probe was calibrated using an electric signal and the zero-value was set during diastole. Fluid-filled pressure-transducers were positioned on the back of the animals and calibrated at mid-chest level. After completing the exercise protocol animals were allowed to rest on the treadmill for 90 min after which the mixed ET_A /ET_B receptor antagonist tezosentan (a gift from Dr Clozel, Actelion Pharmaceuticals Ltd.) was intravenously administered over 10 min in a dose of 3 mg kg^{-1}, followed by a continuous infusion of 6 mg kg^{-1} h^{-1} iv [33], and the exercise protocol was repeated. We have previously shown that this dose of tezosentan abolished the ~35 mmHg increase in arterial pressure in response to administration of exogenous endothelin in a dose of 50 ng kg^{-1} min^{-1} [33].

Prostanoids and endothelin. Ninety min after 10 Normal and 9 MI swine had undergone a control exercise trial (as described above), animals received the cyclo-oxygenase inhibitor indomethacin ((Sigma) 10 mg kg^{-1} iv over 10 min [34]) and 5 min later underwent a second exercise trial. Ninety min later, animals received indomethacin in a dose of 5 mg kg^{-1} iv, which resulted in hemodynamic conditions that were identical to those following administration of 10 mg kg^{-1} prior to the second exercise trial [19]. Subsequently, animals received tezosentan (3 mg kg^{-1} iv + 6 mg kg^{-1} h^{-1} iv) and underwent a third exercise trial.

NO and endothelin. Ninety min after 10 Normal and 7 MI swine had undergone a control exercise trial, animals received the NO-synthase inhibitor N^{ω}-nitro-L-arginine (NLA (Sigma), 20 mg kg^{-1} iv; [30, 34]), and underwent a second exercise trial. Ninety min later, animals received tezosentan (3 mg kg^{-1} iv + 6 mg kg^{-1} h^{-1} iv) and underwent a third exercise trial.

NO, prostanoids and endothelin. Ninety min after 7 Normal and 7 MI swine underwent an exercise trial in the presence of NLA (20 mg kg^{-1} iv) and indomethacin (10 mg kg^{-1} iv), animals received indomethacin (5 mg kg^{-1} iv) and tezosentan (3 mg kg^{-1} iv and 6 mg kg^{-1} h^{-1} iv) and underwent a second exercise trial [19].

Blood gas measurements

Blood samples were kept in iced syringes until the conclusion of each exercise trial. Measurements of PO_2 (mmHg), PCO_2 (mmHg) and pH were then immediately performed with a blood gas analyser (Acid-Base Laboratory Model 505, Radiometer, Copenhagen, Denmark), and corrected for body temperature. O_2 saturation (%) and haemoglobin (grams per 100 ml) were measured with a hemoximeter (OSM3, Radiometer). Blood O_2 content (µmol ml^{-1}) was computed as (Hb 0.621 O_2-saturation) + (0.00131 PO_2). Body O_2 consumption (BVO_2) was calculated as the product of cardiac output and the difference in O_2 content between arterial and mixed venous blood [30, 35].

Table 1: Schematic representation of the overlap of animals used in the various protocols

Protocol	Con-Tezo	Indo-Tezo	NLA-Tezo	NLA-Indo-Tezo	*Total*
Con-Tezo	**17N / 9MI**	8N	9N	7N	
Indo-Tezo	8MI	**10N / 9MI**	3N	3N	
NLA-Tezo	6MI	7MI	**10N / 7MI**	6N	
NLA-Indo-Tezo	5MI	6MI	6MI	**7N / 7MI**	
Total					***20N / 11MI***

Data analysis

Digital recording and off-line analysis of hemodynamics have been described previously [30, 35]. Systemic vascular resistance (SVR) was computed as mean aortic blood pressure divided by cardiac output. Pulmonary vascular resistance (PVR) was computed as mean pulmonary arterial pressure minus mean left atrial pressure divided by cardiac output [34].

Statistical analysis

Hemodynamic data were digitally recorded and analysed off-line. Hemodynamic variables were analysed using analysis of variance (ANOVA) for repeated measures. Post-hoc testing was done using Dunnett's test. The relationships between BVO_2 and SVR and PVR were analysed using multiple regression analysis with BVO_2, tezosentan, NLA, indomethacin and their interactions as independent variables and with each animal as a dummy variable for normal animals and animals with MI separately (Statview). Subsequently, interaction between the drugs and MI was included in the regression analysis. Statistical significance was accepted when $P \leq 0.05$. Data are presented as mean ± S.E.M. Since no significant differences were found between male and female swine, data from both sexes were pooled.

Results

Hemodynamic responses to exercise in normal and MI swine

Exercise up to 4 km h^{-1} in normal swine resulted in a tripling of BVO_2 which was met by a doubling of cardiac output and an increase in O_2 extraction from 47±1% to 67±1% (Fig. 1). The increase in cardiac output was principally due to an increase in heart rate, as stroke volume increased by only 7%. Mean aortic blood pressure was minimally affected, implying that the increase in cardiac output was balanced by a similar decrease in systemic vascular resistance (Table 2). In contrast, mean pulmonary arterial pressure almost doubled in normal swine during exercise (Fig. 1). The transpulmonary pressure gradient (pulmonary arterial pressure minus left atrial pressure) increased commensurate with cardiac output, so that PVR did not change during exercise.

The 18% lower stroke volume in MI compared to normal swine was accompanied by an increase in heart rate. Yet, the increase in heart rate did not fully compensate for the decrease in stroke volume. Hence, cardiac output was slightly lower at all levels of treadmill exercise, which necessitated a small increase in O_2-extraction that resulted in a further decrease in mixed venous O_2 saturation during exercise in MI compared to normal swine (Fig. 1, Table 3). However, aerobic metabolism

was still maintained as arterial (Table 4 and 5) and mixed venous (not shown) pH and HCO_3^- were not different between normal swine and swine with MI. Pulmonary arterial pressure was significantly elevated in MI swine both at rest and during exercise, which was principally due to a marked increase in left atrial pressure but also due to a ~20% increase in PVR (Fig. 1).

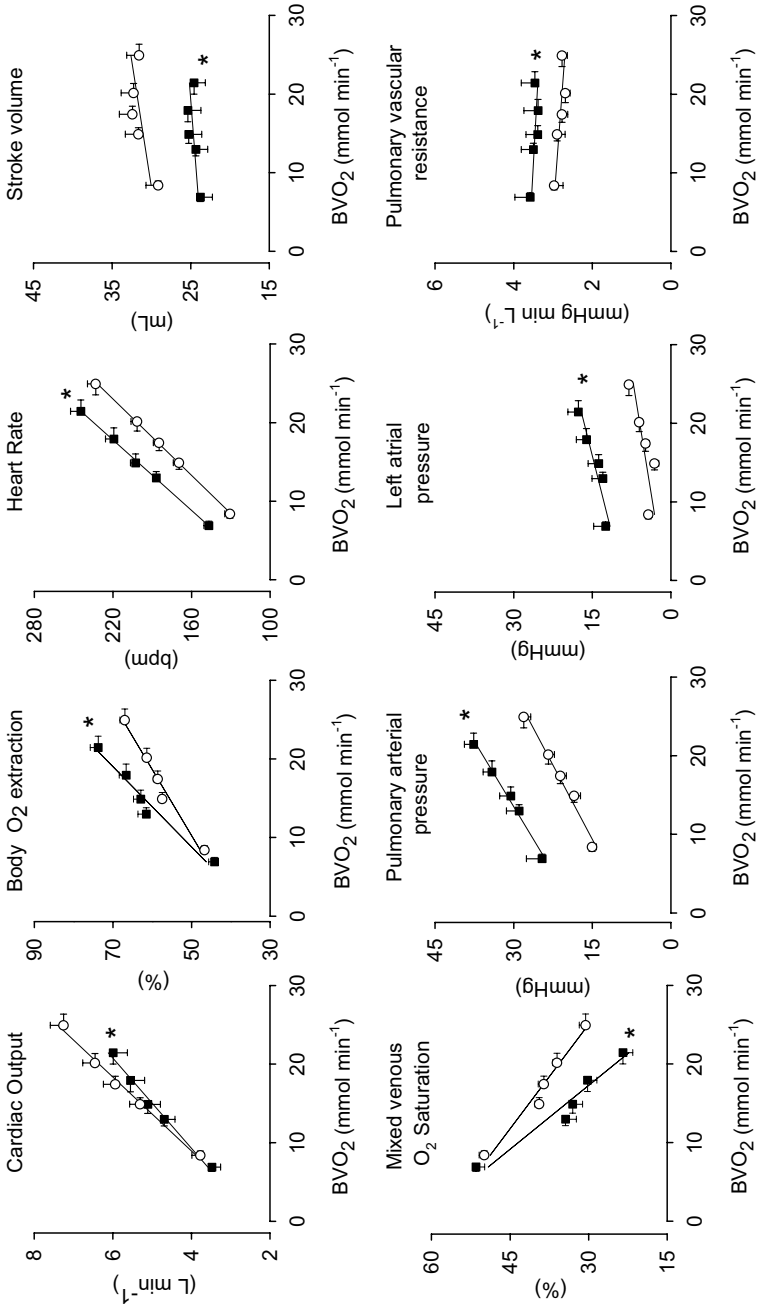

Figure1: *The responses to exercise of various parameters in relation to the body oxygen consumption (BVO_2) under control conditions in 16 normal swine (open circles) and 9 MI swine (closed squares). *P≤0.05 MI versus Normal (change in intercept and/or slope)*

Role of NO and prostanoids in the regulation of vascular tone

Systemic circulation. Administration of the eNOS inhibitor NLA or the cyclo-oxygenase inhibitor indomethacin resulted in a pressor response due to systemic vasoconstriction as evidenced by marked increases in mean arterial pressure and SVR in both normal and MI swine (Fig. 2, Table 2 and 3). As a result heart rate decreased, while stroke volume was not altered, resulting in a decrease in cardiac output. During exercise the systemic vasoconstriction and the pressor response to indomethacin were progressively blunted, whereas the pressor response and systemic vasoconstriction in response to NLA was unaltered (Fig. 2,Table 2 and 3).

Pretreatment with NLA enhanced the vasoconstriction induced by indomethacin particularly at rest, as evidenced by an exaggerated increase in SVR in normal swine, indicating that NO and prostanoids act synergistically to maintain a low vasomotor tone. Yet, despite the enhanced response at rest, exercise-induced vasodilation was unmitigated in the presence of both NLA and indomethacin (Fig. 2). In contrast to the findings in normal swine, the vasoconstrictor response to indomethacin was not altered by pretreatment with NLA in swine with MI either at rest or during exercise.

Pulmonary circulation. Indomethacin had no effect on the PVR either during rest or exercise in normal swine; however PVR increased by 42±8% in response to indomethacin at rest, but not during exercise in MI swine ($P \leq 0.05$). Administration of NLA produced a similar rise in PVR in normal and MI swine at rest, which persisted during exercise (Fig. 3). Subsequent inhibition of COX with indomethacin resulted in a 71±18% increase in PVR in MI swine at rest ($P \leq 0.05$), but did not increase PVR in either normal or MI swine during exercise (Fig. 3).

The pulmonary hemodynamic effects of indomethacin and NLA did not result in impaired pulmonary gas exchange in either normal (Table 4) or MI swine (Table 5). Thus, arterial PO_2 was not altered by NLA in either group, while indomethacin actually produced a small increase in arterial PO_2 that was however similar in both groups of swine. In view of the small indomethacin-induced decrease in arterial PCO_2, it is likely that this was caused by mild hyperventilation.

The role of endothelin in the regulation of vascular tone

Systemic circulation. Administration of the mixed ET_A/ET_B antagonist tezosentan resulted in a decrease of mean arterial pressure and SVR in both normal and MI swine. The systemic vasodilation resulted in a compensatory, probably baroreflex mediated increase in heart rate and cardiac output (Table 2 and 3). The tezosentan-induced vasodilator response diminished with increasing exercise levels (Fig. 2). Pretreatment with the eNOS inhibitor NLA enhanced the vasodilator response

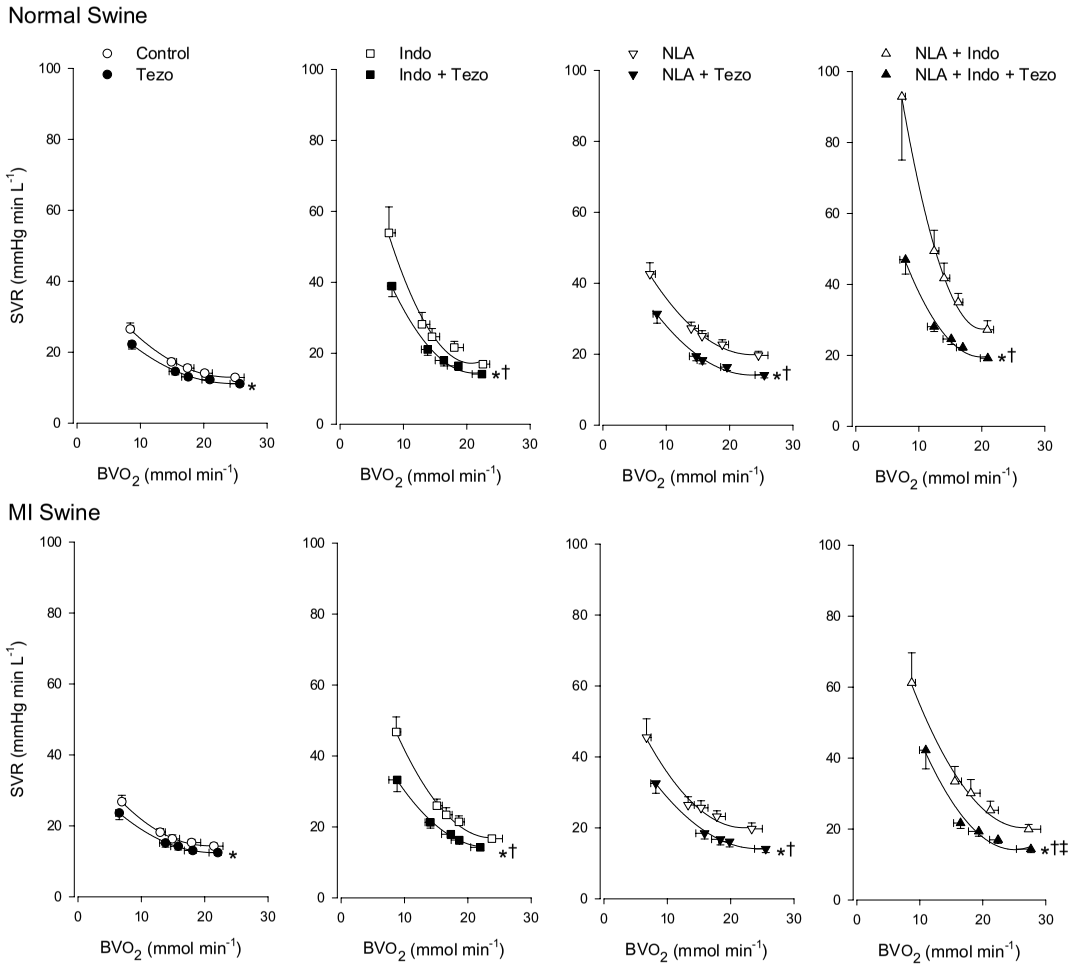

Figure 2: *From left to right: effect of endothelin receptor blockade (tezosentan (Tezo); 16 normal and 9 MI swine), cyclo-oxygenase inhibition and endothelin receptor blockade (Indo + Tezo; 9 normal and 8 MI swine), NO synthase inhibition and endothelin receptor blockade (NLA + Tezo; 10 normal and 7 MI swine) and combined inhibition of cyclo-oxygenase and NO synthase and endothelin receptor blockade (NLA + Indo + Tezo; 6 normal and 6 MI swine) on the relation between body oxygen consumption (BVO_2) and systemic vascular resistance (SVR) in normal swine (upper panels) and swine with MI (lower panels). Significance symbols denote a change in intercept or intercept and slope. *P≤0.05 effect of Tezo vs corresponding control, †P≤0.05 effect of Tezo in presence of Indo, NLA or NLA+Indo vs Tezo alone, ‡P≤0.05 effect of Tezo is different after MI.*

to tezosentan at rest and during exercise in both normal and MI swine (Fig. 2). Administration of COX-inhibitor indomethacin prior to tezosentan, increased the vasodilator response to tezosentan at rest, but only in MI swine, however this effect waned with exercise (Fig. 2). In normal swine, combined administration of indomethacin and NLA increased the vasodilator responses to tezosentan even further, particularly at rest (Fig. 2). These observations suggest that both NO and prostanoids blunt the vasoconstrictor effects of ET in the systemic circulation. Although plasma-ET levels were increased following MI (3.9±0.5 pM vs 2.3±0.3 pM in normal swine, $P \leq 0.05$), myocardial infarction did not change the vasodilator effect of tezosentan on the systemic circulation after administration of NLA or indomethacin alone (Fig. 2), indicating that after MI, NO and prostanoids still exert part of their vasodilator effect through inhibition of ET-induced vasoconstriction. However, the effect of tezosentan after combined administration of NLA and indomethacin was reduced in swine with MI as compared to normal swine, and was similar to the effect of tezosentan in the presence of NLA or indomethacin alone (Fig. 2). Thus, the vasoconstrictor effect of ET in the systemic circulation is limited by NO and prostanoids, but loss of both NO and prostanoids does not result in further activation of the ET system as compared to loss of one of these compounds (Fig. 2).

Pulmonary circulation. Administration of tezosentan did not exert any effect on the pulmonary circulation of normal swine at rest. However, during exercise pulmonary arterial pressure and pulmonary vascular resistance decreased in response to administration of tezosentan (Fig. 3). In agreement with our previous observations [27], the vasodilator response to tezosentan was more pronounced in MI swine as compared to normal swine (Fig. 3). Tezosentan had no effect on arterial blood gas values in either normal (Table 4) or MI swine (Table 5).

Pretreatment with NLA markedly augmented the vasodilator effect of tezosentan at rest as well as during exercise in normal swine and swine with MI (Fig. 3), indicating that NO limits the ET-induced pulmonary vasoconstriction to the same extent in both groups. Pretreatment with indomethacin did not affect vasodilation in response to tezosentan in normal swine. However, indomethacin resulted in an increase in the vasodilator response to tezosentan in MI swine particularly at rest (Fig. 3), indicating that prostanoids limit ET-induced pulmonary vasoconstriction in swine with MI. In the presence of NLA, the pulmonary vasodilator effect of tezosentan in normal swine was not affected by administration of indomethacin (Fig. 3). In contrast, in swine with MI, the effect of tezosentan was smaller in the presence of NLA and indomethacin as compared to the presence of NLA alone (Fig. 3).

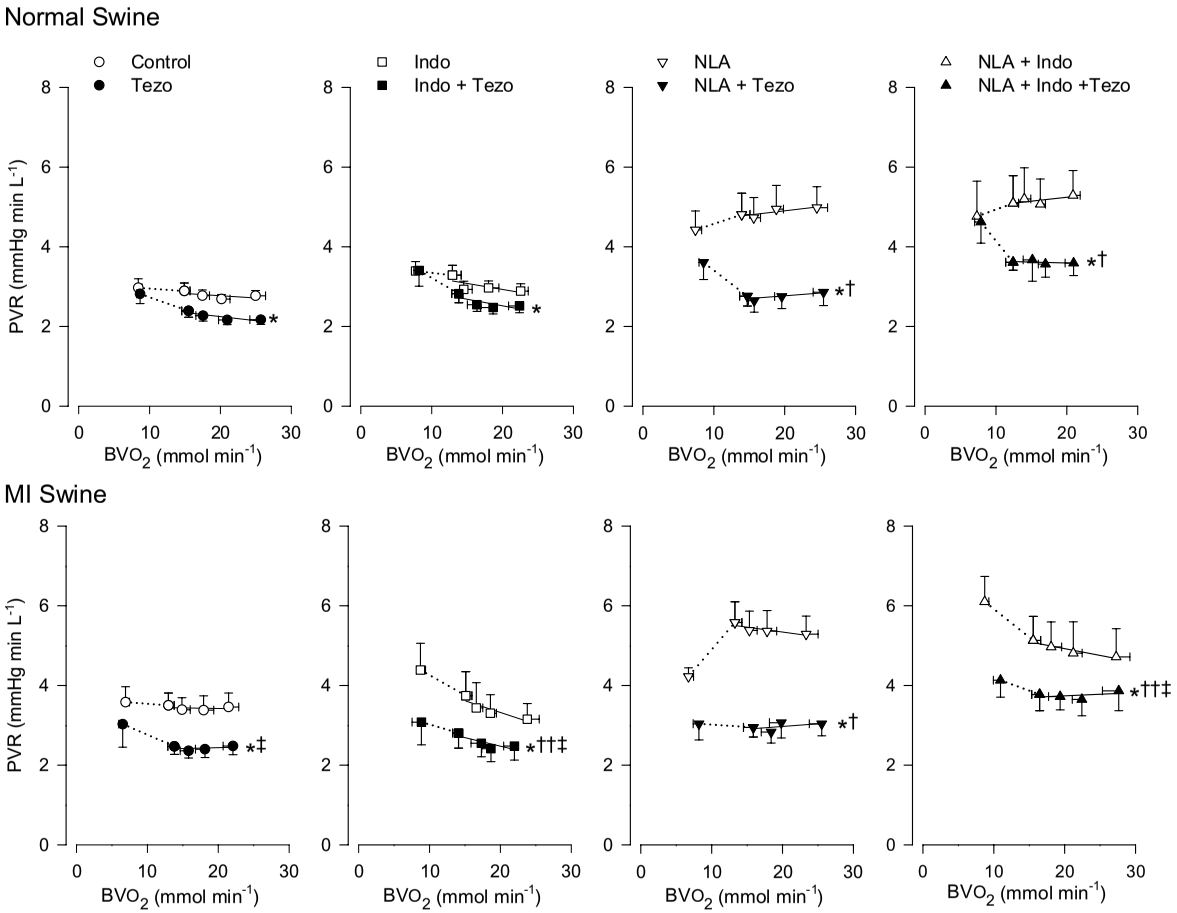

Figure 3: *From left to right: effect of endothelin receptor blockade (tezosentan (Tezo); 16 normal and 9 MI swine), cyclo-oxygenase inhibition and endothelin receptor blockade (Indo + Tezo; 9 normal and 8 MI swine), NO synthase inhibition and endothelin receptor blockade (NLA + Tezo; 10 normal and 7 MI swine) and combined inhibition of cyclo-oxygenase and NO synthase and endothelin receptor blockade (NLA + Indo + Tezo; 6 normal and 6 MI swine) on the relation between body oxygen consumption (BVO_2) and pulmonary vascular resistance (PVR) in normal swine (upper panels) and swine with MI (lower panels). Significance symbols denote a change in intercept or intercept and slope. *P≤0.05 effect of Tezo vs corresponding control, †P≤0.05 effect of Tezo in presence of Indo, NLA or NLA+Indo vs Tezo alone,, ††P≤0.10 effect of Tezo in presence of Indo, NLA or NLA+Indo vs Tezo alone, ‡P≤0.05 effect of Tezo is different after MI.*

Table 2: *Hemodynamic variables in normal swine at rest and during exercise before and after single or combined blockade of eNOS, cyclooxygenase and/or ET_A/ET_B receptors*

	Rest		Exercise level (km/h)			
Treatment	bl	standing	1	2	3	4
HR (bpm)						
Control	131 ± 4	148 ± 4*	170 ± 4*	185 ± 4*	202 ± 5*	233 ± 6*
Tezo	150 ± 4‡	162 ± 4*‡	185 ± 5*‡	185 ± 7*‡	215 ± 6*‡	244 ± 7*‡
Indo	86 ± 7†	97 ± 5*†	121 ± 6*†	134 ± 6*†	150 ± 6*†	174 ± 5*†
Indo + Tezo	97 ± 7†‡	112 ± 5*†‡	128 ± 6*†	141 ± 6*†	157 ± 7*†	178 ± 8*†
LNNA	103 ± 6†	120 ± 4*†	132 ± 5*†	142 ± 5*†	159 ± 5*†	191 ± 6*†
LNNA + Tezo	118 ± 5‡	141 ± 6*‡	152 ± 6*†‡	160 ± 6*†‡	179 ± 6*†‡	204 ± 6*†‡
LNNA + Indo	82 ± 4†	95 ± 6*†	112 ± 6*†	121 ± 8*†	134 ± 7*†	164 ± 9*†
LNNA + Indo + Tezo	101 ± 8	116 ± 7*‡	128 ± 6*‡	141 ± 8*	155 ± 10*†‡	179 ± 9*†
CO (l/min)						
Control	3.8 ± 0.2	4.6 ± 0.2*	5.3 ± 0.3*	5.9 ± 0.3*	6.5 ± 0.3*	7.3 ± 0.3*
Tezo	4.2 ± 0.2‡	4.8 ± 0.3*	5.7 ± 0.3*	6.2 ± 0.3*	6.9 ± 0.4*‡	7.7 ± 0.4*‡
Indo	2.6 ± 0.3†	3.2 ± 0.3*†	4.1 ± 0.3*†	4.5 ± 0.3*†	5.1 ± 0.3*†	6.2 ± 0.3*†
Indo + Tezo	3.0 ± 0.2†	3.8 ± 0.3*†‡	4.4 ± 0.3*†	5.2 ± 0.4*‡	5.6 ± 0.4*†‡	6.4 ± 0.4*†
LNNA	3.1 ± 0.3†	3.8 ± 0.2*†	4.5 ± 0.3*†	4.9 ± 0.4*†	5.5 ± 0.4*†	6.4 ± 0.4*†
LNNA + Tezo	3.8 ± 0.3‡	4.8 ± 0.4*‡	5.3 ± 0.4*†‡	5.6 ± 0.4*†‡	6.4 ± 0.4*‡	7.3 ± 0.5*†‡
LNNA + Indo	1.9 ± 0.2	2.4 ± 0.2*	3.1 ± 0.2*†	3.5 ± 0.3*	4.0 ± 0.2*†	4.9 ± 0.4*
LNNA + Indo + Tezo	2.8 ± 0.3‡	3.5 ± 0.3*‡	4.0 ± 0.2*‡	4.6 ± 0.3*‡	5.0 ± 0.3*‡	5.7 ± 0.2*‡
SV (l/min)						
Control	29 ± 2	31 ± 2*	32 ± 2*	32 ± 2*	32 ± 2*	32 ± 2*
Tezo	28 ± 2	30 ± 2*	31 ± 2*	31 ± 2*	33 ± 2*	32 ± 2*
Indo	30 ± 2	32 ± 2*	34 ± 2*	34 ± 2*	35 ± 2*†	36 ± 1*†
Indo + Tezo	31 ± 2†	34 ± 2*	34 ± 1*	37 ± 1*†	36 ± 2*†	36 ± 1*†
LNNA	30 ± 3	32 ± 2*	34 ± 2*	34 ± 2*	35 ± 2*	34 ± 2
LNNA + Tezo	32 ± 3	34 ± 3*	35 ± 3*	35 ± 3*	36 ± 2‡	36 ± 3*‡
LNNA + Indo	23 ± 1	25 ± 1	27 ± 1*	28 ± 1*	29 ± 1*	30 ± 1*†
LNNA + Indo + Tezo	27 ± 1	30 ± 1‡	31 ± 1*‡	32 ± 2*	32 ± 1*†‡	31 ± 1*†
MAP(mmHg)						
Control	95 ± 2	89 ± 3*	87 ± 2*	88 ± 2*	88 ± 2*	90 ± 2*
Tezo	89 ± 2‡	79 ± 2*‡	79 ± 2*‡	79 ± 2*‡	81 ± 2*‡	82 ± 1*‡
Indo	124 ± 7†	120 ± 7†	107 ± 6*†	106 ± 6*†	107 ± 5*†	104 ± 5*†
Indo + Tezo	111 ± 3†	93 ± 3*‡	89 ± 2*‡	89 ± 3*‡	89 ± 3*‡	88 ± 3*‡
LNNA	122 ± 2†	120 ± 3†	118 ± 3†	118 ± 2†	121 ± 2†	123 ± 2†
LNNA + Tezo	111 ± 4†‡	104 ± 2†‡	98 ± 3*†‡	99 ± 2*†‡	102 ± 3†‡	101 ± 2*†‡
LNNA + Indo	161 ± 7†	156 ± 8†	146 ± 6*†	142 ± 5*†	137 ± 4*†	132 ± 4*†
LNNA + Indo + Tezo	131 ± 7‡	121 ± 7*‡	114 ± 5*‡	114 ± 5*‡	115 ± 5*‡	113 ± 5*‡
PAP(mmHg)						
Control	15 ± 1	15 ± 1	18 ± 1*	21 ± 1*	23 ± 1*	28 ± 1*
Tezo	15 ± 1	13 ± 1	17 ± 1‡	17 ± 1*‡	21 ± 1*‡	25 ± 1*‡
Indo	19 ± 1†	18 ± 2	18 ± 2	20 ± 2	22 ± 2*	26 ± 2*
Indo + Tezo	17 ± 2	14 ± 2*‡	15 ± 2†	17 ± 2†	20 ± 2†	23 ± 2*†
LNNA	23 ± 2†	23 ± 2†	27 ± 3†	29 ± 3†	34 ± 3*†	41 ± 2*†
LNNA + Tezo	16 ± 2‡	17 ± 2	17 ± 2†‡	19 ± 2‡	25 ± 2*†‡	29 ± 3*‡
LNNA + Indo	24 ± 2†	26 ± 3	27 ± 3	29 ± 3*	32 ± 3*	37 ± 3*
LNNA + Indo + Tezo	21 ± 2	18 ± 3	20 ± 2‡	24 ± 3	26 ± 3*	31 ± 3*†‡
LAP(mmHg)						
Control	4 ± 1	2 ± 1	3 ± 1	5 ± 1	6 ± 1*	8 ± 1*
Tezo	4 ± 1	0 ± 1*	3 ± 1	3 ± 1	6 ± 1*	8 ± 1*
Indo	10 ± 2†	7 ± 2	3 ± 2*	6 ± 1*	6 ± 1*	7 ± 1
Indo + Tezo	8 ± 1†	2 ± 1*‡	3 ± 1*	4 ± 1*	6 ± 1*	7 ± 1
LNNA	10 ± 1†	5 ± 1	7 ± 1	8 ± 1	9 ± 1	10 ± 1
LNNA + Tezo	2 ± 2	3 ± 2	4 ± 1	6 ± 1	9 ± 1*	10 ± 1*
LNNA + Indo	15 ± 2†	13 ± 3	12 ± 3†	12 ± 2*†	13 ± 2*	12 ± 2*
LNNA + Indo + Tezo	8 ± 2‡	4 ± 3	5 ± 2‡	8 ± 2‡	9 ± 1	11 ± 2

*HR, heart rate; CO, cardiac output; MAP, mean arterial pressure; PAP, pulmonary arterial pressure; LAP, left atrial pressure; Data are mean ± S.E.M. * P≤0.05 versus Rest (Lying); † P≤0.05 versus corresponding Control; ‡ P≤0.05 effect of Tezo*

Table 3: *Hemodynamic variables in swine with MI at rest and during exercise before and after single or combined blockade of eNOS, cyclooxygenase and/or ET_A/ET_B receptors*

	Rest		Exercise level (km/h)			
	bl	standing	1	2	3	4
HR (bpm)						
Control	147 ± 4§	158 ± 5*	187 ± 2*§	203 ± 4*§	219 ± 6*§	244 ±8*
Tezo	157 ± 4‡	169 ± 4*‡	190 ± 4*	205 ± 4*	222 ± 7*	243 ±6*
Indo	104 ± 3†§	124 ± 3*†§	139 ± 3*†§	143 ± 4*†	150 ± 6*†	181 ±5*†
Indo + Tezo	112 ± 7†	125 ± 2†§	135 ± 7†	148 ± 5*†	159 ± 4*†	175 ±7*†
LNNA	111 ± 4†	139 ± 6	155 ± 7*†§	167 ± 6*†§	180 ± 6*†§	209 ±8*
LNNA + Tezo	131 ± 5‡	152 ± 5	165 ± 5*	176 ± 8	190 ± 9	216 ±10
LNNA + Indo	91 ± 6	99 ± 6*	115 ± 6*	125 ± 6*	142 ± 5*	165 ±5*
LNNA + Indo + Tezo	86 ± 5	100 ± 4*	122 ± 4*	133 ± 4*	148 ± 4*	173 ±3*‡
CO (l/min)						
Control	3.5 ± 0.2	4.0 ± 0.3*	4.7 ± 0.3*	5.1 ± 0.3*	5.6 ± 0.4*	6.0 ±0.4*§
Tezo	3.6 ± 0.3	4.6 ± 0.4*‡	5.2 ± 0.4*‡	5.6 ± 0.4*‡	6.0 ± 0.4*‡	6.4 ±0.4*‡
Indo	2.8 ± 0.2†	3.6 ± 0.3*†	4.2 ± 0.3*†	4.6 ± 0.3*†	4.9 ± 0.3*†	5.8 ±0.3*†
Indo + Tezo	3.4 ± 0.3‡	4.2 ± 0.3*‡	4.7 ± 0.3*†	5.2 ± 0.3*†‡	5.6 ± 0.3*†‡	6.2 ±0.3*†
LNNA	3.0 ± 0.3†	3.9 ± 0.3	4.4 ± 0.3*†	4.6 ± 0.3*†	5.1 ± 0.3*†	5.8 ±0.4*†
LNNA + Tezo	3.7 ± 0.2‡	4.8 ± 0.3	5.2 ± 0.3*‡	5.8 ± 0.4	6.1 ± 0.5	6.8 ±0.4
LNNA + Indo	2.6 ± 0.3†	3.3 ± 0.3†	4.2 ± 0.3*†	4.6 ± 0.3*†	5.2 ± 0.3*†	6.2 ±0.4*†
LNNA + Indo + Tezo	3.4 ± 0.3‡	4.5 ± 0.3*‡	5.1 ± 0.2*‡	5.6 ± 0.3*‡	6.2 ± 0.3*‡	7.1 ±0.4*‡
MAP(mmHg)						
Control	90 ± 2	83 ± 2*	81 ± 2*§	81 ± 2*§	82 ± 3*	83 ±3
Tezo	80 ± 2‡§	76 ± 2‡	75 ± 2‡	76 ± 2‡	76 ± 2‡	77 ±2‡
Indo	126 ± 5†	114 ± 4†	104 ± 4*†	101 ± 4*†	99 ± 4*†	94 ±5*†
Indo + Tezo	103 ± 4†‡	97 ± 5†‡	94 ± 3*†‡	90 ± 4*‡	87 ± 4*‡	85 ±4*‡
LNNA	125 ± 5†	112 ± 5	113 ± 4*†	115 ± 4*†	115 ± 3*†	111 ±3*†
LNNA + Tezo	115 ± 5†‡	97 ± 4	94 ± 5*†‡	94 ± 5*	94 ± 5*‡	93 ±4*
LNNA + Indo	147 ± 5†	141 ± 8†	133 ± 7*	132 ± 7*	127 ± 7*	118 ±5*
LNNA + Indo + Tezo	129 ± 4‡	117 ± 4*‡	112 ± 5*‡	109 ± 5*‡	108 ± 5*	105 ±5*
SV(ml)						
Control	24 ± 2	26 ± 2*	24 ± 1	25 ± 2	25 ± 2*	25 ±1
Tezo	23 ± 1	27 ± 2*	27 ± 2*	27 ± 2*‡	27 ± 2*‡	27 ±2*‡
Indo	30 ± 2	32 ± 2*	34 ± 2*	34 ± 2*	35 ± 2*†	36 ±1*†
Indo + Tezo	31 ± 2†	34 ± 2*	34 ± 1*	37 ± 1*†	36 ± 2*†	36 ±1*†
LNNA	26 ± 2	28 ± 2	29 ± 2*†	28 ± 2†§	29 ± 2	28 ±2
LNNA + Tezo	28 ± 1	32 ± 2	32 ± 2*‡	33 ± 3	33 ± 3	32 ±3
LNNA + Indo	27 ± 1	33 ± 1†	36 ± 1	36 ± 1	36 ± 1	37 ±1
LNNA + Indo + Tezo	39 ± 2†‡	43 ± 2*†‡	41 ± 1†‡	42 ± 1†‡	42 ± 1†‡	41 ±2†‡
PAP(mmHg)						
Control	25 ± 3§	24 ± 2§	29 ± 2*§	31 ± 2*§	34 ± 2*§	38 ±2*§
Tezo	21 ± 2§	21 ± 2‡§	25 ± 2‡§	28 ± 2*‡§	31 ± 2*§	33 ±2*‡§
Indo	28 ± 3†§	29 ± 3†§	32 ± 3§	33 ± 3§	35 ± 3*§	37 ±3*§
Indo + Tezo	21 ± 2‡	22 ± 3‡§	28 ± 4*‡§	29 ± 3*§	29 ± 3*†‡§	33 ±3*†‡§
LNNA	29 ± 2†	35 ± 2	38 ± 3*†§	41 ± 3*†§	44 ± 3*†§	48 ±2*†§
LNNA + Tezo	25 ± 2	24 ± 3	26 ± 2‡§	30 ± 2‡	33 ± 3‡	37 ±3‡
LNNA + Indo	35 ± 4†§	34 ± 4	37 ± 5	39 ± 5	43 ± 5*	45 ±4*
LNNA + Indo + Tezo	29 ± 3‡	27 ± 4*‡	31 ± 3*‡§	34 ± 4*‡	38 ± 4*‡§	42 ±4*§
LAP(mmHg)						
Control	12 ± 2§	10 ± 2§	13 ± 2§	14 ± 2§	16 ± 2*§	18 ±2*§
Tezo	11 ± 2§	10 ± 2§	12 ± 2§	15 ± 2*§	17 ± 2*§	18 ±2*§
Indo	17 ± 3†	15 ± 3†	18 ± 3†§	20 ± 3§	20 ± 3§	20 ±2§
Indo + Tezo	11 ± 2‡	11 ± 3§	15 ± 2*†§	16 ± 2*†§	17 ± 2*§	19 ±2*§
LNNA	17 ± 1†§	14 ± 2§	14 ± 1§	16 ± 1§	17 ± 0§	18 ±1§
LNNA + Tezo	13 ± 1‡§	9 ± 2*	10 ± 1*†‡§	13 ± 1	15 ± 1	17 ±1
LNNA + Indo	14 ± 3†	14 ± 3	15 ± 3	16 ± 3	17 ± 3	18 ±3
LNNA + Indo + Tezo	15 ± 3†‡	13 ± 2	14 ± 2§	15 ± 3	18 ± 3	18 ±3

HR, heart rate; CO, cardiac output; MAP, mean arterial pressure; PAP, pulmonary arterial pressure; LAP, left atrial pressure; Data are mean ± S.E.M. P≤0.05 versus Rest (Lying); † P≤0.05 versus corresponding Control; ‡P≤0.05 effect of Tezo; § P≤0.05 MI vs Normal*

***Table 4**: Arterial blood gas values in normal swine at rest and during exercise before and after single or combined blockade of eNOS, cyclooxygenase and/or ET_A/ET_B receptors*

	Rest	Exercise level (km/h)			
	Lying	1	2	3	4
PO2 (mmHg)					
Control	97 ± 2	96 ± 2	95 ± 2	95 ± 2	91 ± 2*
Tezo	98 ± 2	95 ± 4	95 ± 2	93 ± 2*	92 ± 2*
Indo	115 ± 5†	110 ± 5†	116 ± 4†	109 ± 7	107 ± 5†
Indo + Tezo	115 ± 4†	104 ± 4*	107 ± 5†	105 ± 5	100 ± 5*†
NLA	98 ± 2	163 ± 68	94 ± 3	92 ± 4	93 ± 5
NLA + Tezo	95 ± 3	92 ± 3	92 ± 4	92 ± 4	88 ± 2
NLA + Indo	125 ± 6†	128 ± 8†	120 ± 3†	112 ± 3†	106 ± 3*†
NLA + Indo + Tezo	111 ± 8	114 ± 11	109 ± 4‡	106 ± 3	103 ± 5
SO2 (%)					
Control	93 ± 1	92 ± 1	93 ± 1	93 ± 1	93 ± 1
Tezo	93 ± 1	92 ± 1*	93 ± 1	92 ± 1	92 ± 1
Indo	93 ± 1†	93 ± 1†	94 ± 1†	93 ± 1†	93 ± 1†
Indo + Tezo	93 ± 1†	92 ± 1†	93 ± 1†	93 ± 1†	93 ± 1†‡
NLA	93 ± 1	92 ± 1	93 ± 1	93 ± 1	93 ± 1
NLA + Tezo	93 ± 1	92 ± 1	93 ± 1	93 ± 1	93 ± 1
NLA + Indo	94 ± 1†	95 ± 1	95 ± 1	95 ± 1	94 ± 1
NLA + Indo + Tezo	94 ± 1	93 ± 1	94 ± 1	94 ± 1	94 ± 1
pH					
Control	7.439 ± 0.019	7.467 ± 0.008	7.474 ± 0.007	7.484 ± 0.007	7.490 ± 0.007*
Tezo	7.454 ± 0.006	7.455 ± 0.006	7.473 ± 0.008*	7.481 ± 0.008*	7.490 ± 0.007*
Indo	7.500 ± 0.008†	7.501 ± 0.007†	7.509 ± 0.008†	7.505 ± 0.009†	7.511 ± 0.007†
Indo + Tezo	7.486 ± 0.007†	7.490 ± 0.008†	7.494 ± 0.007	7.502 ± 0.007*†	7.503 ± 0.007
NLA	7.495 ± 0.011	7.502 ± 0.014	7.512 ± 0.013†	7.513 ± 0.014	7.527 ± 0.009
NLA + Tezo	7.478 ± 0.009‡	7.488 ± 0.013	7.503 ± 0.010*	7.501 ± 0.007	7.516 ± 0.011*
NLA + Indo	7.494 ± 0.011	7.493 ± 0.013	7.495 ± 0.010	7.489 ± 0.012	7.476 ± 0.017
NLA + Indo + Tezo	7.477 ± 0.006	7.484 ± 0.009	7.494 ± 0.008	7.507 ± 0.009	7.502 ± 0.009
PCO2 (mmHg)					
Control	44 ± 1	44 ± 1	43 ± 1	42 ± 1*	41 ± 1*
Tezo	46 ± 1	46 ± 1	45 ± 1	42 ± 1*	42 ± 1*
Indo	39 ± 4	39 ± 4	39 ± 4	39 ± 4	38 ± 4
Indo + Tezo	37 ± 1†	37 ± 1†	36 ± 1†	35 ± 1*†	35 ± 1†
NLA	43 ± 1	42 ± 1	41 ± 1	41 ± 1	37 ± 1
NLA + Tezo	44 ± 1	42 ± 1	41 ± 1*	41 ± 1*	38 ± 1*
NLA + Indo	31 ± 1†	33 ± 2†	32 ± 2†	34 ± 2†	34 ± 1
NLA + Indo + Tezo	37 ± 2†‡	37 ± 1†	37 ± 1†	35 ± 1†	35 ± 1
HCO3 (mmol/L)					
Control	30 ± 1	31 ± 1	31 ± 1	30 ± 1	30 ± 1
Tezo	36 ± 5	36 ± 5	36 ± 5	35 ± 5	35 ± 5
Indo	29 ± 3	30 ± 3	30 ± 4	30 ± 3	30 ± 3
Indo + Tezo	27 ± 1†	27 ± 1*	27 ± 1*	26 ± 1	26 ± 1
NLA	33 ± 1	32 ± 1	32 ± 1	32 ± 1	30 ± 1
NLA + Tezo	32 ± 1	32 ± 1	31 ± 1	31 ± 0	30 ± 0
NLA + Indo	23 ± 1†	24 ± 2†	24 ± 2†	25 ± 2†	25 ± 2†
NLA + Indo + Tezo	27 ± 1†	27 ± 1†	28 ± 1†	27 ± 1†	27 ± 1†

** P≤0.05 versus Rest (Lying); † P≤0.05 versus corresponding Control; ‡ P≤0.05 effect of Tezo*

***Table 5**: Arterial blood gas values in MI swine at rest and during exercise before and after single or combined blockade of eNOS, cyclooxygenase and/or ET_A/ET_B receptors*

	Rest	Exercise level (km/h)			
	Lying	1	2	3	4
PO2 (mmHg)					
Control	101 ± 2	89 ± 5*	86 ± 4*§	90 ± 3*	86 ± 3*
Tezo	93 ± 2†	87 ± 3	87 ± 4*	86 ± 3*	84 ± 3*
Indo	122 ± 7†	121 ± 7	117 ± 5†	109 ± 3†	105 ± 6
Indo + Tezo	109 ± 4	102 ± 5*	108 ± 7‡	106 ± 4	101 ± 3
NLA	95 ± 3	93 ± 4	93 ± 5	93 ± 4	91 ± 5
NLA + Tezo	92 ± 5	90 ± 5	94 ± 3	93 ± 3	85 ± 4
NLA + Indo	117 ± 3†	113 ± 9	104 ± 6*§	103 ± 6	102 ± 5*
NLA + Indo + Tezo	104 ± 3‡	104 ± 5	97 ± 5	99 ± 5	92 ± 7
SO2 (%)					
Control	91 ± 1	89 ± 1§	89 ± 1*§	90 ± 1§	90 ± 1§
Tezo	91 ± 1	89 ± 1§	90 ± 1*§	89 ± 1§	89 ± 1§
Indo	93 ± 1	94 ± 1†	94 ± 1†	94 ± 1†	92 ± 2
Indo + Tezo	93 ± 1	92 ± 1	93 ± 1	94 ± 1†	93 ± 1†
NLA	91 ± 1	92 ± 1	92 ± 1	92 ± 1	92 ± 1
NLA + Tezo	91 ± 1	92 ± 2	94 ± 1	93 ± 2	92 ± 2
NLA + Indo	96 ± 1	96 ± 1	95 ± 1	95 ± 1	95 ± 1
NLA + Indo + Tezo	95 ± 1‡	94 ± 1‡	94 ± 1‡	94 ± 1	92 ± 2
pH					
Control	7.457 ± 0.006	7.457 ± 0.009	7.460 ± 0.009	7.482 ± 0.015*	7.480 ± 0.013*
Tezo	7.455 ± 0.005	7.464 ± 0.010	7.462 ± 0.005	7.474 ± 0.009*	7.474 ± 0.011
Indo	7.479 ± 0.012	7.502 ± 0.013†	7.514 ± 0.012*†	7.511 ± 0.015	7.488 ± 0.026
Indo + Tezo	7.464 ± 0.007	7.487 ± 0.017	7.489 ± 0.014	7.507 ± 0.013*†	7.508 ± 0.015*
NLA	7.467 ± 0.005	7.491 ± 0.009	7.503 ± 0.011*†	7.505 ± 0.008*†	7.516 ± 0.011*†
NLA + Tezo	7.472 ± 0.007	7.490 ± 0.005*	7.495 ± 0.007	7.494 ± 0.011	7.494 ± 0.011
NLA + Indo	7.508 ± 0.016	7.516 ± 0.011	7.504 ± 0.012	7.503 ± 0.013	7.505 ± 0.014
NLA + Indo + Tezo	7.463 ± 0.008‡	7.555 ± 0.060	7.486 ± 0.010	7.500 ± 0.010*	7.489 ± 0.011
PCO2 (mmHg)					
Control	45 ± 1	44 ± 1	45 ± 1	42 ± 1*	41 ± 1*
Tezo	46 ± 1	45 ± 1	44 ± 1	43 ± 1*	42 ± 1*
Indo	33 ± 1†	33 ± 1†	32 ± 1†	34 ± 1†	36 ± 2
Indo + Tezo	39 ± 1†‡	39 ± 1†‡	38 ± 1†‡	37 ± 0†‡	36 ± 1*†
NLA	47 ± 3	44 ± 2*	42 ± 2*	42 ± 2*	40 ± 2*
NLA + Tezo	50 ± 3	48 ± 4*	47 ± 4	48 ± 5	55 ± 9
NLA + Indo	34 ± 3	35 ± 3	36 ± 3*	34 ± 1	33 ± 1
NLA + Indo + Tezo	43 ± 4‡	40 ± 3*‡	41 ± 4‡	40 ± 3*	40 ± 3
HCO3 (mmol/L)					
Control	30 ± 1	30 ± 1	30 ± 1	30 ± 1	29 ± 1*
Tezo	31 ± 1	31 ± 1	30 ± 1	31 ± 1	30 ± 1*
Indo	24 ± 1†	25 ± 1*	25 ± 1	26 ± 1*	25 ± 1
Indo + Tezo	27 ± 1‡	28 ± 1*‡	28 ± 1‡	28 ± 1‡	27 ± 1
NLA	32 ± 2	32 ± 2	91 ± 56	32 ± 2	31 ± 2
NLA + Tezo	35 ± 3	35 ± 3	35 ± 3	36 ± 4	41 ± 7
NLA + Indo	26 ± 2	28 ± 3	28 ± 3	26 ± 1	26 ± 1
NLA + Indo + Tezo	30 ± 3‡	31 ± 4‡	31 ± 3‡	31 ± 3	31 ± 3

** P≤0.05 versus Rest (Lying); † P≤0.05 versus corresponding Control; ‡ P≤0.05 effect of Tezo; § P≤0.05 MI vs Normal*

Discussion

The main findings of the present study are that in swine with pulmonary hypertension secondary to myocardial infarction: (*i*) the contribution of endogenous ET to pulmonary vasomotor tone is enhanced as compared to normal swine, (*ii*) prostanoids exert a vasodilator influence on the pulmonary vasculature and attenuate the vasoconstrictor influence of endogenous ET, but only under resting conditions and not during exercise, while in normal swine prostanoids do not appear to play a role in control of pulmonary resistance vessel tone either at rest or during exercise and do not modulate the pulmonary vasoconstrictor influence of ET; (*iii*) the contribution of endogenous NO to control of pulmonary resistance vessel tone is not altered as compared to normal swine, while endogenous NO blunts the vasoconstrictor influence of ET to a similar extent as in normal swine.

Vasomotor control of the pulmonary vasculature: matching of ventilation to perfusion

For the lungs to perform their gas-exchange function efficiently, the pulmonary circulation must maintain low pulmonary arterial and capillary pressure, while accommodating the entire cardiac output. In addition, perfusion must be distributed among the ventilated units through local variations in pulmonary vascular resistance so that flow matches local ventilation, [36]. The pulmonary vascular resistance is determined by passive distension as well as active regulation of vasomotor tone [36]. During exercise, the increase in cardiac output results in an increased flow through the pulmonary vasculature. Moreover, exercise results in an increase in left atrial pressure that is progressive with exercise intensity and accounts for the majority of the increase in pulmonary arterial pressure. In the human lung with its large base and small top, the well-ventilated base is mainly perfused in the upright posture under resting conditions, while the increase in pressure during exercise recruits perfusion of the smaller top and results in a ~40% decrease in pulmonary vascular resistance [37, 38]. In contrast to humans, quadruped lungs have small ventral volumes but large dorsal volumes. As a result, resting pulmonary pressure is slightly higher in quadrupeds, but ventilation and perfusion are more evenly distributed across the entire lung [38]. During exercise, flows in all lung areas increase proportionally to their resting flows so that flow distribution does not change and hence pulmonary vascular resistance decreases by only 10-20% [38]. Another consequence of the body posture of quadrupeds is that perfusion and ventilation are already matched under resting conditions, with little additional recruitment of lung areas during exercise [38]. This may explain

our observation of a small decrease in arterial PO_2 at the highest level of exercise, which is in accordance with reports from various laboratories [39-41], although this is not a uniform finding [42, 43]. It is important to note that small changes in arterial PO_2 contribute little to the arterial oxygen content, since the arterial oxygen-haemoglobin dissociation curve operates at its upper plateau. Consequently, arterial haemoglobin oxygen saturation was maintained (Table 4 and 5).
The changes in pulmonary vascular resistance induced by ET receptor blockade and eNOS inhibition do not affect arterial PO_2, suggesting that matching between ventilation and perfusion is not affected. In contrast, cyclooxygenase inhibition did not affect pulmonary vascular resistance, but increased arterial PO_2, which may have been the result of the increased pulmonary retention time of the blood due to the decrease in cardiac output, in conjunction with mild hyperventilation.

Vasomotor control of the pulmonary vasculature following MI

MI results in neurohumoral activation, i.e. activation of the sympathetic nervous system and the renin-angiotensin system. In our model, elevations of adrenaline, noradrenaline and angiotensin-II are most pronounced during exercise [31, 41]. Additionally, MI increases LV filling pressures that are transmitted backwards into the pulmonary circulation [1]. Although the secondary increase in pulmonary arterial pressure may initially act to passively decrease PVR, it also results in a 'reactive' increase in PVR and inward remodelling and stiffening of the pulmonary vasculature [1]. This reactive increase in PVR may be caused by increased contribution of vasoconstrictors or a decreased contribution of vasodilators to pulmonary vasomotor tone and may limit the decrease in PVR during exercise [1]. This reduced vasodilator capacity of the pulmonary vasculature results in exacerbation of pulmonary hypertension during exercise, which increases RV afterload and contributes to RV hypertrophy and RV failure [1]. Moreover, it has been reported that exercise capacity is inversely correlated with PVR and with the failure to decrease PVR during exercise in patients with heart failure [6]. The pathogenesis of the increased PVR in pulmonary hypertension is incompletely understood, but may involve alterations in serotonin, thromboxane A_2 and/or angiotensin-II [7]. Additionally, it may be mediated in part by downregulation or desensitization of β-adrenoceptors. In the pulmonary vasculature of healthy swine, the sympathetic nervous system exerts a vasodilator influence that is mediated through b-adrenoceptors [35]. Although the increased sympathetic drive following MI may initially counteract the increase in PVR, β-blockade does not affect pulmonary vascular resistance in patients with chronic heart failure [44, 45], suggesting that prolonged β-adrenergic stimulation may have caused desensitization of the β-

adrenoceptors, thereby contributing to the increase in PVR following pulmonary hypertension. Additionally, endothelial dysfunction may play a role in this process [1, 8, 46]. With endothelial dysfunction, the production of the vasodilators NO and prostacyclin decreases, while the production of the vasoconstrictor ET increases. This imbalance between vasodilators and vasoconstrictors is likely to contribute to an increase in pulmonary vascular tone, thereby increasing PVR. In agreement with a previous study from our laboratory [27], we observed an enhanced ET-mediated vasoconstriction in the present study.

ET and secondary pulmonary hypertension following MI

Activation of the ET-system can contribute to the progression of the disease and has been shown to occur in various animal models as well as patients with pulmonary hypertension. Thus, production of ET is increased in the pulmonary endothelium of subjects with pulmonary hypertension [47], while pulmonary clearance of ET is reduced [48-51]. The increased production and decreased clearance of ET together result in increased circulating levels of ET, which is an independent predictor of clinical prognosis and survival [52]. Moreover, the ET-plasma levels correlate closely with the exercise capacity of patients with pulmonary hypertension [29].

In addition to increased plasma-levels of ET, which in itself could be sufficient to explain the increased vasoconstrictor influence of ET on the pulmonary circulation, we found that the sensitivity of the pulmonary circulation to administration of exogenous ET was increased after MI [27]. Moreover, while the response to endogenous ET was exclusively ET_B-receptor mediated in normal swine, an ET_A-receptor mediated component emerged in swine with MI [27]. In contrast, studies on isolated pulmonary resistance vessels from rats [53] and rabbits [54] with and without MI show no difference in response to ET or in ET_A or ET_B receptor expression [53]. However, the response of isolated arterioles to administration of ET may be different from the response of the intact vasculature [55]. This could be due to factors such as NO [9, 11] and possibly prostacyclin, that are capable of modifying ET receptor sensitivity and are present in vivo, while they are reduced in the in vitro preparations.

Prostanoids and pulmonary hypertension

Prostacyclin has very potent pulmonary vasodilator properties [56]. Moreover, both cyclooxygenase-1 and 2 have been shown to be expressed in the pulmonary vasculature of rats [57] and mice [58]. Yet, in agreement with other studies [34, 56, 59], endogenous prostanoids did not contribute to regulation of basal pulmonary tone and did not influence ET-mediated pulmonary vasoconstriction

[19], suggesting that endogenous prostanoid production is absent in the normal healthy pulmonary circulation. However, after MI, inhibition of cyclooxygenase resulted in pulmonary vasoconstriction at rest both in the absence and presence of inhibition of NO-synthesis, suggesting that after MI, prostanoids exert a basal vasodilator influence and therefore act to limit the pulmonary vasoconstriction that occurs after MI. These data are in accordance with a study on isolated pulmonary smooth muscle cells, showing that stimulation of these cells with cytokines, to mimic pathological pulmonary conditions can induce COX-2 expression, and thereby increase production of prostanoids [13]. Because prostanoids have been shown to improve the balance between ET-clearance and production [60], and because blockade of cyclooxygenase has been shown to affect pulmonary vascular tone only when ET-receptors are unblocked [61], this slight increase in pulmonary prostanoids may have acted to limit ET-induced pulmonary vasoconstriction [13] rather than to induce pulmonary vasodilation itself. Indeed, the present study also shows that, in the presence of tezosentan, PVR is not different before and after inhibition of cyclooxygenase, suggesting that the main action of prostanoids in the pulmonary vasculature is to suppress the ET-system.

In contrast to the findings under resting conditions, there was no effect of cyclooxygenase inhibition on pulmonary vasomotor tone during exercise in the presence or absence of eNOS inhibition, suggesting that the vasodilator effect of prostanoids is lost during exercise. In accordance with these findings, the pulmonary vasodilator effect of tezosentan during exercise was similar in the presence and absence of cyclooxygenase inhibition.

NO and pulmonary hypertension

NO is a very potent vasodilator of the pulmonary vasculature and endogenous NO contributes to the regulation of pulmonary vascular tone in normal swine [30, 56]. Physiologically, NO is produced by eNOS in response to increases in shear stress and/or in response to receptor activation and produces vasodilation through activation of soluble guanylyl cyclase in vascular smooth muscle, followed by reduction in intracellular calcium concentrations as well as reduction in myosin light chain phosphorylation, both of which are essential for smooth muscle cell contraction [62]. Besides its direct vasodilator effect on the pulmonary vasculature, NO can also cause vasodilation through inhibition of the ET system. Thus, NO inhibits the production of ET through endothelin converting enzyme [17] and modulates ET_A receptor sensitivity [11]. Indeed, the vasodilation in response to ET_A/ET_B receptor blockade with tezosentan is larger after inhibition of NO-synthesis (present study, [19], suggesting that part of the vasodilator effect of NO is exerted through

inhibition of ET-mediated vasoconstriction.

The direct vasoconstrictor effect of inhibition of NO-synthesis within the pulmonary vascular bed may be modified by the central effects of inhibition of eNOS. Thus, inhibition of central NO will result in an increase in sympathetic outflow [63]. Conversely, the systemic pressor response was accompanied by a decrease in heart rate, likely mediated via the baroreceptor reflex. In both normal and MI swine NLA resulted in similar decrements in heart rate, indicating that in both groups the baroreceptor reflex outweighed any effects of central eNOS inhibition. The resultant attenuated sympathetic activity will result in blunted β-receptor mediated pulmonary vasodilation [35], thereby potentially enhancing the vasoconstriction produced by inhibition of endothelial NO-production. However, the increase in pulmonary vascular resistance produced by NLA was much more pronounced than that previously observed following complete β-adrenergic receptor blockade [35], suggesting that the NLA-induced increase in pulmonary vascular resistance was principally the result of inhibition of local NO synthesis.

Studies on the role of NO in regulation of pulmonary vasomotor tone in pulmonary hypertension have yielded variable results in that the contribution of NO is either increased, decreased or unaltered, depending on the model of pulmonary hypertension as well as the severity and duration of the disease. In early stages of pulmonary hypertension, pulmonary vasoconstriction may increase shear stress, thereby increasing NO production and counteracting the development of pulmonary hypertension [62]. However, when pulmonary hypertension persists for longer periods of time, the chronically elevated pressure may contribute to endothelial dysfunction, thereby decreasing NO production and promoting the development of pulmonary hypertension [24, 25, 64]. The present study shows that, in accordance with a previous study from our laboratory [28], the pulmonary vasoconstriction induced by inhibition of eNOS with NLA was similar in swine with MI as compared to normal swine, suggesting that basal NO-production is maintained ~2 weeks after induction of MI. Apparently, the MI-induced pulmonary hypertension is not severe enough and/or did not last long enough to provoke perturbations in the NO system. These data are in accordance with a study in humans, showing that basal NO-activity is reduced in patients with congestive heart failure and severely elevated levels of pulmonary arterial pressure and pulmonary vascular resistance but not in patients with mildly elevated levels of pulmonary arterial pressure and pulmonary vascular resistance [24].

In accordance with the maintained basal NO production in swine with MI [28], the present study shows that the vasodilation induced by ET_A/ET_B receptor blockade in MI swine was increased after inhibition of NO-synthesis to a similar

extent as in normal swine. These findings indicate that NO acts to limit the ET-induced pulmonary vasoconstriction to a similar degree in MI and normal swine. Thus, the increased sensitivity of the pulmonary vasculature to ET as observed in our swine model [27] cannot be explained by a loss of NO-mediated inhibition of ET-induced vasoconstriction.

Endothelial control of the systemic resistance vessels after MI

NO and prostanoids exert a vasodilator influence on the systemic vasculature of normal swine, while ET exerts a vasoconstrictor influence. The effects of endogenous NO, prostanoids and ET on systemic vasomotor tone are similar in normal swine and swine with MI. Moreover, in both normal swine and swine with MI, the vasodilation induced by endogenous NO and prostanoids is in part mediated through inhibition of ET-mediated vasoconstriction [12, 13, 17, 60] as evidenced by a larger effect of ET_A/ET_B receptor blockade after blocking NO or prostanoid production and in part a direct effect, as SVR does not return to baseline levels after ET_A/ET_B receptor blockade.

In normal swine, the vasoconstrictor effect of combined inhibition of NO and prostanoids is larger than the sum of the individual effects, suggesting that inhibition of one endogenous vasodilator is compensated by increased production of the other dilator [34]. These data are in accordance with findings in isolated endothelial cells as well as in vivo [20, 65, 66]. Additionally, the effect of ET_A/ET_B receptor blockade is significantly enhanced when production of both NO and prostanoids is inhibited, suggesting that NO and prostanoids act in concert to limit ET-induced vasoconstriction. In contrast, in swine with MI the effects of inhibition of NO and prostanoid production on systemic vasomotor tone are additive. Moreover, the effect of ET_A/ET_B receptor blockade is not further enhanced after combined inhibition of NO and prostanoids. These findings could be interpreted to suggest that after MI, the effect of loss of NO or prostanoids on vasomotor tone and on ET-mediated vasoconstriction is not compensated by increased production of the other endothelial vasodilator.

Conclusion

The present study demonstrates that 2-3 weeks after MI, there is an increased pulmonary vasoconstrictor influence of endogenous ET. We have previously shown that this increased pulmonary vasoconstrictor influence is likely due to increased plasma ET levels in combination with an increased sensitivity of the pulmonary vasculature to ET [27]. In contrast, the pulmonary vasodilator influence of NO was

well maintained after MI, while the inhibition of the ET-mediated vasoconstrictor influence by NO was also unmitigated. Moreover, increased production of prostanoids after MI acted to limit the increase in pulmonary vascular resistance under resting conditions. This effect of prostanoids was mediated by a reduction of the ET-induced pulmonary vasoconstriction. Taken together these findings indicate that the increased vasoconstrictor influence of ET in the pulmonary circulation after MI is not the result of a loss of vasodilator influence of NO or prostanoids. Moreover, our findings provide a rationale for the therapeutic use of endothelin receptor blockers to alleviate pulmonary hypertension.

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Chapter 7

Pulmonary hemodynamic effects of the phosphodiesterase-5 inhibitor EMD360527 in awake swine at rest and during treadmill exercise

Birgit Houweling, Daphne Merkus, Johan Quispel, Pieter D Verdouw and Dirk J Duncker

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Abstract

Pulmonary hypertension is a major risk factor for right-sided heart failure. Phosphodiesterase (PDE) 5 inhibition has emerged as a promising novel vasodilator therapy by prolonging the half-life of cGMP. In this study we assessed the pulmonary vasodilator effects of PDE5 inhibitor EMD360527 in chronically instrumented awake swine at rest and during exercise. Since pulmonary hypertension is often associated with endothelial dysfunction, and hence a reduced bioavailability of nitric oxide (NO), we also studied the effect of EMD360527 in the presence of NO synthase inhibition. The PDE5 inhibitor EMD360527 was infused intravenously at 10, 30, 100 and 300 μg min kg^{-1} in resting pigs while the highest dose was also administered during exercise in the absence and presence of the NO synthase inhibitor N^{ω}- nitro-L-arginine (NLA, 20 mg min kg^{-1}). Finally vascular responsiveness to the NO donor nitroprusside in the absence and presence of NLA was investigated.

EMD360527 decreased dose-dependently pulmonary and systemic vascular resistance in resting pigs, by up to 16±8% and 18±5%. The vasodilator responses were maintained during exercise. Prior inhibition of NO enhanced the EMD360527-induced decreases in pulmonary and systemic vascular resistance (32±12% and 23±5% respectively), which correlated well with the basal level of vasomotor tone. Furthermore, NLA, increased the pulmonary and systemic vasodilator responses to nitroprusside. In conclusion, EMD360527 causes vasodilation in the pulmonary and systemic vascular bed, which is maintained during exercise. The level of dilatation depends on the initial level of vasomotor tone, indicating that this vasodilator therapy might be most effective in patients with severe pulmonary hypertension.

Introduction

Pulmonary hypertension is a major risk factor for right-sided heart failure, and constitutes a life-threatening disease with a poor prognosis. [1-3]. The elevation in pulmonary pressure is caused, at least in part, by an elevated pulmonary vascular resistance [4]. Consequently, therapy is principally aimed at reducing pulmonary vascular resistance. Currently available therapies include inhaled nitric oxide (NO), Ca^{2+}-channel blockers, prostacyclin and endothelin-receptor blockade. However, these therapies have short-duration, limited efficacy, are expensive and/or associated with significant side-effects, indicating that there is a continued need for novel vasodilator therapies of pulmonary hypertension [5]. In recent years, phosphodiesterase (PDE) 5 inhibition has emerged as a promising novel vasodilator therapy [5-7]. The vasodilator influence of PDE5 inhibition is achieved by prolonging the half-life of cyclic guanosine monophosphate (cGMP), a second messenger which results in vasodilation through various pathways [8]. Since PDE5 is abundantly expressed in lung vascular smooth muscle [9, 10], PDE5 inhibition may provide a powerful tool to selectively reduce pulmonary vascular resistance, while leaving systemic vascular resistance unperturbed [6]. In support of this notion, a number of patient studies reported a significant vasodilator effect within the pulmonary vascular bed, with minimal systemic effects [7, 11, 12]. However, some PDE5 inhibitors appear to be more selective for the pulmonary circulation than others, which may depend in part on the dosage used [13, 14]. Therefore, the first aim of the present study was to assess the vasodilator effects of various dosages of the novel PDE5 inhibitor EMD360527 [15] and to assess its selectivity in the pulmonary versus the systemic vascular bed of chronically instrumented awake swine.

The pulmonary circulation is a low-pressure system due to a low resistance. As a result, it has less capacity for additional vasodilation as compared to the systemic circulation, which is especially notable during exercise, when the increased cardiac output causes a marked increase in pulmonary artery pressure [16]. Since in patients with pulmonary hypertension exercise can lead to exaggerated increases in pulmonary pressure, it is important to know the efficacy of vasodilator therapy not only under resting conditions but particularly during exercise [17]. Consequently, the second aim of the present study was to investigate the pulmonary and systemic vasodilator effect of EMD360527 during exercise.

NO is considered as one of the major sources of cGMP [18]. Since pulmonary hypertension has been proposed to be associated with endothelial dysfunction, and reduced endothelial NO synthase (eNOS) activity [19-21], it is eminent to

determine to what the extent the efficacy of PDE5 inhibitors depends on the activity of endothelial NO synthase. Consequently, the third aim of the study was to evaluate the vasodilator effect of EMD360527 in the presence of NO synthase inhibition.

Methods

Studies were performed in accordance with the "Guiding Principles in the Care and Use of Laboratory Animals" as approved by the Council of the American Physiological Society, and with approval of the Animal Care Committee of the Erasmus MC Rotterdam. Fifteen crossbred Landrace x Yorkshire swine of either sex (2-3 months old) entered the study. Daily adaptation of animals to laboratory conditions started 1 wk before surgery and continued during the first week after surgery.

Surgical procedures

Swine were sedated with ketamine (30 mg kg^{-1} im), anaesthetized with thiopental (10 mg kg^{-1} iv), intubated and ventilated with a mixture of O_2 and N_2O (1:2) to which 0.2-1% (v/v) isoflurane was added [22-24]. Anaesthesia was maintained with midazolam (2 mg/kg + 1 mg kg^{-1} per hour iv) and fentanyl (10 µg kg^{-1} per hour iv). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid-filled polyvinylchloride catheter was inserted into the aortic arch and pulmonary artery for blood pressure measurement (Combitrans pressure transducers, Braun, Melsungen, Germany) and blood sampling. An electromagnetic flow probe (14-15 mm, Skalar) was positioned around the ascending aorta for measurement of cardiac output. A polyvinylchloride catheter was also inserted into the left atrium to measure pressure. Catheters were tunnelled to the back and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine im) for 2 days and antibiotic prophylaxis (25 mg kg^{-1} amoxicillin and 5 mg kg^{-1} gentamycin iv) for 5 days. Studies were performed 1-2 weeks after surgery.

Experimental protocols

Effect of EMD360527 in resting swine. With swine (n=10) resting quietly in a cage, the vasodilator responses in the systemic and pulmonary circulation to the PDE5 inhibitor EMD360527 [15] were studied. EMD360527 was administered in consecutive 15 min infusions of 10, 30, 100 and 300 µg min kg^{-1} iv, and heart rate, cardiac output, and aortic, pulmonary arterial and left atrial blood pressures were recorded. EMD360527 was dissolved in a final concentration of 0.1 mg min ml^{-1} in

a 1:1 (v:v) mixture of saline and $NaHCO_3$ (4.2%). We have previously shown that saline infusions do not affect hemodynamic variables in awake resting swine [25, 26].

Effect of EMD360527 during treadmill exercise. After hemodynamic measurements (lying and standing), consisting of left atrial, aortic and pulmonary arterial blood pressures, heart rate and cardiac output, blood samples (lying) and rectal temperature (standing) had been obtained, swine (n=11, of which 6 had also participated in the resting study) were subjected to a five-stage exercise protocol (1-5 km h^{-1}) on a motor driven treadmill. Hemodynamic variables were continuously recorded and blood samples collected during the last 60 s of each 3-min exercise stage, at a time when hemodynamics had reached a steady state. After 90 minutes of rest, the EMD360527 was infused continuously in a dose of 300 μg min kg^{-1} iv, and 10 minutes after starting the infusion the exercise protocol was repeated. We have previously observed excellent reproducibility of two consecutive exercise protocols [23, 27].

Effects of EMD360527 during treadmill exercise in the presence of NO synthase inhibition. On a different day, the eNOS inhibitor N^{ω}-nitro-L-Arginine (NLA) was administered to swine (n=8, all of which had participated in the above described protocol) in a dose of 20 mg kg^{-1} iv prior to the first exercise protocol [24, 27]. After 90 minutes of rest, EMD360527 (300 μg min kg^{-1} iv) was administered as described above and the five-stage exercise protocol was repeated.

Effects of SNP in the presence of NO synthase inhibition. With swine (n=4) resting quietly, the NO donor nitroprusside was infused in consecutive doses of 0.5, 1.0, 2.0 and 3.0 μg min kg^{-1} lasting 10 min per dose, in the absence and presence of eNOS inhibitor N^{ω}-nitro-L-Arginine (NLA, 20 mg min kg^{-1}iv) and systemic and pulmonary hemodynamic responses were studied.

Data Analysis

Digital recording and off-line analysis of hemodynamics have been described previously [23, 27]. Systemic vascular resistance (SVR) was computed as mean aortic blood pressure (MAP) divided by cardiac output (CO). Pulmonary vascular resistance (PVR) is defined as mean pulmonary artery pressure (PAP) minus mean left atrial pressure (LAP) divided by cardiac output.

Statistical analysis

The effects of EMD360527 in resting swine were assessed using analysis of variance (ANOVA) for repeated measures followed by post-hoc testing using Scheffe's test. The effects of EMD360527 during exercise were analyzed using two-way ANOVA (exercise level x EMD360527) for repeated measures. Post-hoc testing for exercise and drug effect was performed using Scheffe's test. The effect of NO-synthase inhibition on the responses to EMD360527 during exercise, were tested using three-way ANOVA (exercise level x EMD360527 x NLA). Linear regression analysis was used to test the dependency of the effect of EMD360527 on the initial level of pressure and resistance in the pulmonary and systemic vascular bed. Finally, the effect of NO-synthase inhibition on the vasodilator responses to SNP was assessed by two-way ANOVA (SNP dose x NLA). Statistical significance was accepted when P≤0.05. Data are presented as mean± S.E.M.

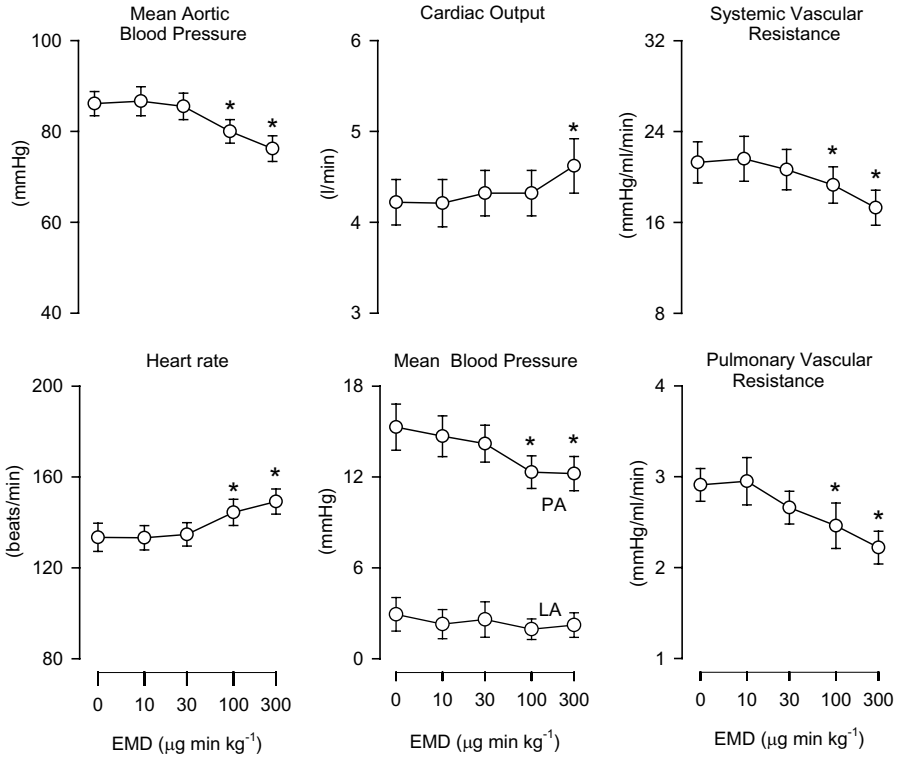

***Figure 1**: Effect of different doses of PDE5 inhibitor EMD360527 (EMD) on different hemodynamic parameters in awake swine (n=10, except for LA pressure and PVR: n=7). PA: pulmonary artery; LA: left atrium *P<0.05 vs baseline (0 mg min kg^{-1}).*

Results

Effects of EMD360527 in resting swine

Intravenous infusion of EMD360527 resulted in a dose-dependent decrease in AoP of up to 12±2%, which was the result of a 19±3% decrease in SVR, as cardiac output increased by up to 10±3% due to a, probably baroreflex-mediated, increase in heart rate (Fig. 1). EMD360527 also caused a dose-dependent decrease in PAP of up to 17±7%, which was due to a decrease in PVR of up to 24±4% as LAP was not affected and CO increased (Fig. 1). The relative decreases in arterial pressure and vascular resistance in response to EMD360527 were slightly larger in the pulmonary than in the systemic vascular bed (Fig. 2).

Effects of EMD360527 during treadmill exercise

Exercise up to 5 km h^{-1} in swine resulted in a 105±5 % increase in cardiac output that was principally due to an increase in heart rate (Table 1). AoP was minimally affected, implying that the increase in CO was accompanied by an equivalent decrease in SVR (Table 1). PAP almost doubled during exercise. However, the transpulmonary pressure gradient (PAP minus LAP) increased to the same extent as cardiac output, resulting in an unchanged PVR during exercise (Table 1).

Ten min after the onset of intravenous infusion of EMD360527, in a dose of 300 μg min kg^{-1}, systemic and pulmonary pressures and vascular resistances had decreased significantly (Table 1). During the subsequent exercise protocol, the vasodilator effect of EMD360527 on the pulmonary vasculature tended to increase (P=0.08 by ANOVA), while the vasodilator effect in the systemic vasculature decreased (P<0.05 by ANOVA), compared to the effects of EMD365027 during resting conditions (Fig. 3).

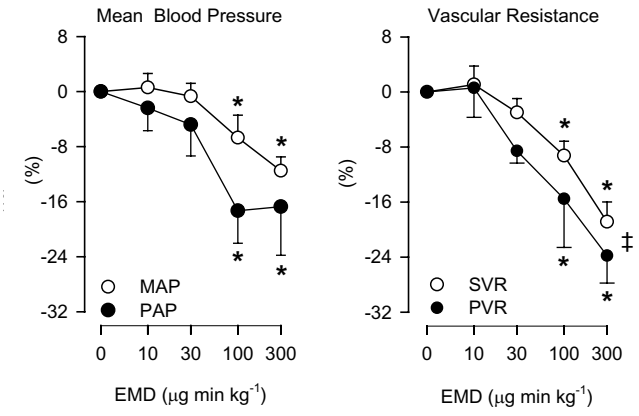

Figure 2: *Relative effect of different doses of PDE5 inhibitor EMD360527 (EMD) on different hemodynamic parameters in awake swine (n=10, except for PVR: n=7). PAP: pulmonary artery pressure; MAP: mean arterial pressure, SVR: systemic vascular resistance; PVR: pulmonary vascular resistance. *P<0.05 vs baseline (0 mg min kg^{-1}); †P<0.05 by ANOVA, effect PDE5 inhibition on MAP vs PAP; ‡P=0.054 by ANOVA, effect PDE5 inhibition on SVR vs PVR for dosages 30-300 mg min kg^{-1}.*

***Table 1**: Hemodynamics of swine at rest and during exercise before and after administration of EMD360527 and NLA*

		Rest		Exercise level (km·h^{-1})				
	Treatment	lying	standing	1	2	3	4	5
HR	Control	124 ± 1	139 ± 3*	172 ± 3*	185 ± 4*	204 ± 7*	242 ± 8*	263 ± 7*
(bpm)	EMD	143 ± 6†	156 ± 5*†	179 ± 5*	194 ± 6*	220 ± 8*†	245 ± 9*	270 ± 8*
	NLA	91 ± 4	113 ± 4*	127 ± 3*	132 ± 3*	151 ± 4*	197 ± 8*	234 ± 7*
	NLA+EMD	105 ± 8	125 ± 7*	138 ± 7*†	151 ± 6*†	168 ± 7*†	203 ± 8*	240 ± 7*
MAP	Control	87 ± 2	85 ± 3	83 ± 2*	84 ± 2	85 ± 2	87 ± 3	89 ± 3
(mmHg)	EMD	80 ± 2†	75 ± 3†	74 ± 2*†	76 ± 3†	78 ± 2†	79 ± 2†	82 ± 3†
	NLA	120 ± 3	112 ± 3*	111 ± 3*	112 ± 3*	114 ± 4	115 ± 3	115 ± 3
	NLA+EMD	105 ± 5†	105 ± 5	104 ± 4†	105 ± 4†	105 ± 4†	106 ± 4†	105 ± 4†
CO	Control	5.0 ± 0.3	5.4 ± 0.3*	6.4 ± 0.3*	7.0 ± 0.3*	7.4 ± 0.3*	8.4 ± 0.3*	9.0 ± 0.3*
(l·min^{-1})	EMD	5.0 ± 0.3	5.7 ± 0.3*	6.6 ± 0.3*	7.0 ± 0.3*	8.2 ± 0.3*†	9.0 ± 0.3*†	9.5 ± 0.4*†
	NLA	3.1 ± 0.2	4.1 ± 0.3*	4.9 ± 0.4*	5.4 ± 0.4*	6.1 ± 0.4*	7.2 ± 0.4*	7.9 ± 0.5*
	NLA+EMD	3.6 ± 0.2†	4.9 ± 0.4*	5.6 ± 0.4*	6.2 ± 0.4*†	6.9 ± 0.5*†	8.0 ± 0.5*†	8.8 ± 0.5*†
SVR	Control	20 ± 1	16 ± 1*	13 ± 1*	12 ± 1*	11 ± 0*	10 ± 0*	10 ± 0*
(mmHg min l^{-1})	EMD	16 ± 1†	13 ± 1*†	11 ± 1*†	11 ± 0*†	9 ± 0*†	9 ± 0*†	8 ± 0*†
	NLA	39 ± 3	28 ± 2*	23 ± 2*	21 ± 2*	19 ± 1*	16 ± 1*	15 ± 1*
	NLA+EMD	30 ± 3†	22 ± 2*†	19 ± 2*	17 ± 1*†	15 ± 1*†	13 ± 1*†	12 ± 0*†
PAP	Control	17 ± 1	19 ± 1*	22 ± 1*	25 ± 1*	27 ± 1*	34 ± 1*	36 ± 1*
(mmHg)	EMD	15 ± 1†	14 ± 1†	17 ± 1*†	19 ± 1*†	23 ± 1*†	26 ± 1*†	30 ± 1*†
	NLA	22 ± 3	26 ± 2*	28 ± 2*	28 ± 2*	34 ± 3*	40 ± 4*	43 ± 3*
	NLA+EMD	15 ± 2†	17 ± 2†	19 ± 2†	22 ± 2*†	24 ± 2*†	29 ± 2*†	33 ± 2*†
LAP	Control	7 ± 1	5 ± 1	6 ± 1	7 ± 1	9 ± 1*	11 ± 1*	13 ± 1*
(mmHg)	EMD	4 ± 1	3 ± 1†	5 ± 1	7 ± 1*	9 ± 1*	11 ± 1*	13 ± 1*
	NLA	8 ± 1	7 ± 1	7 ± 1	9 ± 1	10 ± 1	10 ± 1	11 ± 1
	NLA+EMD	6 ± 1	6 ± 2	7 ± 1	9 ± 1*	9 ± 1*	11 ± 1*	12 ± 1*
PVR	Control	2.5 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.7 ± 0.2	2.7 ± 0.2
(mmHg min l^{-1})	EMD	2.1 ± 0.2	2.0 ± 0.2†	1.9 ± 0.2†	1.8 ± 0.2†	1.8 ± 0.2†	1.8 ± 0.2†	1.9 ± 0.2†
	NLA	4.1 ± 0.6	4.7 ± 0.6	4.3 ± 0.7	4.1 ± 0.7	4.3 ± 0.8	4.4 ± 0.8	4.4 ± 0.7
	NLA+EMD	2.7 ± 0.6	2.3 ± 0.4†	2.2 ± 0.3†	2.2 ± 0.3†	2.2 ± 0.3†	2.5 ± 0.4†	2.5 ± 0.4†

*HR: Heart Rate, MAP: Mean Arterial Pressure, CO: Cardiac Output, SVR: Systemic Vascular Resistance, PAP: Mean Pulmonary Arterial Pressure, LAP: Mean Left Atrial Pressure, PVR: Pulmonary Vascular Resistance, NLA: NO synthase inhibitor N$^{\omega}$- nitro-L-arginine, EMD: PDE5 inhibitor EMD360527 Data are mean ± S.E.M.; *P≤0.05 vs rest (lying); †P≤0.05 effect of PDE5 inhibition with EMD360527*

Effects of EMD360527 during exercise in the presence of NO synthase inhibition

Administration of the NO synthase inhibitor NLA resulted in marked increases in aortic (37±2%) and pulmonary arterial (41±14%) blood pressures, which were the result of a 94±9% and 90±23% increase in SVR and PVR, respectively (Table 1). The vasoconstrictor responses to NLA were similar at rest and during exercise.

Subsequent infusion of EMD360527 caused vasodilator responses in the systemic (23±5%) and pulmonary (31±12%) circulations that were enhanced compared to

the vasodilator responses to EMD360527, in the absence of NLA (Fig. 3). During exercise, the pulmonary vasodilation by EMD360527 was maintained, while the systemic vasodilation by EMD360527 decreased, compared to the vasodilation by EMD365027 during resting conditions (Fig. 3).

The vasodilator responses of individual animals to EMD360527 in the systemic and pulmonary vascular beds, both at rest and during exercise and both in absence or presence of NLA, correlated well with the baseline values obtained just prior to administration of EMD360527, while the depressor responses correlated much less, reaching statistical significance only in the pulmonary circulation (Fig. 4).

Effects of SNP in the presence of NO synthase inhibition

To investigate whether the vasculature becomes more sensitive to the vasodilator influence of NO/cGMP after increased inhibition of NO synthase, the NO donor SNP was infused intravenously under control conditions and after NO synthase inhibition with NLA. Under control conditions, SNP in a dose range of 0.5-3.0 μg min kg^{-1} iv resulted in mild vasodilator responses in systemic (16±5%) and pulmonary (4±8%) circulation (Fig. 5). Conversely, following NLA, the vasodilator responses were enhanced in both the systemic (47±5%) and pulmonary (34±13%) vascular bed (all $P<0.05$ by ANOVA).

Discussion

The main findings of the present study are that (*i*) the PDE5-inhibitor EMD360527 resulted in dose-dependent vasodilation in the pulmonary and systemic circulation of awake swine under resting conditions; (*ii*) the pulmonary circulation displayed slightly higher sensitivity to the vasodilator influence of EMD360527 than the systemic circulation; (*iii*) the effect of EMD360527 in the pulmonary circulation was well maintained during exercise, while it decreased slightly in the systemic circulation; (*iv*) inhibition of NO-synthase increased the vasodilator effect of EMD360527 and of nitroprusside in both pulmonary and systemic circulation. The implications of these findings will be discussed.

Effects of PDE5-inhibition on pulmonary vascular resistance

Many vasoactive substances influence vascular resistance by increasing the production of the second messengers cAMP and/or cGMP within vascular smooth muscle cells. These second messengers are short-lived because they are degraded by PDE allowing tight control of vascular tone [28]. At least eleven different gene families of PDE are currently known to exist in mammalian tissues but the tissue

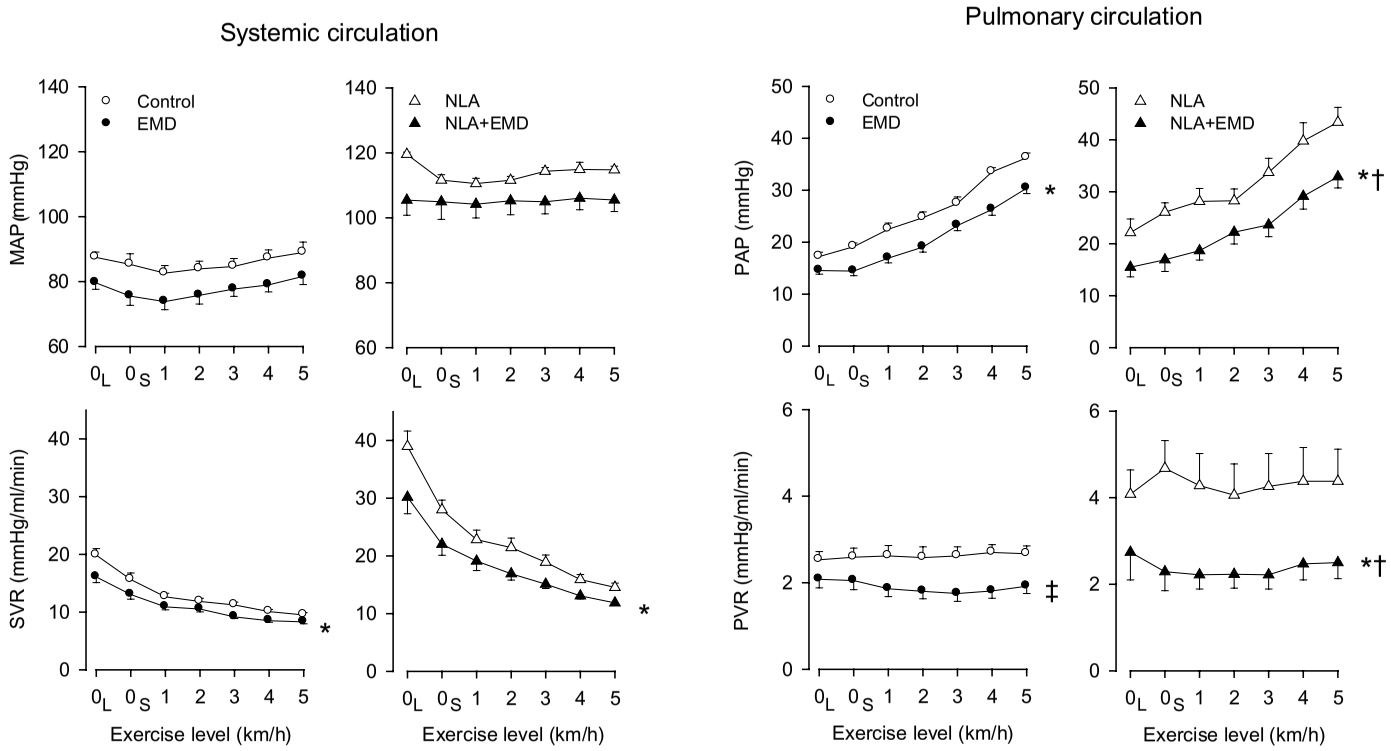

***Figure 3**: The effects of the PDE5 inhibitor EMD360527 alone and after prior administration of the NO synthase inhibitor NLA in swine on mean arterial blood pressure (MAP, n=11 without NLA and n=9 with NLA), systemic vascular resistance (SVR, n=9 without NLA and n=6 with NLA), pulmonary artery pressure (PAP, n=11 without NLA and n=9 with NLA) and pulmonary vascular resistance (PVR, n=9 without NLA and n=6 with NLA) at rest (0_S: standing and 0_L: lying) and during exercise (1-5 km h^{-1}). * P<0.05 by ANOVA, effect of PDE5 inhibition different during exercise, ‡ P=0.08 by ANOVA, effect of PDE5 inhibition different during exercise, †P<0.05 by ANOVA, effect of PDE5 inhibition after NLA vs effect of PDE5 inhibition alone.*

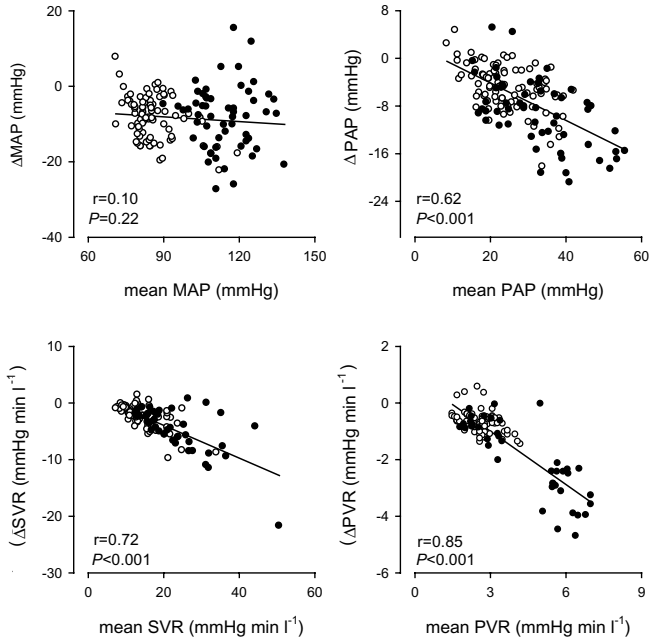

Figure 4: *Responses of mean arterial pressure (MAP), systemic vascular resistance (SVR), pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR) to PDE5 inhibitor EMD360527 in the absence (open symbols) and presence (closed symbols) of inhibition of NO synthase. Each point in the figure shows a data point of an individual animal at 1 exercise level. Regression lines are shown as solid lines. The relation between parameters within one figure was assessed using linear regression.*

distribution of the PDE-isoforms as well as their specificity for cAMP and cGMP varies [29]. Inhibition of PDE in vascular smooth muscle can provide a powerful tool to reduce vascular resistance, by prolonging the half-life of cAMP and/or cGMP. The PDE isoforms that are predominantly present in vascular smooth muscle are PDE1, 3, 4, 5, 7 and 9. [30, 31]. PDE1, PDE4 and PDE7 are cAMP-specific, while PDE3 has a 4-10 times higher affinity for cAMP than for cGMP. PDE5 and PDE9 are cGMP-specific [29]. However, PDE9 is abundantly expressed throughout the body [29], whereas PDE5 expression is 10 times more abundant in the pulmonary vasculature as compared to the heart [9, 10]. Thus, PDE5 inhibition may provide a tool to preferentially reduce pulmonary vascular resistance, while leaving systemic vascular resistance principally unchanged.

In the present study we found that the PDE5 inhibitor EMD360527 decreased both systemic and pulmonary vascular resistance. Yet, the pulmonary circulation appeared to be slightly more sensitive to EMD360527, as the relative decreases in PAP and PVR were greater than the decreases in MAP and SVR. These findings are in agreement with several other experimental studies [32, 33]. Similarly, most studies in patients with pulmonary hypertension show that pulmonary pressure decreases more in response to PDE5 inhibition as compared to systemic arterial pressure [7, 11, 12]. The degree of selectivity may depend, at least in part, on the particular PDE5 inhibitor and/or dosage that is used [13, 14].

Effects of PDE5 inhibition on pulmonary vascular resistance during treadmill exercise

In agreement with previous observations from our laboratory [24, 34], we found that exercise had minimal effects on PVR. Consequently, in parallel to the increase in cardiac output, PAP almost doubled during exercise, whereas the exercise-induced systemic vasodilation maintained MAP constant. The modest exercise-induced pulmonary vasodilation is enhanced by endothelin receptor blockade [35], and is blunted by NO synthase inhibition [24, 27, 34], demonstrating that pulmonary vasomotor tone is the resultant of an interplay between vasodilator and vasoconstrictor influences. The pulmonary vasodilator effect of PDE5-inhibition observed under resting conditions was well maintained during exercise. Since the exercise capacity in the patients with pulmonary hypertension is inversely correlated with PVR [36], these observations, may explain the PDE5-inhibitor-induced improvement in exercise capacity that is observed in patients with pulmonary hypertension [14, 37].

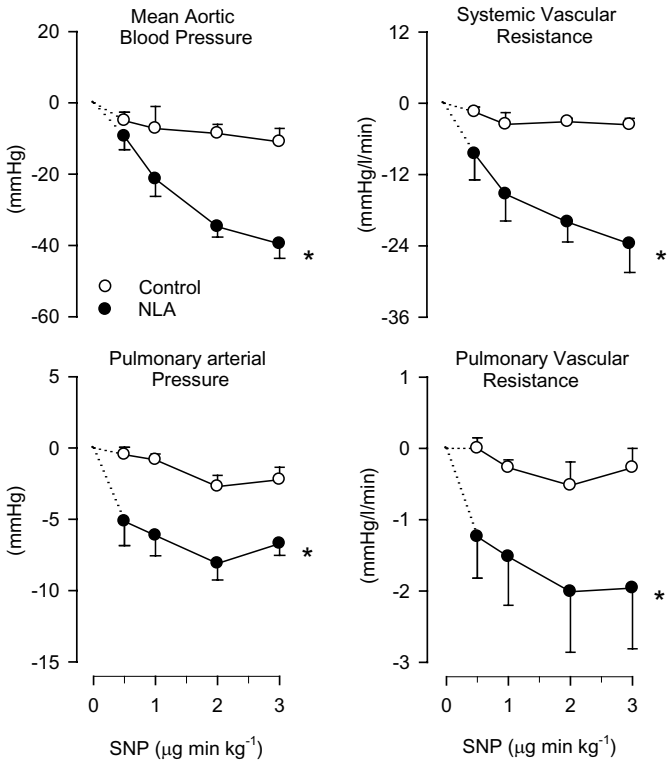

Figure 5: *Responses of mean aortic blood pressure, systemic vascular resistance, pulmonary arterial pressure and pulmonary vascular resistance to different doses of nitro prusside (SNP) in the absence (open symbols) and presence (closed symbols) of inhibition of NO synthase (NLA). *P<0.05 by ANOVA, effect of NLA*

Effects of PDE5 inhibition on pulmonary and systemic resistance in the presence of NO inhibition

Pulmonary hypertension may be accompanied by endothelial dysfunction, resulting in decreased eNOS activity and consequently cGMP production may decrease [19-21]. To simulate this condition we administered NLA to block endothelial NO synthase and compared the effect of PDE5 inhibition before and after NLA. We found that the effect of EMD360527 inhibition both at rest and during exercise was larger after prior inhibition of NO synthase. This observation appears at odds with several studies in anesthetized animal preparations, which reported that inhibition of NO synthase blunted the pulmonary vasodilator responses PDE5-inhibition. For example, Mc Mahon et al reported that the NO synthase inhibitor L-NAME blunted the vasodilator effect of zaprinast in the cat pulmonary vascular bed [38]. Also, in lambs with acute pulmonary hypertension produced with either the thromboxane A_2 analogue U46619 or the NO synthase inhibitors L-NAME or NLA, the vasodilator effects of PDE5 inhibition by zaprinast or sildenafil were reported to be significantly lower in the presence of NO synthase inhibition as compared to U46619 [32, 33]. An explanation for these disparate results is not readily found, but may be due to the type of PDE5 inhibitor or may be species-dependent. This is supported by observations in anaesthetized rabbits, in which the vasodilation produced by EMD360527 was not affected by pretreatment with L-NAME (personal communication with Dr. P. Schelling, E. Merck Darmstadt).

Several reasons could be forwarded to explain the unperturbed vasodilator response to EMD360527 in the presence of NLA. First, a large number of other mediators cause vasodilation via stimulation of sGC, including carbon monoxide [39] and hydrogen peroxide [40, 41]. Furthermore, there is evidence that blockade of NO production may result in increased carbon monoxide [42] and hydrogen peroxide [40] production, which could act to compensate for the loss of NO-induced cGMP production. Second, the natriuretic peptide family of proteins (consisting of atrial, brain and C-terminal natriuretic peptide (ANP, BNP and CNP)) cause increases in cGMP by activating particulate GC [43] and may exert an increased vasodilator influence after NO synthase inhibition [44]. Third, it has been suggested that inhibition of NO synthase results in supersensitization of the NO/cGMP pathway [45], thereby resulting in enhanced response to vasodilators acting via the NO/cGMP pathway [46, 47]. This concept is supported by our observation that the pulmonary and systemic vasodilator responses to the NO donor nitroprusside were enhanced by pretreatment with NLA and could in part explain the enhanced vasodilator response to PDE5. Finally, it is also possible that the increased vasodilator response to EMD360527 was increased following NLA, simply due to an increase in basal

tone. This is illustrated in figure 4, which suggests that the initial level of PVR is a strong determinant of the degree of vasodilation produced by EMD360527.

Conclusions

The PDE5 inhibitor EMD360527 produced pulmonary vasodilation in awake resting swine that was maintained during treadmill exercise. Inhibition of NO synthase enhanced the vasodilator response not only to nitroprusside, but also to EMD360527. Patients with severe pulmonary hypertension, often have endothelial dysfunction and therefore a decreased NO synthase activity [19-21]. The observation that inhibition of PDE5 was enhanced following NO synthase inhibition and correlated well with the basal level of pulmonary resistance vessel tone, suggests that PDE5 inhibition may be most effective in patients with the severest degree of pulmonary hypertension.

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Chapter 8

Pulmonary vasodilator effect of phosphodiesterase 5 inhibition is enhanced in swine with pulmonary hypertension after a recent myocardial infarction

Abstract

Myocardial infarction (MI) results in secondary pulmonary hypertension, which poses an increased workload for the right ventricle and may ultimately lead to right ventricular failure. In swine, after induction of MI, pulmonary artery pressures are increased reflecting pulmonary hypertension. Inhibition of phosphodiesterase 5 (PDE5), the enzyme responsible for the breakdown of cGMP in vascular smooth muscle, has been proposed to be of benefit for the alleviation of pulmonary hypertension. In the present study, we investigated the pulmonary hemodynamic effect of PDE5 inhibition in swine with pulmonary hypertension secondary to MI.

Nitric oxide (NO) is an important source of cGMP via soluble guanylate cyclase, while other sources can produces cGMP also, for example atrial natriuretic peptides (ANP) produce cGMP via particulate guanylate cylase. Since NO production can be enhanced in early pulmonary hypertension and decreased in severe pulmonary hypertension, we investigated the role of NO as a source of cGMP in the pulmonary circulation in swine with pulmonary hypertension after MI. The PDE5 inhibitor EMD360527 (300 μg min kg^{-1} iv) was administered in chronically instrumented swine with (n=8) and without (n=13) MI.

Inhibition of PDE5 resulted in pulmonary vasodilation as evidenced by a decrease in pulmonary arterial pressure as well as a decrease in pulmonary vascular resistance (PVR). This response was slightly larger in MI compared to normal swine. Inhibition of NO resulted in similar increases in PVR in normal and MI swine. Furthermore, prior NO synthase inhibition enhanced pulmonary vasodilation by PDE5 inhibition in both normal and MI swine so that a larger effect of PDE5 inhibition was still apparent in swine with MI. This suggests that the increase in vasodilator response to PDE5 inhibition after MI was not due to an increase in NO mediated cGMP production. In conclusion, PDE5 inhibition may be a useful strategy to treat pulmonary hypertension secondary to MI. The greater vasodilator response to PDE5 inhibition in MI swine does not appear to be the result of an increased NO production early after MI.

Introduction

Pulmonary hypertension after myocardial infarction is a severe disease with a poor prognosis if untreated [1]. In pulmonary hypertension, endothelial dysfunction accounts for an imbalance between vasodilators (nitric oxide (NO) and prostacyclin) and vasoconstrictors (endothelin) resulting in impaired vasodilation and exaggerated vasoconstriction [2-4]. This imbalance between production of vasodilators and vasoconstrictors likely contributes to the sustained state of pulmonary vasoconstriction after MI.

However, endothelial dysfunction appears to be present in severe pulmonary hypertension, while in the early stages NO production can be enhanced. In these early stages the pulmonary vasoconstriction may increase shear stress, thereby increasing NO production and counteracting the development of pulmonary hypertension [5-7]. This is in accordance with a study in humans, showing that basal NO-activity is reduced in patients with severe pulmonary hypertension, but not in patients with mild pulmonary hypertension [8]. These observations suggest that the normal response of the pulmonary circulation to pulmonary hypertension is to increase the synthesis of NO in an attempt to restore normal tone until sustained vasoconstriction eventually results in endothelial dysfunction. Indeed, in our swine model of pulmonary hypertension as a result of a recent MI, NO-induced vasodilation appears to be unaltered [9], although we have recently shown that endothelin-induced pulmonary vasoconstriction [10] and prostanoid-induced vasodilation [Chapter 6] are enhanced after MI.

NO-induced vasodilation is mediated through production of cGMP by soluble gyanylate cyclase (GC) and reduction in intracellular Ca^{2+} concentration. NO signaling is terminated through removal of cGMP by phosphodiesterase 5 (PDE5), which removes cGMP by hydrolyzing it to 5' GMP [11]. Therefore, inhibition of PDE5 has been proposed to be of benefit for the alleviation of pulmonary hypertension by prolonging the action of NO. In a previous study we showed that in normal swine, inhibition of PDE5 results in vasodilation of the pulmonary vascular bed [Chapter 7]. Another enzyme that produces cGMP is particulate GC, which is activated through atrial natriuretic peptide (ANP) [12]. ANP levels are elevated in patients with cardiovascular diseases and pulmonary hypertension [13, 14] and in our animal model after MI [15, 16]. This could lead to increased levels of cGMP after MI thereby further enhancing the potential beneficial effects of PDE5 inhibition.

The increased ANP levels after MI, in combination with the unaltered NO-induced vasodilation, led us to hypothesize that the vasodilator effect of inhibition of PDE5 in the pulmonary circulation would be increased after MI. Results of the

present study indeed showed an increased vasodilator effect of inhibition of PDE5 in the pulmonary circulation. Hence, since we hypothesized that this increased response was not the result of altered NO induced cGMP production, we secondly investigated the role of NO by inhibiting NO synthase with N^{ω}-nitro-L-Arginine (NLA) prior to inhibition of PDE5.

Methods

Studies were performed in accordance with the "Guiding Principles in the Care and Use of Laboratory Animals" as approved by the Council of the American Physiological Society, and with approval of the Animal Care Committee of the Erasmus MC Rotterdam. Twenty-one crossbred Landrace x Yorkshire swine of either sex (2-3 months old) entered the study.

Surgical procedures

Swine were sedated with ketamine (30 mg kg^{-1} im), anaesthetized with thiopental (10 mg kg^{-1} iv), intubated and ventilated with a mixture of O_2 and N_2O (1:2) to which 0.2-1% (v/v) isoflurane was added [17, 18]. Anaesthesia was maintained with midazolam (2 mg/kg + 1 mg kg^{-1} per hour iv) and fentanyl (10 µg kg^{-1} per hour iv). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid-filled polyvinylchloride catheter was inserted into the aortic arch and pulmonary artery for blood pressure measurement (Combitrans pressure transducers, Braun, Melsungen, Germany) and blood sampling.

An electromagnetic flow probe (14-15 mm, Skalar) was positioned around the ascending aorta for measurement of cardiac output. A polyvinylchloride catheter was also inserted into the left atrium to measure pressure. In all 28 swine the proximal part of the left coronary circumflex artery (LCx) was exposed, but only in 12 animals the LCx was permanently occluded with a silk suture to produce an MI [16, 19]. One MI swine died during surgery due to recurrent fibrillation. Catheters were tunnelled to the back and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine im) for 2 days and antibiotic prophylaxis (25 mg kg^{-1} amoxicillin and 5 mg kg^{-1} gentamycin iv) for 5 days.

Experimental protocols

Studies were performed approximately 2 weeks after surgery.

Effect of PDE5-inhibition: After hemodynamic measurements (lying and standing) consisting of left atrial, aortic and pulmonary artery blood pressures, heart rate and cardiac output, blood samples (lying), and rectal temperature (standing)

had been obtained, swine (12 normal and 6 MI) were subjected to a four-stage exercise protocol on a motor driven treadmill (1-4 km h^{-1}). Hemodynamic variables were continuously recorded and blood samples collected during the last 60 s of each 3 min exercise stage, at a time when hemodynamics had reached a steady state. After 90 minutes of rest, the PDE5 inhibitor EMD360527, was administered by an infusion of 300 mg min kg^{-1} iv and after ten minutes of infusion the exercise protocol was repeated, with continuous infusion of the EMD 360527 during the entire protocol [*Chapter 7*].

Effect of PDE5-inhibition after NO-inhibition: On a different day, the eNOS inhibitor N^{ω}-nitro-L-Arginine (NLA) was administered to swine (10 normal and 5 MI, of which 9 normal and 3 MI had also participated in the above described protocol) in a dose of 20 mg kg^{-1} iv prior to the first exercise protocol. After 90 minutes of rest, the PDE5 inhibitor was administered as described above and the exercise protocol was repeated.

Blood Gas Measurements

Blood samples were kept in iced syringes until the conclusion of each exercise trial. Measurements of PO_2 (mmHg), PCO_2 (mmHg) and pH were then immediately performed with a blood gas analyser (Acid-Base Laboratory Model 505, Radiometer, Copenhagen, Denmark). Oxygen saturation and hemoglobin (grams per 100 ml) were measured with a hemoximeter (OSM2, Radiometer). Blood O_2 content (mmol/ml) was computed as (Hb·0.621·O_2-saturation) + (0.00131·PO_2). Body O_2 consumption (BVO_2) was calculated as the product of cardiac output and the difference in O_2 content between arterial and mixed venous blood [17, 18].

Data Analysis

Digital recording and off-line analysis of hemodynamics have been described previously [18, 20]. Systemic vascular resistance (SVR) was computed as mean aortic blood pressure (MAP) divided by cardiac output (CO). Pulmonary vascular resistance (PVR) is defined as mean pulmonary artery pressure (PAP) minus mean left atrial pressure (LAP) divided by cardiac output.

Statistical analysis

To test for the effects of MI and drug treatment (EMD 360527 in presence or absence of NLA) on the relation between BVO_2 and PVR, regression analysis was performed using MI, drug treatment and BVO_2 as well as their interaction as independent variables and assigning a dummy variable to each animal. Statistical significance was accepted when $P<0.05$. Data are presented as mean±S.E.M.

Results

Effects of EMD 360527 during exercise

Exercise up to 4 km h^{-1} in normal swine resulted in an increase in cardiac output of 105±5% that was principally due to an increase in heart rate by 114±6% (table 1). Mean aortic blood pressure was minimally affected (Table 1), implying that the increase in cardiac output was balanced by a similar decrease in systemic vascular resistance. Mean pulmonary artery pressure almost doubled in normal swine during exercise. However, the transpulmonary pressure gradient (pulmonary artery pressure minus left atrial pressure) increased in approximately the same amount as cardiac output, reflecting an unchanged PVR during exercise (Fig. 1).

After MI, mean resting pulmonary arterial pressure increased from 17 ±1 to 23±2 mmHg (table 1), which was in part the result of the increased mean left atrial pressure (normal swine, 6±1 mmHg; MI swine, 12±2 mmHg, table 1). Cardiac output was virtually maintained both at rest and during treadmill exercise in MI compared to normal swine (table 1) despite a 21% lower stroke volume. Pulmonary arterial pressure was elevated by 35%, which was partly due to an increase in left atrial pressure of 50% and partly due to a 37% increase in PVR

Administration of the PDE5 inhibitor EMD360527 caused vasodilation in the systemic and pulmonary bed of normal and MI swine (table 1, Fig. 1). Mean arterial pressure decreased and cardiac output increased both in normal and MI swine, resulting in a decreased systemic vascular resistance at rest (18±5% in normal and 20±5% in MI swine), which slighly waned during exercise (respectively to 15±2% and 17±3% at 4 km h^{-1}) Pulmonary artery pressure and pulmonary vascular resistance decreased after administration of the PDE5 inhibitor EMD360527, indicating vasodilation in the pulmonary vascular bed (table 1, Fig. 1), which was significantly larger in the MI swine than in normal swine. During exercise the vasodilator effect of EMD360527 on the pulmonary vascular resistance increases in normal swine (from 15±9% at rest to 29±3% at 4 km h^{-1}) and was maintained in MI swine (Fig. 1).

Effects of combined NLA and EMD 360527 during exercise

Administration of the NO synthase inhibitor NLA resulted in a marked increase in aortic blood pressure, which resulted from systemic vasoconstriction in both normal and MI swine (table 1). This vasoconstriction was maintained during exercise. In the pulmonary circulation NO inhibition also resulted in marked vasoconstriction, demonstrated by an increase in pulmonary vascular resistance, which was maintained in normal and increased in MI swine during exercise (Fig.1).

Administration of the PDE5 inhibitor EMD360527 after prior administration of

***Table 1**: Hemodynamics of swine at rest and during exercise before and after administration of EMD360527 and NLA*

				Rest		Exercise level (km h^{-1})			
	n	Treatment	Infarct	lying	standing	1	2	3	4
HR (bpm)	12	Control	-	124 ± 1	139 ± 3*	170 ± 3*	183 ± 4*	202 ± 7*	240 ± 8*
		EMD	-	144 ± 5†	156 ± 5*†	178 ± 4*	193 ± 5*	218 ± 8*†	244 ± 8*
	6	Control	+	142 ± 5‡	155 ± 7*‡	181 ± 3*	188 ± 3*	206 ± 3*	233 ± 8*
		EMD	+	150 ± 8	165 ± 3	187 ± 3* †	199 ± 3* †	215 ± 4* †	232 ± 6*
	10	NLA	-	89 ± 4	112 ± 3*	125 ± 3*	132 ± 2*	150 ± 3*	193 ± 8*
		NLA+ EMD	-	102 ± 8†	122 ± 6*	135 ± 7*	147 ± 7*†	164 ± 7*†	199 ± 8*
	5	NLA	+	106 ± 6‡	128 ± 8*	149 ± 6*‡	158 ± 6*‡	179 ± 9*‡	195 ± 9*
		NLA+ EMD	+	121 ± 6‡	149 ± 6*†‡	155 ± 4†‡	176 ± 12‡	183 ± 9‡	204 ± 10‡
CO (l/min)	10	Control	-	4.4 ± 0.2	5.4 ± 0.3*	6.4 ± 0.3*	6.9 ± 0.3*	7.4 ± 0.3*	8.5 ± 0.3*
		EMD	-	5.0 ± 0.2†	5.7 ± 0.3*	6.6 ± 0.3*	7.0 ± 0.3*	8.2 ± 0.3*†	9.1 ± 0.3*†
	6	Control	+	4.1 ± 0.1	4.9 ± 0.1*	5.8 ± 0.2*	6.2 ± 0.2*	6.8 ± 0.2*	7.5 ± 0.2*
		EMD	+	4.6 ± 0.2	5.7 ± 0.2* †	6.3 ± 0.1* †	6.8 ± 0.2* †	7.5 ± 0.2* †	8.0 ± 0.2*
	7	NLA	-	3.1 ± 0.2	4.3 ± 0.3*	5.0 ± 0.3*	5.6 ± 0.4*	6.2 ± 0.4*	7.3 ± 0.4*
		NLA+ EMD	-	3.6 ± 0.2†	5.0 ± 0.3*	5.6 ± 0.4*	6.1 ± 0.4*†	6.9 ± 0.4*†	8.1 ± 0.4*†
	4	NLA	+	3.1 ± 0.2	4.2 ± 0.2	4.8 ± 0.2*	5.1 ± 0.1*	5.7 ± 0.4*	6.1 ± 0.2*
		NLA+ EMD	+	4.0 ± 0.2‡	5.5 ± 0.4†‡	5.6 ± 0.2†‡	6.6 ± 0.7‡	6.7 ± 0.4‡	7.3 ± 0.6‡
MAP (mmHg)	12	Control	-	89 ± 2	87 ± 3	84 ± 2*	85 ± 2*	86 ± 3	89 ± 3
		EMD	-	80 ± 2†	76 ± 3*†	74 ± 2*†	76 ± 2†	78 ± 2†	80 ± 2†
	6	Control	+	89 ± 5	81 ± 5*	79 ± 3	77 ± 2	79 ± 2	79 ± 2
		EMD	+	80 ± 4†	67 ± 2*†	69 ± 2*†	69 ± 2*†	70 ± 2†	69 ± 2†‡
	10	NLA	-	121 ± 3	111 ± 3*	111 ± 2*	112 ± 3*	115 ± 3	116 ± 3
		NLA+ EMD	-	106 ± 4†	105 ± 5	104 ± 4†	106 ± 4†	105 ± 3†	106 ± 3†
	5	NLA	+	116 ± 3	113 ± 3	113 ± 4	114 ± 2	112 ± 2	110 ± 3
		NLA+ EMD	+	108 ± 4‡	101 ± 3‡	100 ± 2†‡	99 ± 2†‡	100 ± 3†‡	100 ± 3†
SVR (mmHg/l/min)	10	Control	-	20.3 ± 1.1	16.0 ± 1.1*	13.0 ± 0.6*	12.1 ± 0.5*	11.4 ± 0.5*	10.3 ± 0.4*
		EMD	-	16.1 ± 0.9†	13.1 ± 0.7*†	10.9 ± 0.5*†	10.5 ± 0.4*†	9.2 ± 0.3*†	8.6 ± 0.3*†
	6	Control	+	21.9 ± 0.7	16.7 ± 1.0*	13.9 ± 0.8*	12.5 ± 0.5*	11.7 ± 0.4*	10.5 ± 0.4*
		EMD	+	17.7 ± 1.5†	12.0 ± 0.6*†	10.8 ± 0.4*†	10.2 ± 0.4*†	9.4 ± 0.2*†	8.6 ± 0.2*†
	7	NLA	-	39.3 ± 2.3	27.0 ± 1.7*	22.6 ± 1.4*	20.9 ± 1.5*	18.7 ± 1.1*	15.9 ± 0.7*
		NLA+ EMD	-	30.7 ± 2.3†	21.7 ± 1.7*†	19.1 ± 1.4*	17.1 ± 1.0*†	15.0 ± 0.6*†	12.9 ± 0.5*†
	4	NLA	+	38.2 ± 2.6	27.1 ± 1.1	23.9 ± 1.7	22.2 ± 0.7*	19.6 ± 1.0*	17.9 ± 0.7*
		NLA+ EMD	+	27.1 ± 0.3‡	18.3 ± 0.8†‡	18.0 ± 0.3†‡	15.6 ± 1.6‡	15.3 ± 1.1‡	14.0 ± 1.1*†‡
PAP (mmHg)	12	Control	-	17 ± 1	18 ± 1	22 ± 1*	24 ± 1*	27 ± 1*	34 ± 1*
		EMD	-	15 ± 1†	14 ± 1†	17 ± 1*†	19 ± 1*†	23 ± 1*†	26 ± 1*†
	6	Control	+	23 ± 2‡	23 ± 3‡	28 ± 3*‡	29 ± 2*‡	33 ± 3*‡	36 ± 2*
		EMD	+	19 ± 2†‡	17 ± 2†	22 ± 2†‡	23 ± 2*†‡	26 ± 2*†‡	29 ± 3*†
	10	NLA	-	22 ± 2	25 ± 2	27 ± 2	28 ± 2	33 ± 2*	39 ± 3*
		NLA+ EMD	-	15 ± 2†	16 ± 2†	18 ± 2†	21 ± 2*†	23 ± 2*†	29 ± 2*†
	5	NLA	+	27 ± 4	30 ± 4	38 ± 5	40 ± 5*‡	44 ± 4*‡	46 ± 3*
		NLA+ EMD	+	20 ± 3†‡	20 ± 4†‡	22 ± 4†‡	29 ± 4†‡	30 ± 4†‡	34 ± 3†‡
LAP (mmHg)	10	Control	-	6 ± 1	5 ± 1*	6 ± 1	7 ± 1	9 ± 1*	11 ± 1*
		EMD	-	4 ± 1	3 ± 1	5 ± 1	6 ± 1*	9 ± 1*	11 ± 1*
	6	Control	+	12 ± 2‡	11 ± 2‡	14 ± 2‡	15 ± 2‡	17 ± 2*‡	19 ± 2*
		EMD	+	11 ± 2‡	9 ± 2*‡	13 ± 2†‡	15 ± 2*‡	17 ± 2*‡	19 ± 2*
	9	NLA	-	8 ± 1	6 ± 1	7 ± 1	8 ± 1	10 ± 1	10 ± 1
		NLA+ EMD	-	6 ± 1	6 ± 2	7 ± 1	8 ± 1*	9 ± 1*	10 ± 1*
	4	NLA	+	13 ± 2‡	13 ± 2‡	16 ± 2‡	16 ± 1‡	17 ± 2‡	17 ± 1
		NLA+ EMD	+	10 ± 2‡	10 ± 3‡	12 ± 3†‡	16 ± 2*‡	17 ± 2*‡	19 ± 2*‡

*HR: Heart Rate, CO: Cardiac Output, MAP: Mean Arterial Pressure, SVR: Systemic Vascular Resistance, PAP: Mean Pulmonary Arterial Pressure, LAP: Mean Left Atrial Pressure, Data are mean ± S.E.M.; *P≤0.05 vs rest (lying); †P≤0.05 vs corresponding control, ‡P≤0.05 vs corresponding normal swine*

NLA, caused an increase in cardiac output and a decrease in mean arterial pressure in normal and MI swine, resulting in a decreased systemic vascular resistance at rest (23±5% in normal and 27±6% in MI swine), which slightly waned during exercise (respectively to 17±3% and 22±4% at 4 km h^{-1}) (table 1).

Inhibition of PDE5 also caused vasodilation in the pulmonary vascular bed of normal and MI swine, demonstrated by a decrease in pulmonary vascular resistance at rest (32±12% in normal and 42±5% in MI swine), which increased slightly during exercise (respectively to 41±3% and 57±5% at 4 km h^{-1}) (Fig.1). Surprisingly, the effect of PDE5 inhibition after inhibition of NO synthase is larger than the effect of PDE5 inhibition alone, indicating that even though there is a loss of NO, the availability or sensitivity of cGMP is enlarged. The effect on the pulmonary vascular resistance of MI swine is also slightly larger than the effect on the pulmonary circulation of normal animals and increases with incremental exercise level (Fig.1).

Discussion

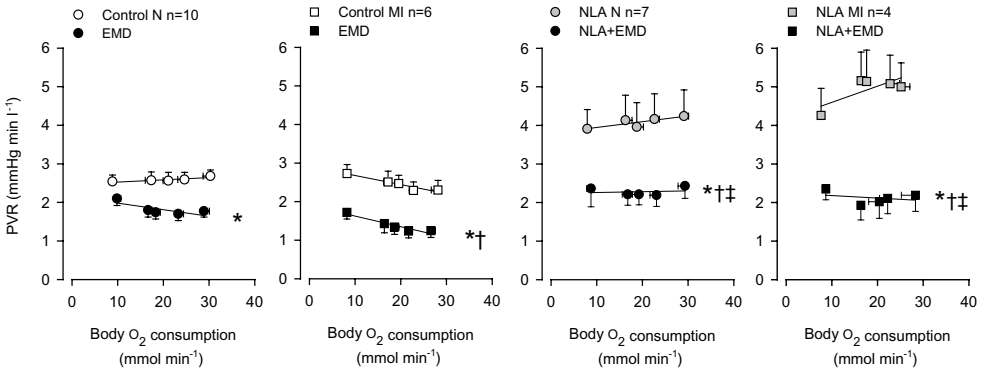

Figure 1: *The effects of the PDE5 inhibitor EMD360527 alone and after prior administration of the NO synthase inhibitor NLA in normal swine (circles) and MI swine (squares) on pulmonary vascular resistance (PVR, upper panels) and pulmonary artery pressure (PAP, lower panels) plotted as a function of body O2 consumption. Control exercise trials are shown as open symbols, exercise trials in the presence of antagonists are shown as solid symbols. *P<0.05 effect of EMD360527 vs corresponding Control, † P<0.05 effect of EMD 360527 different after MI, ‡P<0.05 effect of EMD360527 after NLA vs effect of EMD360527 alone*

The main findings of the present study are that (*i*) PDE5-inhibition results in vasodilation in the pulmonary circulation of normal swine and MI swine both at rest and during exercise; (*ii*) after MI, PDE5-inhibition induced pulmonary vasodilation was slightly enhanced; (*iii*) in both normal and MI swine, the effect of PDE5 inhibition at rest and during exercise was similarly enhanced after prior inhibition of NO synthase.

cGMP/PDE in pulmonary hypertension

In the present study we found that PDE5 inhibition in swine with secondary pulmonary hypertension after MI has a larger effect than in the normal swine under resting conditions, which indicates that there are increased cGMP-levels or an increased PDE5 activity. Increased levels of cGMP can be the result of increased NO synthase, which is found in early stages of pulmonary hypertension [6, 7] as is likely present in our model 2-3 weeks after MI. In our model we have previously shown that the vasodilator response of NO is maintained in pulmonary hypertension after myocardial infarction, but it is possible that increased NO synthase was accompanied by a similar increase in PDE5 activity. This is in accordance with studies that found that the NO-cGMP pathway is altered in pulmonary hypertension, demonstrated by different observations in animal models that cGMP activity and PDE5 activity are increased [21-24]. However, two studies showed that it leads to a net result of increased levels of cGMP [22, 23].

cGMP can also be produced through activation of particulate GC by ANP. ANP is stored within atrial secretory granules and is released in response of increased wall stress or pressure [25]. In pulmonary hypertension after myocardial infarction atrial pressures are increased and can thereby contribute to increased levels of ANP that is found in patients with pulmonary hypertension [26]. In accordance with these finding, we have previously shown that ANP levels are in increased in our model of pulmonary hypertension [15, 16]. Since increased levels of ANP can lead to increased production of cGMP, the increased vasodilator effect on PDE5 inhibition in MI swine could be the result of these increased levels of ANP. Future studies with an ANP receptor antagonist [27] are needed to further explore this hypothesis. However, inhibition of PDE5 after inhibition of NO, led to a decrease in pulmonary vascular resistance that was larger in swine with MI in comparison with normal swine. Thus, the increased response to PDE5 inhibition in pulmonary hypertension is preserved after inhibition of NO, indicating that this increased response was not NO-mediated.

Conclusions and clinical relevance

Inhibition of PDE5 is often used to alleviate PAP and PVR in patients with pulmonary hypertension who suffer from endothelial dysfunction and therefore decreased availability of NO. Our study showed that patients with early pulmonary hypertension also benefit from the vasodilator capacity of PDE5 inhibition and that the effect is increased after MI in comparison with the healthy pulmonary circulation, probably as a result of increased cGMP availabilty or activity that was not NO-mediated. In addition, when patients develop endothelial dysfunction and decreased NO synthase as a result of aggravation of pulmonary hypertension, inhibition of PDE5 still results in pulmonary vasodilation and decreases PAP and PVR.

Patients with pulmonary hypertension have a decreased exercise capacity because of the increased afterload of the right ventricle. Indeed, Franciosa et al found that it is the right ventricle and PAP that limit the exercise capacity in patients with left ventricular failure [28]. The present study showed that the decreases in PAP and PVR following inhibition of PDE5 are maintained during exercise, speculating that this therapy could increase exercise capacity in patients with pulmonary hypertension after myocardial infarction.

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Chapter 9

General discussion and summary

General discussion and summary

The studies presented in this thesis were carried out to obtain a better understanding of the regulation of pulmonary vascular tone in health and disease. Investigation of the pulmonary circulation is important, because there is increasing evidence that the regulation of vascular tone in the pulmonary and systemic circulation is different [1, 2]. Hence, rational pharmacotherapy of pulmonary vascular diseases needs to be tailored to the pulmonary vascular bed. In particular, the regulation of pulmonary vascular tone during physical exercise has received little attention. This is surprising, in view of reports that the pulmonary circulation and the right ventricle are the main determinants of exercise capacity [3, 4].

Pulmonary hypertension is a disease with different etiologies and a poor prognosis if left untreated. One of the etiologies is myocardial infarction (MI)-induced left ventricular dysfunction. The incidence of post-MI pulmonary hypertension is increasing because of considerably improved survival after acute MI. The pulmonary vascular mechanisms that contribute to post-MI pulmonary hypertension and the optimal therapy for patients with pulmonary hypertension secondary to MI have received little attention. These therapies are important to prevent right ventricular failure due to the high pulmonary pressure. There are studies in patients with heart failure, for example to investigate endothelin receptor blockers, where they also looked at the pulmonary circulation but the main goal in these studies is to alleviate heart failure [5, 6]. More studies should be conducted to investigate specifically the pulmonary vascular regulation in patients with heart failure. In addition, patients with pulmonary hypertension are particularly vulnerable to the exercise-induced additional elevations in pulmonary artery pressure and exercise capacity is often reduced [3, 4]. Therapy therefore should be aimed to increase exercise capacity. Indeed one of the endpoints often used in clinical trials is the six-minute-walk. However, there is little literature about the regulation of pulmonary vascular tone in pulmonary hypertension during exercise.

In light of these considerations, we investigated the regulation of pulmonary vascular tone at rest and during exercise in our swine model. We used healthy swine and swine with MI-induced pulmonary hypertension. This thesis consists of two parts. Part one (*Chapter 2-4*), describes the regulation of pulmonary vascular tone under physiological conditions. Specifically, we investigated the integrative control of pulmonary vascular resistance by endothelium dependent vasodilators and vasoconstrictors, at rest and at various levels of physical activity. Part two (*Chapter 5-8*), describes the alterations in the regulation of pulmonary vascular

tone in swine with pulmonary hypertension secondary to an MI.

Regulation of pulmonary vascular tone in swine under physiological conditions.

The endothelium plays a crucial role in the regulation of vascular tone by producing both vasodilating (nitric oxide (NO) and prostacyclin) and vasoconstricting (endothelin (ET)) substances. In a previous study reported by our group it has been shown that endogenous NO exerts a vasodilator influence on the systemic and pulmonary vasculature of awake swine at rest, while the exercise-induced vasodilation was only slightly blunted after inhibition of NO production [7]. Hence, other vasodilators, such as prostacyclin, might be involved in exercise-induced vasodilation and act to compensate when NO synthase (NOS) activity is blunted. In *Chapter 2* of this thesis the contribution of prostanoids to the regulation of systemic, as well as pulmonary vascular tone was investigated in chronically instrumented swine performing treadmill exercise. Furthermore we studied whether the contribution of prostanoids to the regulation of vascular tone is enhanced after inhibition of NOS in exercising swine. The results of *Chapter 2* demonstrate that prostanoids are involved in the regulation of vascular tone in the systemic circulation and that the contribution of prostanoids to vasodilation is increased when NOS activity is inhibited. In contrast, prostanoids do not contribute to the regulation of pulmonary vascular tone, even not when NO production is attenuated.

The endothelium produces not only vasodilating but also vasoconstricting substances, such as ET [8]. ET exerts its influence on the vasculature via the ET_A and ET_B receptor. Both receptors are located on vascular smooth muscle cells where they induce vasoconstriction, while ET_B receptors are also located on the endothelial cells where they induce vasodilation and regulate clearance of ET. The net effect of ET is the result of its action on binding to both receptors [9]. In *Chapter 3* we investigated the role of ET and its receptors in regulation of systemic and pulmonary vascular tone of swine at rest and during exercise, using a selective ET_A as well as a mixed ET_A/ET_B receptor antagonist. The most important findings are that ET contributes to basal resting tone in the systemic, but not in the pulmonary circulation. During treadmill exercise, the ET_A-mediated constriction of the systemic vascular bed wanes, thereby facilitating vasodilation. In contrast, an ET-mediated constriction becomes apparent in the pulmonary bed during exercise, which appears to be ET_B-mediated.

Since ET can increase the production of NO and prostanoids, which in turn can blunt the release of ET and/or modify the responsiveness of its receptors,

we investigated the integrated vasomotor control of systemic and pulmonary vascular resistance by NO, prostanoids and ET in *Chapter 4*. The results show that prostanoids blunt the vasoconstrictor influence of ET in the systemic but not in the pulmonary circulation while NO blunts the vasoconstrictor influence of ET in both the systemic and pulmonary circulation, particularly during exercise. Prostanoids and NO blunt the vasoconstrictor influence of ET on the systemic vascular bed by an additive manner, but loss of NO does not unmask a role of prostanoids in blunting the vasoconstrictor influence of ET in the pulmonary circulation. Thus, under conditions of endothelial dysfunction, in particular reduced NO bioavailability, the exercise-induced pulmonary vasodilation is impaired, due to "unopposed" ET-mediated pulmonary vasoconstriction.

Regulation of pulmonary vascular tone in healthy swine at rest and during exercise as studied in this thesis is schematically presented in figure 1.

Regulation of pulmonary vascular tone in swine with pulmonary hypertension after myocardial infarction.

Congestive heart failure is the only major cardiovascular disorder of which the incidence has increased over the past decade, which is principally related to a reduction in mortality associated with acute MI [10]. The loss of viable myocardial

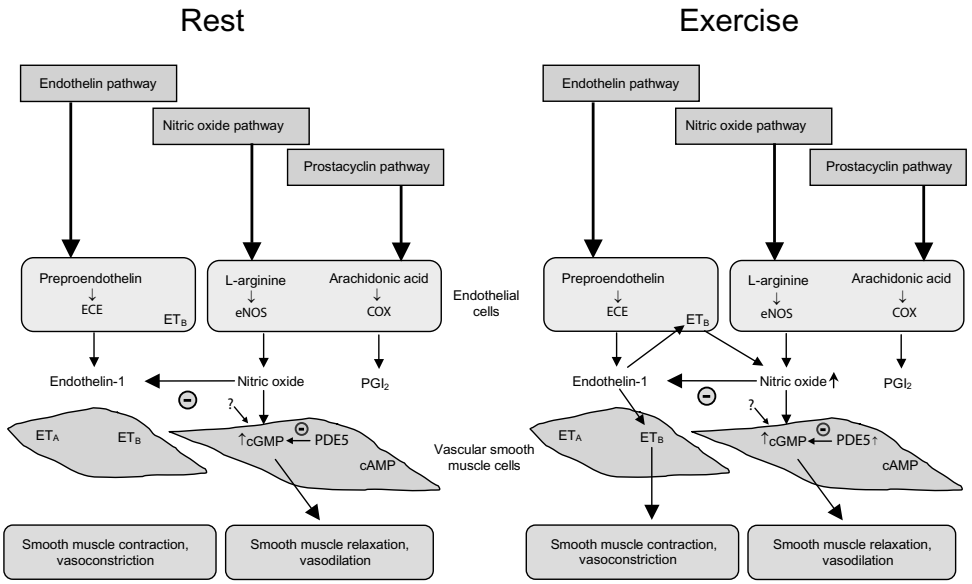

***Figure 1**: Regulation of pulmonary vascular tone at rest and during exercise in healthy swine. ET_A: Endothelin A receptor; ET_B: ET B receptor; cGMP: cyclic guanosine monophosphate; cAMP: cyclic adenosine monophospate; PDE5: phosphodiesterase 5*

tissue after MI causes left ventricular dysfunction. This leads to an increase in left ventricular filling pressure and neurohumoral activation particularly during exercise [11-13]. The increase in left ventricular filling pressure is transmitted backwards into the pulmonary circulation, resulting in pulmonary congestion and an increase in pulmonary arterial blood pressure, thereby elevating right ventricular afterload [11, 14]. The resultant right ventricular hypertrophy is a risk factor for the development of right-sided heart failure [15]. Pulmonary hypertension is often accompanied by endothelial dysfunction resulting in impaired vasodilation and exaggerated vasoconstriction [16-18], which can be the result of activation of the ET system. Elevated plasma ET-1 levels found in patients with pulmonary hypertension correlate with their New York Heart Association (NYHA) functional class [19] suggesting a role for ET-1 in pulmonary hypertension.

In *Chapter 5* we investigated whether increased ET vasoconstrictor tone in the pulmonary circulation contributes to secondary pulmonary hypertension in swine with a recent MI and assessed the role of ET_A and ET_B receptor subtypes in this process. Since in healthy swine a pulmonary ET –dependent vasoconstriction emerges during exercise, but is negligible under resting conditions (*Chapter 3*), the vasoconstrictor influence of endogenous ET in MI swine was studied both at rest and during treadmill exercise. The results show that plasma ET levels as well as the pulmonary vascular responsiveness to exogenous ET-1 are increased after MI and that this increase is principally mediated by the ET_A receptor. The pulmonary vasoconstriction of ET mediated by the ET_B receptor is not altered as compared to healthy swine. Thus, although the ET_A receptor does not contribute to the regulation of pulmonary vasomotor tone in healthy swine at rest or during exercise, an ET_A-mediated vasoconstriction emerged after pulmonary hypertension secondary to an MI. The enhanced vasodilator effect of combined ET_A/ET_B receptor blockade after MI was confirmed recently in a mouse model of MI [20]. It has been proposed that for treatment of pulmonary hypertension ET_A receptor blockade is more favourable than combined ET_A/ET_B receptor blockade because single blockade of ET_A receptor leaves both the ET_B-receptor-mediated clearance of ET and the endothelium ET_B-receptor-mediated pulmonary vasodilation unaffected [21]. However, our results suggest that patients with pulmonary hypertension secondary to an MI may benefit more from combined ET_A/ET_B than from selective ET_A receptor blockade. To date, only one clinical trial compared the treatment of pulmonary hypertension with a combined ET_A/ET_B blocker and with a selective ET_A receptor blocker [22]. This trial reported a significant improvement in exercise capacity and World Health Organisation Functional Class (WHO FC) following ET_A/ET_B blockade, whereas the effects of selective ET_A receptor blockade reached statistical significance only at

the highest dose [22].

The increased ET-dependent vasoconstriction in swine with a recent MI can be the result of decreased vasodilation by NO and/or prostanoids since they are both capable of limiting the ET-production or receptor sensitivity to ET [23-25]. Based on the observations in *Chapter 4*, we hypothesized that the increased ET-dependent vasoconstriction was the result of a decreased NO- (but not prostanoid-) mediated inhibition on ET-vasoconstriction. Therefore, we investigated the alterations in integrated endothelium dependent control of pulmonary vascular tone in swine with pulmonary hypertension following an MI in *Chapter 6*. In contrast to our hypothesis, we found that the pulmonary vasodilator influence of NO was well maintained after MI and that the inhibition of the ET-mediated vasoconstrictor influence by NO was unmitigated. In contrast, an increased production of prostanoids after MI appeared to limit the ET-induced pulmonary vasoconstriction and contributed to lower pulmonary vascular resistance under resting conditions. Taken together these findings indicate that the increased ET-dependent vasoconstriction in the pulmonary circulation after an MI is not explained by a loss of vasodilator influence of NO or prostanoids.

Regulation of pulmonary vascular tone in swine with post-MI pulmonary hypertension at rest and during exercise as studied in this thesis is schematically presented in figure 2.

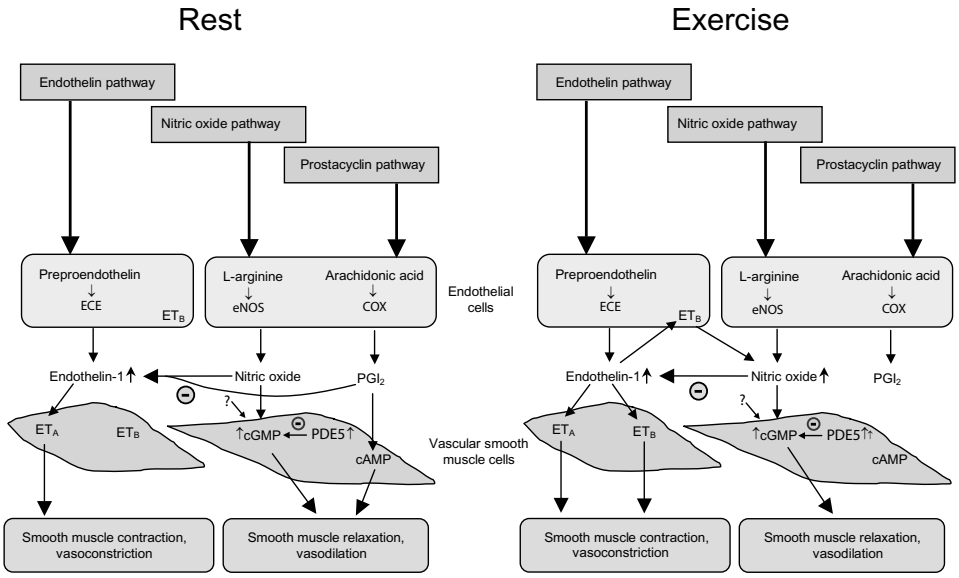

Figure 2: *Regulation of pulmonary vascular tone at rest and during exercise in swine with pulmonary hypertension after myocardial infarction. ET_A: Endothelin A receptor; ET_B: ET B receptor; cGMP: cyclic guanosine monophosphate; cAMP: cyclic adenosine monophospate; PDE5: phosphodiesterase 5*

Vasodilator therapy of pulmonary hypertension

Vasodilator therapy for patients with pulmonary hypertension is aimed at reducing pulmonary artery pressure both at rest and during exercise. Currently available therapies include inhalation of NO and administration of Ca^{2+}-channel blockers, exogenous prostacyclin or combined ET-receptor blockers. However, these therapies have short-duration, limited efficacy, are expensive and/or are associated with significant side effects. Thus there is room for improvement of vasodilator therapie in pulmonary hypertension [26]. In recent years, phosphodiesterase (PDE) 5 inhibition has emerged as a promising novel vasodilator therapy [26-28]. In *Chapters 7 and 8*, we assessed the vasodilator profiles of the novel PDE5 inhibitor EMD360527 [29] in healthy swine (*Chapter 7*) and swine with a recent MI (*Chapter 8*).

In *Chapter 7*, we investigated the selectivity of EMD360527 for the pulmonary vascular bed as compared to the systemic vascular bed. Furthermore, since NO is considered as one of the major sources of cGMP [30] and pulmonary hypertension has shown to be associated with endothelial dysfunction, and reduced endothelial NOS (eNOS) activity [31], we determined to what extent the action of the PDE5 inhibitor EMD360527 depends on the activity of endothelial NOS. Our results show that administration of EMD360527 results in a dose-dependent vasodilation of the pulmonary and systemic circulation under resting conditions. The vasodilation is slightly higher in the pulmonary than in the systemic circulation. During exercise, the vasodilator effects of EMD360527 in the pulmonary circulation were well maintained, but decreased slightly in the systemic circulation. Unexpectedly, inhibition of eNOS increased the vasodilator effect of EMD360527 as well as of nitroprusside in both the pulmonary and systemic circulation (Fig. 1). Several reasons might explain the unperturbed vasodilator response to EMD360527 in the absence of NO, for example, increased activity of other vasoactive mediators that stimulate the production of cGMP or supersensitization of the NO/cGMP pathway [32-34]. The observation that the vasodilator effect of inhibition of PDE5 was enhanced following eNOS inhibition, and that it correlated well with the basal level of pulmonary vasoconstrictor tone, suggests that PDE5 inhibition will be most effective in patients with the most severe degree of pulmonary hypertension.

In post-MI pulmonary hypertension, one of the other main stimulators of cGMP, atrial natriuretic peptide (ANP), has shown to be increased in patients with heart failure and pulmonary hypertension [35, 36] and in our swine model [11, 14]. This possibly result in an increased availability of cGMP in post MI pulmonary hypertension and consequently an enhanced vasodilator effect of EMD360527. The increased

ANP levels after MI, in combination with the unaltered NO-induced vasodilation (*Chapter 6*), led us to hypothesize that the vasodilator effect of inhibition of PDE5 in the pulmonary circulation would be increased after MI. In *Chapter 8* we found that pulmonary vasodilation after PDE5-inhibition was slightly enhanced in swine with MI-induced pulmonary hypertension (Fig. 2). To test the hypothesis that an increased vasodilator response was not the result of altered NO bioavailability, we also studied the vasodilator effects of EMD360527 following inhibition of eNOS. The results demonstrated that in both healthy and in MI swine, the effect of PDE5 inhibition at rest and during exercise was similarly enhanced after prior inhibition of eNOS. These findings indicate that the enhanced vasodilator effect of PDE5 inhibition after MI is not the result of altered NO-bioavailabilty.

Future perspectives

In our studies, the underlying mechanism of two important, currently used and investigated, vasodilator therapies for pulmonary hypertension were studied: ET receptor blockade and PDE5 inhibition. The findings demonstrate that both pulmonary vasodilation after PDE5 inhibition and vasoconstriction by endogenous ET are increased in post-MI pulmonary hypertension. Since both vasodilator and vasoconstrictor effects are enhanced, it can be hypothesized that these two effects balance each other and that combination therapy results in an improved vasodilator effect. This hypothesis can be tested by using a combination of an ET receptor blocker and PDE5 inhibitor in our swine model with pulmonary hypertension. At the moment, some clinical studies have already combined both therapies [37-39]. This combination therapy is initiated when monotherapy with one vasodilator is not effective. The combination therapy appears to be safe and on average is associated with an imrovement in 6 min walk distance, pulmonary artery pressure and oxygen uptake [37-39].

From a clinical point of view, long-term studies to investigate vasodilator therapies are more relevant than the single dose studies employed in the described studies of this thesis. Our animal model of post-MI pulmonary hypertension is well suited to study long-term effect of various vasodilator therapies on the systemic and pulmonary circulation both at rest and during exercise. With this approach pulmonary arterial pressure and pulmonary vascular resistance can be monitored continuously and the effects of therapy can be followed for prolonged periods. This approach makes it also possible to evaluate the effect of different therapies on vascular remodelling in ex-vivo experiments using isolated pulmonary blood vessels. In addition, histology might be used to investigate the remodelling of

small pulmonary vessels [40]. Histological images of small pulmonary arteries of our animal model are presented in figure 3.

The animal model used in our studies is a model of pulmonary hypertension after a MI. Pulmonary hypertension has many different etiologies. Unfortunately, little epidemiologic information is available on the prevalence of post-MI pulmonary hypertension in patients. Moreover pulmonary hypertension in association with left-sided heart failure is usually either mild or moderate, though it can be severe in up to a third of patients. The speculation is that significant pulmonary hypertension may be present in up to 250,000 heart failure patients in the United States, which is far greater than the reported prevalence of pulmonary hypertension associated with other condition [41]. Yet, for a better understanding of pathophysiology of pulmonary hypertension of other etiologies and response to therapy other animal models of pulmonary hypertension have to be developed. There are genetically modified small animals like mice and rats that develop pulmonary hypertension. However, the advantage of a large animal model is that its (patho)physiology usually more closely resembles human (patho)physiology and that more parameters can be monitored for prolonged periods of time. In our swine model pulmonary hypertension can be induced by injecting microparticles into the pulmonary circulation to cause small pulmonary embolies. In addition monocrotaline injections and hypoxia may be other possibilities to induce pulmonary hypertension.

A. Control Swine **B. Swine with MI**

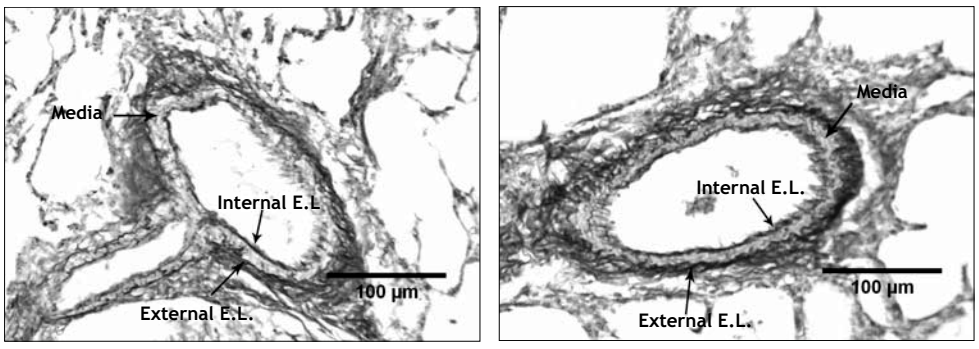

***Figure 3**: Small pulmonary arteries in Control Swine (A) and Swine with MI (B). E.L.: Elastica Lamina. Elastin stain (Resorcin/fuchsin).*

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Algemene discussie en samenvatting

De studies in dit proefschrift zijn uitgevoerd om beter begrip te krijgen over hoe de pulmonale vaattonus gereguleerd wordt onder gezonde omstandigheden en tijdens ziekte. Het is belangrijk om de pulmonale circulatie te bestuderen omdat het steeds duidelijker wordt dat de pulmonale circulatie verschillend is van bijvoorbeeld de systemische circulatie [1, 2]. Dit betekent ook dat geneesmiddelentherapieën voor ziektes van de pulmonale circulatie aangepast moeten worden aan deze specifieke circulatie. Er is vooral weinig bekend over de pulmonale circulatie tijdens fysieke inspanning, terwijl uit verschillende studies is gebleken dat de pulmonale circulatie en de rechter ventrikel de belangrijkste determinanten zijn van inspanningscapaciteit [3, 4].

Pulmonale hypertensie is een ziekte met veel mogelijke oorzaken en deze ziekte heeft een slechte prognose wanneer de patient niet behandeld wordt. Eén van de oorzaken van pulmonale hypertensie is linkerventrikel falen, bijvoorbeeld als gevolg van een myocard infarct (MI). Pulmonale hypertensie na een MI komt steeds vaker voor aangezien er steeds meer patiënten een MI overleven. Deze hebben allen kans om pulmonale hypertensie te ontwikkelen. Tot nu toe is er weinig bekend over de vasculaire mechanismen van de pulmonale circulatie die bijdragen aan deze vorm van pulmonale hypertensie en er ook is er weinig bekend over therapieën voor patiënten met pulmonale hypertensie als gevolg van een MI. Deze therapieën zijn wel belangrijk om rechterventrikelfalen en daarmee verslechteren van de toestand van de patiënt te voorkomen. Er zijn wel studies in patiënten met hartfalen, bijvoorbeeld endotheline receptor blokkers te bestuderen, waarbij ook naar de pulmonale circulatie is gekeken, maar het doel van deze studies was altijd om het hartfalen te verminderen. [5, 6]. Er moeten dus meer studies gedaan worden die specifiek onderzoek doen naar de pulmonale vaattonus regulatie in patiënten met hartfalen.. Aangezien patiënten met pulmonale hypertensie al een verlaagde inspanningscapaciteit hebben [3, 4]. is het belangrijk dat therapieën ook tijdens inspanning werken. Daarom is het van belang dat ook naar de vaattonus regulatie tijdens inspanning onderzoek gedaan wordt.

Dit alles heeft geleid tot dit proefschrift, welke bestaat uit twee delen. In het eerste deel (*Hoofstuk 2-4*) is de regulatie van pulmonale vaattonus onder normale fysiologische omstandigheden bestudeerd. In het bijzonder is de rol van verschillende vaatverwijders (stikstofoxide (NO) en prostacyline) en vaatvernauwers (endotheline) bestudeerd. Deze onderzoeken zijn gedaan in varkens, tijdens rust en tijdens verschillende nivo's van fysieke inspanning. In deel twee van dit proefschrift (*Hoofdstuk 5-8*) zijn de veranderingen in pulmonale vaattonus

regulatie die optreden bij pulmonale hypertensie als gevolg van MI bestudeerd. Deze onderzoeken zijn ook gedaan in varkens tijdens rust en verschillende nivo's van inspanning.

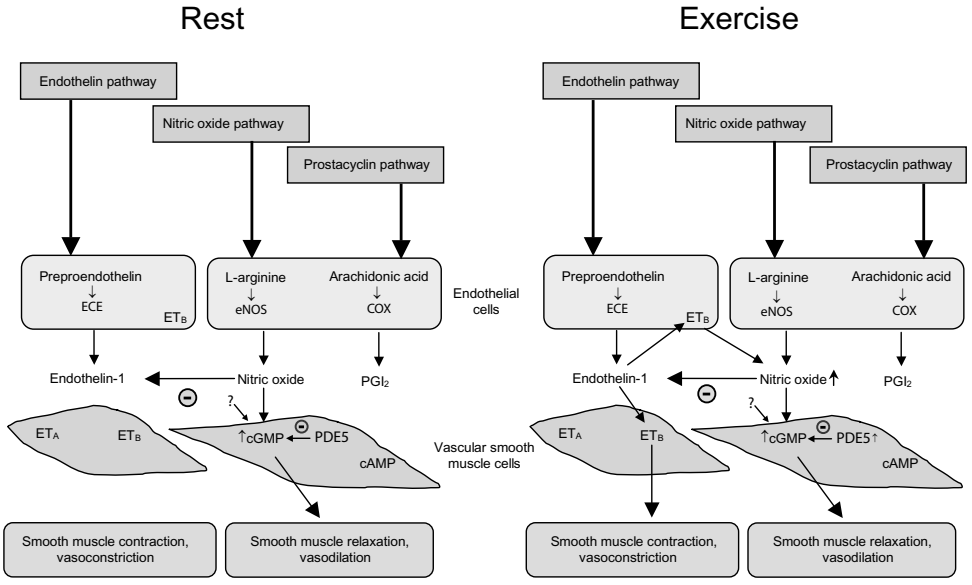
Regulatie van de pulmonale vaattonus in varkens onder gezonde condities

Het endotheel speelt een belangrijke rol in de vaattonus regulatie door het produceren van verschillende vaatverwijders zoals NO en prostacycline. Het endotheel produceerd ook vaatvernauwers zoals bijvoorbeeld endotheline. Een eerdere studie in ons laboratorium heeft laten zien dat lichaamseigen NO een vaatverwijdende invloed heeft in de lichaams- (systemische) en de pulmonale circulatie van wakkere varkens. Tijdens inspanning bleef het effect van NO nagenoeg gelijk want het blokkeren van NO resulteerde slechts in een kleine daling van de vaatverwijding die door inspanning ontstaat [7]. Dit betekent dus dat de vaatverwijding die door inspanning ontstaat niet het gevolg is van een grotere invloed van NO maar dat dit door andere vaatverwijders, zoals misschien prostacycline ontstaat. Of, dat het blokkeren van de invloed van NO ervoor zorgt dat andere vaatverwijders het overnemen. Om dit te onderzoeken hebben we in *Hoofdstuk 2* van dit proefschrift gekeken naar de bijdrage van prostacycline aan de systemische en pulmonale vaattonus regulatie in chronische geïnstrumenteerde varkens in rust en tijdens inspanning op een loopband. Tevens hebben we bestudeerd of de rol van prostacycline verhoogd is na het blokkeren van NO. Uit de resultaten van *Hoofstuk 2* blijkt dat prostacycline betrokken is bij de regulatie van het systemische vaatbed en dat de vaatverwijdende invloed verhoogd is wanneer NO geblokkeerd is. In het pulmonale vaatbad daarentegen heeft prostacycline geen rol in het reguleren van de vaattonus, zelfs niet wanneer een andere belangrijke vaatverwijder (NO) is uitgeschakeld.

Het endotheel produceerd niet alleen de vaatverwijders zoals NO en prostacycline, maar ook vaatvernauwers zoals endotheline [8]. Endotheline werkt via twee receptor subtypes, de ET_A en de ET_B receptor. Beide receptoren bevinden zich op de gladde spiercellen van de bloedvaten en wanneer endotheline aan een van deze receptoren bindt zorgt dit voor vaatvernauwing. Er bevinden zich echter ook ET_B receptoren op de endotheelcellen van de vaatwand en wanneer endotheline aan deze receptoren bindt zorgt dit voor vaatverwijding. De ET_B receptoren op de endotheelcellen zijn ook verantwoordelijk voor de klaring van endotheline. Het netto effect van endotheline op de vaatwand is dus afhankelijk van de mate van binding aan alle receptoren. [9]. In *Hoofdstuk 3* hebben we de rol van endotheline en de receptoren bestudeerd in zowel de systemische als de pulmonale circulatie. We hebben daarvoor gebruik gemaakt van een selectieve ET_A antagonist en een

gecombineerde ET_A/ET_B antagonist. De belangrijkste bevindingen uit deze studie is dat endotheline bijdraagt aan de basale vaattonus van de systemische circulatie. Tijdens inspanning verdwijnt de ET_A gemedieerde constrictie van het systemsiche vaatbed, wat bijdraagt aan de inspannings geinduceerde vaatverwijding. Deze bevindingen staan in tegenstelling tot de bevindingen in het pulmonale vaatbed, waar endotheline niet bijdraagt aan de basale vaattonus, maar tijdens inspanning een vaatvernauwende rol heeft . Deze vaatvernauwende rol tijdens inspanning is gemedieerd door de ET_B receptoren.

Endotheline kan de productie van NO en prostacycline verhogen en deze kunnen op hun beurt het vrijkomen van endotheline of de gevoeligheid van de endotheline receptoren verlagen. Om deze redenen hebben wij de rol van NO, prostacycline en endotheline samen onderzocht in zowel de systemische als de pulmonale circulatie in *Hoofstuk 4*. Uit deze resulaten blijkt dat prostacycline het vaatvernauwende effect van endotheline verlaagd in het systemische vaatbed, maar niet in het pulmonale vaatbed. NO verlaagd ook het vaatvernauwende effect van endotheline in het systemische vaatbed, maar doet dit ook in het pulmonale vaatbed. Beide met name tijdens inspanning. Na het blokkeren van NO wordt de rol van prostacycline groter in het systemische vaatbed wat betekent dat



***Figuur 1**: Regulatie van de pulmonale vaattonus in rust en tijdens inspanning in gezonde varkens. ET_A: Endothelin A receptor; ET_B: ET B receptor; cGMP: cyclic guanosine monophosphate; cAMP: cyclic adenosine monophospate; PDE5: phosphodiesterase 5*

NO en prostacycline elkaar aanvullen en over kunnen nemen. In het pulmonale vaatbed daarentegen speelt prostacycline ook geen vaatverwijdende rol wanneer NO geblokkeerd is. Dus, uit dit hoofdstuk blijkt dat onder omstandigheden van endotheeldysfunctie, met name wanneer de beschikbaarheid van NO verlaagd is, dat de pulmonale vaatverwijding tijdens inspannings verdwijnt als gevolg van een verhoogde endotheline invloed doordat de NO deze niet afremt.

De regulatie van het pulmonale vaatbed in gezonde varkens tijdens rust en inspanning is weergegeven in figuur 1.

Regulatie van pulmonale vaattonus in varkens met pulmonale hypertensie na een hartinfarct.

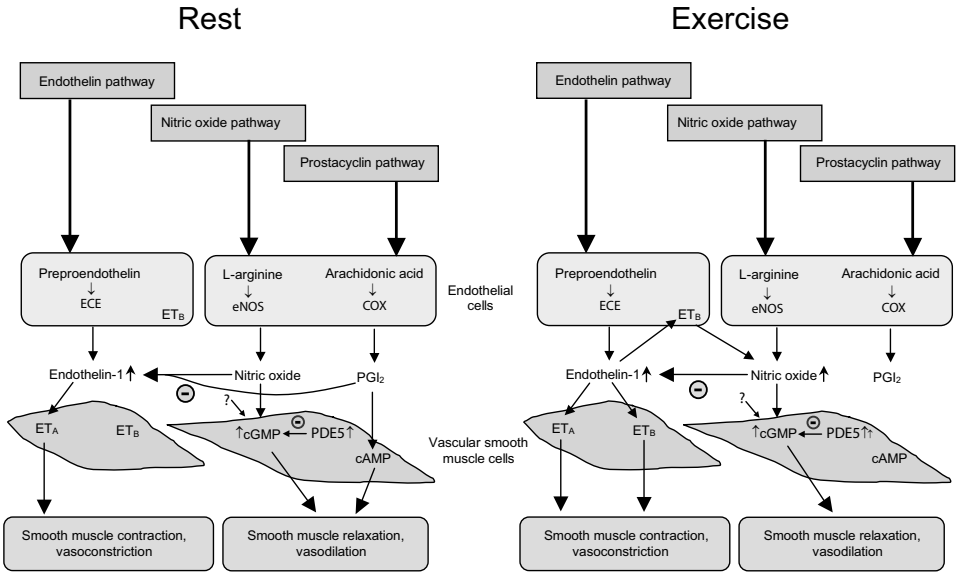
Hartfalen is de enige grote cardiovasculaire aandoening waarvan het aantal patiënten verhoogd is in de afgelopen jaren. Deze verhoging is grotendeels veroorzaakt door een afname in het aantal patiënten dat een hartinfarct overleefd. [10]. Bij deze patiënten is na het hartinfarct echter wel een deel van het hartweefsel afgestorven waardoor het voor het resterende levende weefsel lastiger is om de juiste hoeveelheid bloed rond te pompen. Patiënten krijgen linkerventrikel falen wat leidt tot een hogere druk in de linkerventrikel en een veranderde neurohumorale activatie met name tijdens inspanning [11-13]. De verhoogde linkerventrikeldruk wordt overgedragen naar de pulmonale circulatie waardoor er longoedeem en een hoge bloeddruk in het longvaatbed kan onstaan wat leid tot een verhoogde druk in de rechterventrikel [11, 14]. Deze verhoogde druk in de rechterventrikel is een risicofactor voor het intwikkelen van rechterventrikel falen [15]. Pulmonale hypertensie gaat vaak gepaard met endotheeldysfunctie wat leid tot verslechterde vaatverwijding en een verhoogde vaatvernauwing [16-18], wat het resultaat van activatie van het endotheline systeem zou kunnen zijn. In een patiëntenonderzoek met patiënten met pulmonale hypertensie was de mate van pulmonale hypertensie gecorreleerd aan de hoogte van hun (verhoogde) endotheline-1 plasmaspiegels [19] wat aangeeft dat endotheline een rol speelt bij pulmonale hypertensie.

In *Hoofdstuk 5* hebben we de onderzocht of deze verhoogde vasoconstrictie door endotheline bijdraagt aan secundaire pulmonale hypertensie in de pulmonale circulatie in varkens met een recent MI. Ook hebben we de rol van ET_A en de ET_B receptor subtypes in dit proces onderzocht. Aangezien in gezonde varkens de rol van endotheline toeneemt tijdens inspanning, maar verwaarloosbaar is onder rust condities (*Hoofstuk 3*) hebben we de vasoconstrictor invloed van endogeen endotheline in MI varkens bestudeerd, in rust en tijdens inspanning. De resultaten van dit hoofstuk laten zien dat zowel de circulerende endotheline-levels als de gevoeligheid ten opzichte van exogeen endotheline verhoogd zijn na een MI en

dat deze verhoogde vasoconstrictieve invloed door de ET_A receptor geregeld is. De vaatvernauwende invloed van endotheline die door de ET_B receptor geregeld wordt, is niet veranderd ten opzichte van gezonde varkens. Dus, de ET_A receptor draagt niet bij aan de regulatie van pulmonale vaattonus in gezonde varkens, maar wel bij MI varkens. Het verhoogde vaatverwijdende effect van een gecombineerde ET_A/ET_B receptor blokkade na een MI is onlangs bevestigd in een studie met een muismodel met MI [20]. Een belangrijke gedachte in de behandeling van pulmonale hypertensie is dat ET_A blokkade beter zou zijn dan een gecombineerde ET_A/ET_B blokkade, omdat je dan de ET_B geregelde vaatverwijding en de klaring van endotheline ongemoeid laat [21]. De resultaten van *Hoofdstuk 5* suggereren echter dat patiënten met pulmonale hypertensie na een MI meer gebaat zouden zijn met een gecombineerde ET_A/ET_B therapie. Tot nu toe is er slechts één klinische trial die de effecten van gecombineerde ET_A/ET_B blokkade vergelijkt met ET_A blokkade in patiënten met pulmonale hypertensie [22]. In deze trial is een significante verbetering in de mate van pulmonale hypertensie en in de inspanningscapaciteit na de gecombineerde therapie terwijl slechts de hoogste dosering van de ET_A blokkade significante verbeteringen gaf. [22].

De verhoogde vasoconstrictie in varkens met een MI kan voortkomen uit een verlaagde vaatverwijdende invloed van NO en/of prostanoiden, aangezien beide verantwoordelijk kunnen zijn voor het verlagen van de productie en/of verlagen van de gevoeligheid van de receptoren van endotheline [23-25]. Gebaseerd op de observaties in *Hoofdstuk 4* hebben we de hypothese gevormd dat de verhoogde vaatvernauwende invloed van endotheline het gevolg is van een verlaagde NO- (maar niet prostanoid-) gemedieerde remming van endotheline. Daarom hebben we de veranderingen in de gezamenlijke rol van deze endotheliale stoffen, in de vaattonus regulatie van het pulmonale vaatbed onderzocht in *Hoofstuk 6*. In tegenstelling tot de hypothese vonden we dat de NO gemedieerde vaatverwijding in het pulmonale vaatbed na een MI gehandhaafd blijft en dat de remmende invloed van NO op endotheline ook onveranderd is. Onverwacht bleek er een verhoogde productie van prostanoiden na een MI te zijn, welke bijdraagt aan een lagere weerstand tijdens rust. Alles bij elkaar betekenen deze bevindingen dat de verhoogde vaatvernauwende invloed na een MI niet het gevolg is van een verlaagde remmende werking van NO en/of prostanoiden.

De regulatie van het pulmonale vaatbed tijdens rust en inspanning in varkens met pulmonale hypertensie na een MI is weergegeven in figuur 2.



***Figuur 2**: Regulatie van de pulmonale vaattonus in rust en tijdens inspanning in varkens met pulmonale hypertensie na een myocard infarct. ET_A: Endothelin A receptor; ET_B: ET B receptor; cGMP: cyclic guanosine monophosphate; cAMP: cyclic adenosine monophospate; PDE5: phosphodiesterase 5*

Vaatverwijdende therapie voor pulmonale hypertensie

Vaatverwijdende therapie voor patiënten met pulmonale hypertensie is voornamelijk gericht op het verlagen van de pulmonale druk en de vaatweerstand. Momenteel beschikbare therapieën omvatten onder andere de inhalatie van NO en het toedienen van Ca^{2+} kanaal blokkers, exogene prostacycline en endotheline receptor blokkers. Maar deze therapieën hebben een korte werkingsduur, hebben een beperkt effect, zijn duur en/of hebben ernstige bijwerkingen. Dit geeft al aan dat er ruimte is voor verbetering van deze therapieën of het ontwikkelen van nieuwe therapieën [26]. De afgelopen jaren is het remmen van phosphodiesterase (PDE) 5 als een belangrijke nieuwe vaatverwijdende therapie naar voren gekomen [26-28]. In *Hoofstukken 7 & 8* hebben we het vaatverwijdende profiel van PDE5 remmer EMD 360527 onderzocht in zowel gezonde (*Hoofstuk 7*) als in varkens met een MI (*Hoofstuk 8*).

In *Hoofdstuk 7* hebben we de selectiviteit van EMD 360527 voor het pulmonale versus het systemische vaatbed onderzocht. Verder hebben we onderzocht in welke mate de effectiviteit van PDE5 remmer EMD360527 afhankelijk is van de activiteit van endotheliale NO synthase (eNOS), aangezien NO beschouwd wordt als de belangrijkste bron voor cGMP [30] en pulmonale hypertensie geassocieerd is met endotheel disfunctie en daarmee verlaagde endotheliale eNOS [31]. De resultaten

laten zien dat EMD 360527 een dosis-afhankelijke vaatverwijding in zowel het pulmonale als het systemische vaatbed geeft onder rust omstandigheden, met een iets hogere gevoeligheid voor het pulmonale vaatbed. De vaatverwijdende effecten bleven in het pulmonale vaatbed bestaan tijdens inspanning terwijl deze in het systemische vaatbed afnam. Daarbij namen de vaatverwijdende effecten van zowel EMD 360527 als nitroprusside (NO donor) toe in beide circulaties na het blokkeren van NO (Fig. 1). Verschillende redenen kunnen hiervan de oorzaak zijn, bijvoorbeeld een verhoogde activiteit van een andere bron van cGMP of een verhoogde gevoeligheid van de NO-cGMP pathway [32-34]. De observatie dat PDE5 inhibitie het beste werkt wanneer NO geblokkeerd is en dat het gecorreleerd is aan de basale pulmonale vaatweerstand suggereerd dat PDE5 remming het beste werkt in patiënten met ernstige pulmonale hypertensie.

Bij patiënten met pulmonale hypertensie na een MI was een andere bron van cGMP, atrial natriuretic peptide (ANP) verhoogd [35, 36] en dit was ook het geval in ons varkensmodel [11, 14]. Dit suggereert dat er meer cGMP aanwezig zou zijn en dat het effect van EMD 360527 na een MI verhoogd zou kunnen zijn. De verhoogde ANP spiegels in combinatie met de onveranderde rol van NO (*Hoofstuk 6*) leidde tot onze hypothese dat het effect van EMD 360527 verhoogd zou zijn bij pulmonale hypertensie na een MI. In *Hoofstuk 8* vonden we dat de pulmonale vaatverwijding na blokkade van PDE5 licht verhoogd was in varkens met pulmonale hypertensie na een MI (Fig. 2). Omwille van de hypothese dat de verhoogde vaatverwijding niet het resultaat was van een veranderde invloed van NO, hebben we ook de effecten van EMD 360527 onderzocht na blokkade van NO. De resultaten lieten zien dat in zowel in gezonde als in MI varkens het effect van PDE5 inhibitie gelijk verhoogd was na blokkade van NO en dat de vehoogde vaatverwijding na een MI dus niet het gevolg was van een verandering in de beschikbaarheid van NO.

Toekomst perspectieven

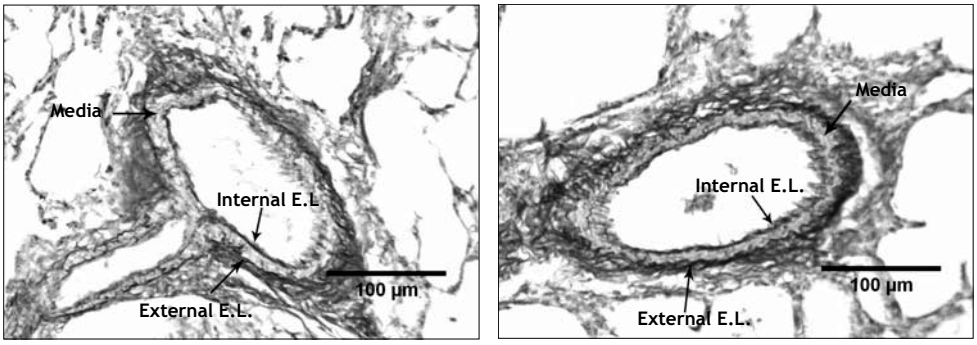
In onze studies zijn de mechanismen van twee belangrijke vaatverwijder therapieën onderzocht, namelijk endotheline receptor blokkade en PDE5 remming. Onze bevindingen laten zien dat zowel de vaatverwijding door PDE5 remming als vaatvernauwing door endogeen endotheline verhoogd zijn in varkens met pulmonale hypertensie na een MI. Aangezien dus een vaatverwijdend en een vaatvernauwend effect verhoogd is, kan het zo zijn dat deze twee effecten elkaar in evenwicht houden en dat een combinatie van beide therapieën een betere vaatverwijding zal geven. Deze hypothese kan getest worden door een endotheline receptor blokker te gebruiken in combinatie met een PDE5 remmer in ons varkensmodel

met pulmonale hypertensie. Op dit moment wordt deze combinatie therapie al bij enkele patiënten studies gebruikt [37-39]. Deze combinatie therapie wordt gestart wanneer therapie met een enkele vaatverwijder niet effectief is. De combinatie therapie lijkt veilig en is gemiddeld geassocieerd met een verbetering in de pulmonale vaatweerstand en de zuurstofopnamen. Ook is de afstand welke binnen 6 minuten door de patient afgelegd kan worden verbeterd [37-39].

Vanuit een klinisch oogpunt zijn langdurige studies om een vaatberwijder te bestuderen meer relevant dan het eenmalig toedienen zoals beschreven in dit proefschrift. Ons diermodel met pulmonale hypertensie na een MI is erg geschikt om de effecten van langdurende toediening van vaatverwijdende therapieën te onderzoeken in de systemische en pulmonale circulatie, in rust en tijdens inspanning. Op deze manier kunnen pulmonale arteriële druk en pulmonale vaatweerstand continu gemeten worden en kunnen de effecten van de therapie gedurende langere tijd bestudeerd worden. Op deze manier kan ook gekeken worden naar effecten van langdurig gebruik van deze therapieën op vaatremodellering in geisoleerde pulmonale vaatjes. Ten slotte kan histologie gebruikt worden om remodellering van kleine pulmonale vaatjes te bestuderen [40]. In figuur 3 zijn histologische afbeeldingen van kleine pulmonale arteriën in ons diermodel weergegeven.

Het diermodel dat wij in onze studies gebruikt hebben is een model voor

A. Controle varken **B. Varken met MI**



Figuur 3: *Kleine pulmonale arteriën in een controle varken (A) en in een varken met pulmonale hypertensie na een MI (B). E.L.: Elastica Lamina. Elastine kleuring (Resorcine/fuchsine).*

pulmonale hypertensie na een MI. Pulmonale hypertensie heeft kan meerdere oorzaken hebben. Jammer genoeg is er weinig informatie over hoe vaak deze vorm van pulmonale hypertensie voorkomt. Pulmonale hypertensie na een MI is meestal mild of matig, maar het kan ernstig zijn in bijna één derde van de patiënten. De speculatie is dat zeer ernstige pulmonale hypertensie aanwezig is in 250.000 hartfalen patiënten in de Verenigde Staten, wat vele malen groter is dan de gerapporteerde epidemiologie van pulmonale hypertensie door andere ziektes [41]. Maar, om de pathofysiologie van pulmonale hypertensie door verschillende oorzaken beter te begrijpen en de respons op therapie goed te onderzoeken zijn er andere modellen van pulmonale hypertensie nodig. Er zijn genetisch gemodificeerde kleine dieren, zoals de muis en rat, welke pulmonale hypertensie ontwikkelen. Er zijn echter grote voordelen aan het gebruiken van grote proefdiermodellen, namelijk dat de (patho)fysiologie meer op die van de mens lijkt en dat er meer parameters gemeten kunnen worden over een langere periode. In ons varkensmodel kan pulmonale hypertensie geïnduceerd worden door micropartikels in de pulmonale circulatie te injecteren die kleine pulmonale emboliën veroorzaken. Daarbij kunnen ook monocrotaline injecties en hypoxie gebruikt worden om pulmonale hypertensie te induceren.

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Curriculum vitae

De auteur van dit proefschrift werd op 30 maart 1979 geboren in Rotterdam. In 1998 behaalde zij haar VWO diploma aan het IJsselcollege te Capelle aan den IJssel. In september 1998 startte ze met haar opleiding 'Hoger Laboratorium Onderzoek' met afstudeerrichting Medische Biologie aan de Hogeschool Rotterdam. In het laatste jaar van deze studie liep ze stage op de afdeling Experimentele Cardiologie van het ErasmusMC waar ze onderzoek deed naar de rol van prostacycline in de vaatbedden van hart, long en lichaam. Eind 2001 studeerde ze af en begin 2002 begon ze als analist op de afdeling Experimentele Cardiologie van het ErasmusMC. Begin 2003 ging ze op diezelfde afdeling het promotietraject in onder leiding van Prof. Dr. D.J. Duncker en Dr. D. Merkus. Het doel van het onderzoek was de pulmonale vaattonus in gezonde toestand en tijdens pulmonale hypertensie na een hartinfarct te onderzoeken. Tijdens deze periode behaalde zij de 'Master of Science in Cardiovascular Research' aan de Cardiovascular Research School 'COEUR'. In augustus 2006 is ze getrouwd met Paul van der Pennen.