REGIONAL MYOCARDIAL PERFUSION AND PERFORMANCE

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. J. SPERNA WEILAND
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 17 DECEMBER 1980 DES NAMIDDAGS
TE 2.00 UUR

DOOR

HENDRIK COENRAAD SCHAMHARDT

GEBOREN TE ZEIST

DRUK WIBRO HELMOND

Promotor : Prof. Dr. P.R. Saxena

Co-referenten : Prof. P.G. Hugenholtz
Prof. Dr. R.S. Reneman

Het verschijnen van dit proefschrift en de uitvoering van de daarin beschreven experimenten werd mede mogelijk gemaakt door de financiële steun van de Nederlandse Hartstichting.

VOORWOORD

Bij het gereed komen van dit proefschrift wil ik iedereen bedanken, die een bijdrage heeft geleverd aan het tot stand komen van dit boekje.

Professor Saxena, beste Pramod, aan jou heb ik te danken, dat ik als fysische eend in de farmacologische bijt heb kunnen rondzwemmen. Voor mij was dit een buitengewoon plezierige en leerzame ervaring, die uiteindelijk heeft geleid tot een aantal publicaties en niet in de laatste plaats tot dit proefschrift. Met plezier denk ik terug aan de vele uren die wij al dan niet gezamenlijk hebben doorgebracht achter diverse computerterminals in een welgemeende poging om die dingen juist datgene te laten doen, wat wij ook wilden. Het opdoen van extra kennis van FORTRAN heeft mij weliswaar een fles whiskey gekost, maar deze kennis is dan ook wel blijvend!

Doctor Verdouw, beste Piet, jou dank ik zeer voor de geboden mogelijkheid om alle in dit proefschrift beschreven experimenten in het lab te kunnen uitvoeren. Daarnaast heb ik veelvuldig en dankbaar gebruik gemaakt van jouw diepgaande kennis van en inzicht in de problematiek van fysiologie, farmacologie and anaesthesiologie en vooral van jouw zeer brede literatuur kennis. Het spijt mij, dat ik je op de baan of op de weg geen partij heb kunnen geven, alhoewel je mij ongetwijfeld, ondanks een zeker anciëniteits verschil, veel te vlug af zou zijn geweest.

Marita Rutteman en Rob van Bremen, jullie "expert technical assistence" vormde een zeer belangrijk aandeel in de practische uitvoering van de experimenten. Marita, de vaardigheid die jij, met de handen gehuld in handschoenen van het formaat vuilniszak, aan de dag weet te leggen in de catheterisatie en de chirurgie, zullen mij ten voorbeeld blijven.. Rob, wij hebben samen de nodige uren gefilosofeerd over de registratie van al dan niet FM-gemoduleerde signalen op een audio-bandrecorder, met als uiteindelijk resultaat, dat zowel op het lab als ook thuis deze registratie perfect verloopt.

Marjan Kroos, helaas niet langer dan ruim een jaar heb jij het merendeel van de rot-klusjes, behorende bij de microsphere techniek opgeknapt en dat met een grote nauwgezetheid en altijd voorzien van een opgewekt humeur. Pas toen jij 16 verdiepingen lager een baan kreeg, heb ik mij ten volle gerealiseerd, hoeveel werk jij mij daarmee uit handen hebt genomen.

Coby de Mik, alhoewel jij nauwelijks bent ingeschakeld bij de tekstverwerking van dit proefschrift, omdat ik dat met alle geweld zelf wilde doen via de computer, is jouw bijdrage in met name het bijhouden van de literatuur voor mij een grote steun geweest.

De groep Automatische Signaal Verwerking onder leiding van Jaap Loeve, met daarin met name te noemen Marco Roede, is de laatste maanden mijn gastheer geweest bij de vele uren van programma

optimalisatie en tekstverwerking. Of het toeval is of niet, wie zal het zeggen, maar ASV kan ook de afkorting zijn van: "Algemene Service Verlening"! Ook zonder deze "bijnaam" was het voor mij een verademing om te werken met een machine die nagenoeg continue draaide, in een afdeling, die de bereidheid had om mee te denken en te helpen.

Tenslotte wil ik mijn ouders bedanken, die mij in de gelegenheid hebben gesteld om een opleiding te volgen, die nu op deze wijze wordt afgerond. Op de laatste plaats, die eigenlijk beter de eerste had kunnen zijn, bedank ik Ank (en de kinderen) voor de steun en het geduld waarmee zij mij gedurende het ontstaan en het afwerken van dit proefwerk heeft opgevangen.

0.1 Survey of this thesis

The function of the heart is to pump blood from the veins into the arteries in response to the need of the tissues for oxygen and substrates. During its action the heart itself needs these nutrients. Factors that mainly determine the myocardial oxygen demand are (fig.0.1): heart rate, myocardial contractile performance (contractility) and ventricular wall tension. The oxygen supply is delivered by the coronary perfusion. Under normal conditions of coronary perfusion an equilibrium between the demand and the supply is present and adaptation is possible during a wide range of circumstances where myocardial oxygen demand is increased.

A limitation of the coronary inflow, due to a coronary stenosis, quickly leads to an oxygen deficit. Even at slight flow reductions a redistribution of blood flow from the endocardium to the epicardium leads to endocardial malfunction. Therefore, the distribution of the blood flow within the myocardium is also a determinant of myocardial oxygen supply (fig.0.1). Acute myocardial ischaemia leads within seconds to an impaired segmental contractile function. When the ischaemic area constitutes a considerable fraction of the left ventricular muscle mass, a decrease in overall contractile function, cardiac output and arterial blood pressure occurs. Furthermore, the cardiac electro-physiological stability is disturbed, which frequently leads to arrhythmias of various severities including ventricular fibrillation.

The pharmacological treatment of ischaemia is directed to restore the myocardial oxygen demand and supply relationship, either by decreasing the demand or by increasing the supply, whereas antiarrhythmic therapy is used to stabilize the haemodynamic condition and to reduce the risk of fatal arrhythmias. Enhancement of the oxygen supply can theoretically be realized by augmentation of perfusion pressure or perfusion time, while another approach is to lower the coronary vascular resistance or to augment the blood flow through interarterial collateral channels. The reduction of myocardial oxygen demand is carried out by reducing heart rate, myocardial contractility or intramyocardial wall tension.

The present thesis, where an attempt is made to gain insight into the transmural distribution of the myocardial blood flow and its relationship with segmental function, has been broadly subdivided into four parts:

(i) Review of literature (chapters 1,2 and 3);
(ii) Radioactive microsphere technique (chapters 4 and 5);
(iii) Effect of some drugs on myocardial blood flow and performance (chapters 6 to 10), and
(iv) Summary and conclusions in English and Dutch (chapters 11 and 12).

The first two chapters present an overview of the normal action of the heart and the effects of coronary flow reduction. Some therapeutic interventions to improve the myocardial oxygen demand and supply relationship during myocardial ischaemia are briefly discussed in chapter 3. The next two chapters deal with the radioactive

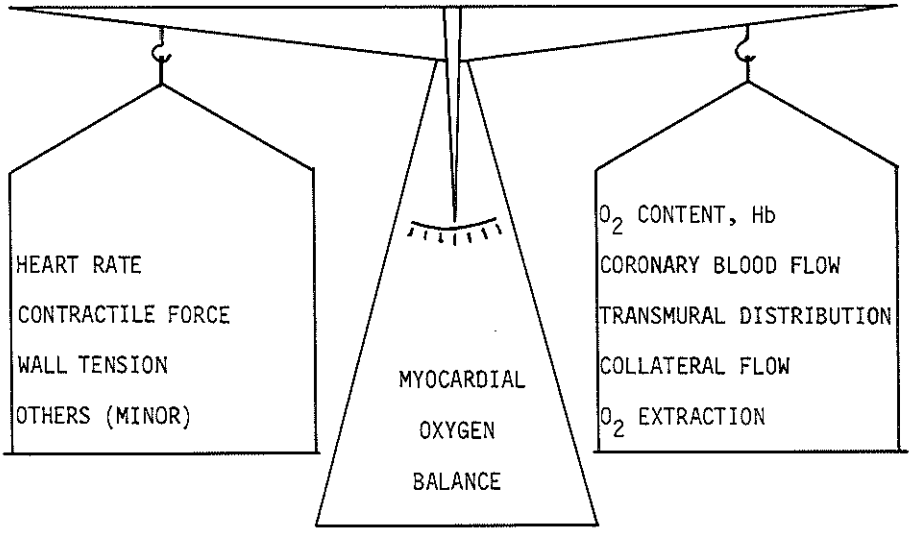

fig.0.1 Determinants of the myocardial oxygen balance. Others: Basal metabolism, activation energy, etc. Hb: haemoglobin content of the blood.

microsphere technique which has been the backbone of the investigations reported in this thesis. Chapter 4 enumerates a review of literature and some general aspects of the above technique whereas our approach to solve the specific problems of data-handling, a necessary evil associated with the radioactive microsphere technique, is described in detail in chapter 5. The last five chapters, 6 to 10, report investigations regarding the effects of (a) oxyfedrine, (b) ergotamine and (c) alinidine, in particular on the regional myocardial blood flow and performance. The drugs selected for investigation have different characteristics (see below) and would, thus, be helpful to gather information about the relation between the regional myocardial circulation and performance under different types of interventions.

The first drug was oxyfedrine, a beta-adrenergic stimulant. Although the use of beta-adrenoceptor agonists in the presence of myocardial ischaemia remains highly controversial, it appears that oxyfedrine may have some potential usage. The increase in myocardial oxygen consumption following its strong positive inotropic and moderate chronotropic actions (Kuehn et al., 1972; Moore and Parratt, 1972; Parratt and Marshall, 1977; Rettig, 1977) seems to be more than sufficiently compensated by an elevation in myocardial perfusion (Ledingham et al., 1972; Parratt and Ledingham, 1972; Raberger et al., 1972; Timogiannakis et al., 1978), since the coronary venous oxygen saturation rises (Moore and Parratt, 1972; Parratt and Ledingham, 1972). Though the total myocardial blood flow invariably increases after oxyfedrine, equivocal results have been obtained as regards the distribution of blood flow to the ischaemic myocardial segment (Szekeres et al., 1972; Parratt and Marshall, 1977; Timogiannakis et al., 1978). For this reason, a study of the effects of oxyfedrine on global haemodynamics and myocardial blood flow, wall function and lactate balance in pigs with a partial stenosis of the left anterior descending coronary artery (LAD) seemed of particular interest.

The second compound selected for study was ergotamine which, even after more than 85 years of use (Thomson, 1894), still remains the most important drug in the treatment of acute attacks of migraine (Berde and Schild, 1978). The therapeutic action of ergotamine may be related to a selective vasoconstriction of the arteriovenous anastomoses (AVAs) in the carotid vascular bed (Saxena and de Vlaam-Schluter, 1974; Johnston and Saxena, 1978; Saxena, 1978; Spierings and Saxena, 1979; 1980). The drug is, however, contraindicated in patients with cardiovascular disease on the premise that it induces coronary vascular spasms and increases arterial blood pressure (Clark et al., 1978). The increase in arterial blood pressure is a property which can augment myocardial tissue perfusion, while the concomitant reduction in heart rate (Johnston and Saxena, 1978; Saxena and Cairo-Rawlins, 1979) reduces oxygen demand and increases diastolic time per minute, that, in turn, may also enhance coronary blood flow. Moreover, the reduction in "wasted" peripheral AVA-flow results in a decrease in the required cardiac output. Our objectives were, therefore, two-fold: (a) since the coronary vascular actions of the

drug, as suggested by its cardiovascular profile, do not a priori seem to be deleterious, the study of the effects of ergotamine on the regional blood flow and performance of normal and ischaemic porcine myocardium was undertaken, and (b) as our preliminary experiments in the pig revealed that a high (about 25%) proportion of cardiac output was shunted through peripheral AVAs, we wanted to confirm the vasoconstrictor activity of the drug on these special vessels.

Alinidine, an N-allyl derivative of clonidine, was the third drug that has been included in this thesis. Unlike clonidine, alinidine does not influence sympathetic neurotransmission but, instead, has a very specific direct salutory action on the sino- auricular node (Kobinger et al., 1979a; 1979b; Lillie et al., 1979; Tritthart and Windisch, 1979). The resultant bradycardia predominates over the negative inotropic effect of alinidine in lower dose ranges (<0.5 $mg.kg^{-1}$). Since this drug provides a novel way to reduce heart rate which, in contrast to beta-adrenoceptor blocking agents, is not accompanied by an elimination of sympathetic neural influences on the heart, we have sought to investigate the effects of this drug on the regional myocardial blood flow and function.

Finally, a summary of the base for the studies, described in this thesis, their results, the conclusions that can be drawn and some perspectives for further investigaton are given. It must be pointed out that chapters 5 to 10 have been, or are to be, published in different Journals. In order to maintain uniformity within this thesis, the format of the papers has been made identical, otherwise these chapters conform to the original text.

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1. MYOCARDIAL FUNCTION DURING NORMAL CORONARY PERFUSION

1.1. Introduction

The normal function of the heart is to pump blood from the veins into the arteries. The resulting arterial blood pressure is determined by the cardiac output and the peripheral vascular resistance. The pressure in the ventricular cavities is generated by the development of tension in the ventricular wall, that is built-up by active muscle contraction. Segmental myocardial function can be determined from changes in the ventricular wall thickness. There exists a close relationship between the regional myocardial performance and the delivery of oxygen and substrates to the tissue (blood flow). A number of control mechanisms are active in the regulation of normal myocardial function. The aspects of overall and segmental myocardial perfusion and function are discussed in the present chapter.

1.2. Haemodynamics

The function of the heart is to eject blood flom the veins into the pulmonary and systemic circulation in response to the needs of the tissues for oxygen and substrates. Like in all muscle contraction processes, the mechanical events are preceded by electrical excitation. One of the differences between heart- and skeletal muscle is that the myocardial cells are able to depolarize spontaneously (Vassalle, 1977). The fastest depolarizing tissue is the sinoauricular node, located in the right atrium near the orifices of the venae cavae. If one site of the heart is depolarized, the remainder follows by cell-to-cell excitation. The depolarization currents cause potential differences which are conducted through the body and can be measured on the skin: the electro-cardiogram (ECG). A redrawn example is shown in fig.1.1.

The depolarization currents that cross the cell membrane are essential for, and closely related to, the generation of force by the heart muscle cells. The coupling between excitation and contraction is very complicated and is incompletely understood (Huxley, 1969; Rich and Langer, 1975; Ebashi, 1976). The general mechanism is that the two protein molecules actin and myosin interact with each other, so that the myosin heads couple with active sites on the actin filaments which, in turn, generate force and produce relative movements of the filaments (Squires, 1975). The presence of calcium-ions plays an essential role in force development (Weber and Murray, 1973; Endo, 1977; Fabiato and Fabiato, 1979); see also 3.3.3).

A few tens of milliseconds before ventricular contraction (systole), the atria push a small additional amount of blood in the nearly maximally filled ventricles. Thereafter, the contraction of

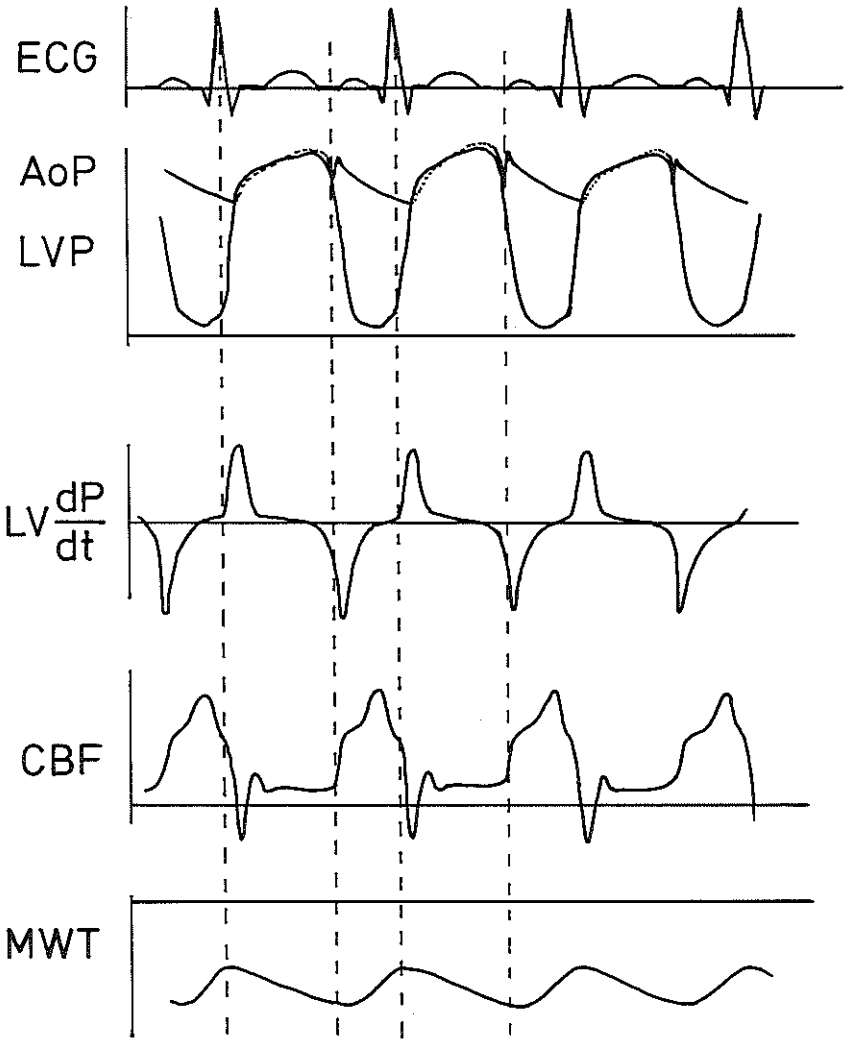

fig.1.1. Events in the cardiac cycle. Note the phase shift between the left ventricular pressure (LVP) and the coronary blood flow (CBF). Abbreviations: ECG, electro cardiogram; AoP, aortic pressure; $LV\frac{dP}{dt}$, rate of change of LVP; MWT, myocardial wall thickness. Vertical lines mark the end-diastole and end-systole, respectively. (Unpublished tracings from this laboratory, redrawn from the original registration)

the ventricles occur while the atrioventricular- and the aortic valves are closed. The rate of pressure development in the left ventricle (LVdP/dt) usually reaches its maximum in the period of isovolumic contraction. LVdP/dtmax can, with a number of restrictions, be used as an index of the contractile performance of the ventricle, usually denoted as myocardial contractility (Mason, 1969; Quinones et al., 1976). As soon as the intraventricular pressure exceeds the aortic pressure, the aortic valves open and blood is pushed into the aorta. The resulting arterial blood pressure is determined by cardiac output (the product of heart rate and stroke volume) and systemic vascular resistance. After closure of the aortic valves, the intraventricular pressure decreases below the atrial pressure at a constant volume. This period is called the isovolumic relaxation phase. The maximum rate of pressure decline (LVdP/dtmin), which is relatively sensitive to myocardial underperfusion (Watanabe et al., 1975), usually falls within this period. In diastole, the ventricle is filled by passive venal inflow and the ventricular blood pressure gradually increases upto its end-diastolic value.

A fraction of the cardiac output is not used for tissue perfusion, but is shunted through arteriovenous anastomoses (AVAs; Daniel and Prichard, 1956; Delaney, 1969; chapter 8). Total body AVA-flow can be measured with the radioactive microsphere technique (see chapters 4 and 5). The nonentrapped microspheres with a diameter larger than 10 micron finally lodge in the lungs (Kaihara et al., 1968; Neutze et al., 1968; Bartrum et al., 1974; Warren and Ledingham, 1974). The microsphere content of the lungs represents both the bronchial artery flow and the total systemic AVA-flow. The former, however, is usually less than 1% of cardiac output (Forsyth et al., 1968) and is, thus, negligible compared with the total AVA-flow, that was reported to be 10 to 50% of cardiac output in young pigs under azaperone and metomidate anaesthesia (Schamhardt et al., 1979; Verdouw et al., 1980b). The anaesthetic agent used had only minor effect on these data, since both halothane and pentobarbital, in combination with N_2O, gave similar total systemic AVA-flow values (unpublished data). Hales (1973; 1974) postulates that peripheral AVAs in the skin of sheep play an important role in the regulation of body temperature, especially in hot environments. The cardiac output can thus be divided in two fractions: total peripheral AVA-flow (non-nutritional) and the remaining nutritional capillary flow, denoted as nutritional cardiac output.

1.3. Regional left ventricular function

1.3.1. Introduction

The most informative way to determine the local myocardial function is to measure the generation of force of an individual muscle fibre in the ventricular wall separate from influences of surrounding tissue. It is obvious that this cannot be realized in the intact heart. Several measuring techniques and devices have been developed which have all their own (dis-) advantages. Using a strain gauge arch

(Boniface et al., 1953) it is possible to measure the force generation within a segment of the ventricular wall. By changing the depth of insertion of the sutures applied to fix the strain gauge arch onto the epicardium, the measurement may reflect the force development in the epicardium or in the endocardium. Since contracting muscle shortens, the shortening of a segment must contain comparable information (Tennant and Wiggers, 1935). Changes in segment length are determined from cineangiography, sometimes after marking the endocardial surface with metallic clips (Mitchell et al., 1969; Heikkila et al., 1972) or after the injection of contrast material (Bove, 1971). It can also be determined from the transit time of ultrasound between two implanted crystals (Theroux et al., 1976). Finally, the expulsion of blood during systole causes a decrease in intracavitary volume and, in turn, an increase in the myocardial wall thickness. The latter can also be determined from cineangiography (Hugenholtz et al., 1969; Mitchell et al., 1969; Bove, 1971; Gould et al., 1976) and ultrasound transit time (Guntheroth, 1974; Heyndrickx et al., 1978), but also directly from a mechanical device plunged through the ventricular wall (Feigl and Fry, 1964; van der Meer et al., 1973; Goldstein and de Jong, 1974), or from the endocardial reflection of ultrasound emitted by an epicardially located crystal (Gaasch and Bernhard, 1977; Kerber et al., 1979; ten Cate et al., 1979; Verdouw et al., 1980a). Since we are using the latter method routinely (see chapters 7 to 10) attention will be paid only to the measurement of wall thickness changes by this means.

1.3.2. Techniques for measuring myocardial wall thickness

1.3.2.1. Mechanical devices

After plunging the harpoon-like tip of the device through the ventricular wall and pushing the other gently on the epicardial surface, the excursions of the epicardium relative to the endocardium can be recorded (Feigl and Fry, 1964; van der Meer et al., 1973; Goldstein and de Jong, 1974). Amongst the disadvantages must be mentioned the trauma of placement, the mechanical load and torsion on the myocardial wall and its limited frequency response (van der Meer et al., 1973). The obtained values for the systolic myocardial wall thickening (swt), computed as the increase in wall thickness during systole divided by its end-diastolic value (EDT), are in the range from 10 to 25%. It must be noted that a significant fraction of swt occurs already during the isovolumic contraction phase. A possible explanation for this observation is the mechanically induced decrease in EDT, which vanishes during systole, giving rise to an apparent increase in EDT. However, concomitantly large values of swt must then be expected and they do not occur.

1.3.2.2. Cineangiography

The contour of the endocardial surface can be made visible after the intracavitary injection of X-ray contrast material, after which the local myocardial wall thickness is determined. A computer algorithm is frequently used to compute the wall thickness from biplane angiograms (Hugenholtz et al., 1969; Bove, 1971; Dumesnil et al., 1974; Gould et al., 1976). The placement of endocardial metallic markers facilitates the recognition of the proper surface (Mitchell et al., 1969; Heikkila et al., 1972). An advantage of cineangiography is the avoidance of acute surgery and the minimal trauma of marker placement. A clear disadvantage is the necessity to reconstruct the wall thickness from biplane films with obvious difficulties in the identification of the endocardial surface. Furthermore, contrast material in the vicinity of papillary muscles may or may not reach the endocardium, giving rise to errors in the detection of the contours. Folding of the endocardium during ejection can also disturb the measurements. Finally, a rather great amount of data must be analyzed, making the use of computer facilities obligatory. In summary, that method must be used with great care to avoid unacceptable measuring errors. The values of swt obtained using cineangiography differed comparing standard angiography and endocardial marker techniques: swt ranged from 80 to 120% and from 25 to 45% in the same animals, respectively (Mitchell et al., 1969). This discrepancy was explained as resulting from an overestimation of the end-systolic wall thickness by the angiographic method due to the thickening of and closure between trabeculae carnae. Hugenholtz et al. (1969) found values from 15 to 90%, averaging 40%, data in the same range as reported by others (Bove, 1971; Heikkila et al., 1972; Gould et al., 1976). The conflicting results on the value of swt has been dicussed in detail by Mitchell et al. (1969), van der Meer et al. (1973) and Gould et al. (1976). Bove (1971) was the only author who described an increase in myocardial wall thickness during the isovolumic contraction phase.

1.3.2.3. Utrasound transit time

The time necessary for ultrasound to travel from a transmitter to a receiver crystal is an accurate measure for the distance between the two crystals. Being in size about 3 mm, they can be placed in the ventricular wall via a stab wound. The induced trauma to the heart is minimal so that this technique can be used in acute as well as in chronic experiments. It is essential that the crystal pair remains faced during the measuring period since otherwise the distance between the crystals seems to increase, giving erroneous data. Representative values for swt were 9% (Guntheroth, 1974), 32% (Sasayama et al., 1976) and 15% (Heyndrickx et al., 1978). No changes in wall thickness during the isovolumic contraction phase were reported (Guntheroth, 1974; Sasayama et al., 1976).

1.3.2.4. Endocardial ultrasound reflection

Miniature ultrasound crystals which can be sutured directly on the epicardium make it possible to register the transmural wall thickness changes continuously with a minimum of trauma to the heart (Gaasch and Bernhard, 1977; ten Cate et al., 1979; Kerber et al., 1979; Verdouw et al., 1980a). The ultrasound echo from an endocardial surface which is not perpendicular to the epicardium (e.g. a papillary muscle) may give rise to badly defined or meaningless signals and, therefore, to unreliable wall thickness data. Furthermore, the "infolding" of the endocardium during ejection may cause the vanishing of the endocardial echo during fractions of the cardiac cycle. A great advantage of this technique is that the endocardial surface remains free from any kind of surgical intervention and that the wall thickness can be registered continuously with a very high frequency response. The relatively high values for swt from 30 to 50% found in our studies (ten Cate et al., 1979; Verdouw et al., 1980a; chapters 7 to 10) may in part be explained by the high cardiac output which, at normal heart rate and end-diastolic volume, leads to a high ejection fraction and, therefore, to large swt values. Moreover, the placement of the wall thickness probe in the lower half of the left ventricular wall may also play a role, since swt values were higher in this region than near the basis (unpublished data), an observation which is consistent with clinical studies.

1.4. Myocardial oxygen balance

The energy for muscle contraction is delivered by the breakdown of adenosine triphosphate (ATP; Katz, 1977). In heart muscle, ATP is synthetized either by glycolysis or by oxydative phosphorylation (Katz, 1977). The latter represents the major energy-producing reaction, but the glycolytic pathways are essential for the aerobic breakdown of carbohydrates to carbondioxide and water. The balance between the uptake of glucose, pyruvate, lactate and free fatty acids is a function of tissue oxygenation and the concentration of these substrates (Hirsche and Lochner, 1961; Bing, 1965; chapter 6). In open chested pigs under azaperone and metomidate anaesthesia, ventilated with $N_2O:O_2=2:1$, having a normal coronary perfusion, the heart extracts lactate and inosine from the coronary blood (de Jong et al., 1977; Verdouw et al., 1979) and does not use glucose or free fatty acids. This unusual finding may be due to the kind of anaesthesia, that causes high arterial lactate levels (Verdouw et al., 1977).

Cardiac muscle exhibits a high oxygen extraction from the coronary blood supply: during normal perfusion, the coronary venous oxygen saturation is as low as 0.3 to 0.4 (Verdouw et al., 1978; Verdouw et al., 1979), which corresponds with an oxygen extraction of 0.6 to 0.7 .

1.5. Myocardial blood supply (Berne and Rubio, 1979)

1.5.1. Techniques for measuring blood flow

Many techniques have been designed to measure blood flow to and within organs. We focus on two methods: a) the electromagnetic flowmeter; b) the microsphere technique. The electromagnetic flowmeter is based on the potential difference, which is generated by moving charges (ions in the blood) in a magnetic field (fig.1.2). The electromagnet and the electrodes for detection of the potential differences are included in the flow probe, that is slipped around the exposed artery. The amplitude of the measured signal is in proportion with the the mean velocity of the blood and the strength of the magnetic field, and is inversely related to the radius of the vessel. The magnetic field can easily be controlled by the current through the electromagnet. The surface area of the artery, which can be determined from the size of the lumen within the flow probe, multiplied by the velocity of the blood, yields the flow.

The advantages of the electromagnetic flowmeter are its purely linear calibration curve, passing through zero flow, the equal sensitivity for forward and backward flows, and its nearly unlimited frequency response. Therefore, the electromagnetic flowmeter permits the recording of the actual phasic blood flow pattern. Finally, it can be used on unopened vessels. Disadvantages are the necessity to expose the vessel, to occlude it frequently to correct for zero flow drift, to calibrate the probe after any measurement, and the lack of information about the regional distribution of the flow within the organ of interest.

The micropshere technique, which is discussed in detail in chapters 4 and 5, makes use of small spherical particles, labelled with gamma radiation emitting radionuclides. The spheres, injected into the arterial blood, distribute in proportion with local blood flow. The size of the micropheres is chosen so that they lodge in first pass in the capillaries or small arterioles of the tissue. The detected radioactivity originating from a certain piece of tissue corresponds with the number of entrapped spheres in that tissue and, thus, with local blood flow.

1.5.2. Blood supply to the ventricles

The perfusion of the ventricles occurs via the right- and the left coronary arteries. In the pig, the anterior descending branch of the left coronary artery delivers the blood to parts of the anterior free wall and the apical region of the left ventricle, and to parts of the intraventricular septum, together about 40% of the left ventricle (unpublished data). The circumflex branch perfuses merely the basal area of the anterior free wall, while the right coronary artery is responsible for the supply of blood to the remainder of the intraventricular septum and to the posterior free wall.

The separation between the coronary arterial branches is not absolute, because anastomoses between these vessels with diameters ranging from 35 to 500 micrometer have been described (Eckstein, 1954;

Baroldi and Scomazzoni, 1967; Schaper, 1971; Downey et al., 1975). These collaterals probably play a role in the survival of myocardium distal to a severely obstructed coronary artery (Becker and Pitt, 1971). The number of collateral vessels and their distribution within the myocardium is largely species-dependent. In the dog heart, numerous collaterals are located preferentially in the epicardium (Schaper, 1971), whereas they are found in the full thickness of the ventricular wall in the human heart (Downey et al., 1975). The collateral circulation in the domestic pig is still less developed than in man, and its function under normal circumstances is of minor importance (Schaper, 1971; Fedor et al., 1978).

1.5.3. Phasic coronary blood flow

The heart supplies the force necessary for its own blood supply. During systole, the pressure in the endocardium exceeds the left ventricular pressure (Kirk and Honig, 1964; Stein et al., 1980). This explains the low coronary flow during systole, which contributes for no more than 10 to 30% of total myocardial perfusion (Fedor et al., 1978; own unpublished observation; fig.2.2). The remaining 70 to 90% of coronary blood supply occurs during diastole, despite the lower perfusion pressure in this period (fig.1.1; Kirk et al., 1972). The systolic flow is influenced by the contractile performance of the heart (Archie, 1975): a depressed contractility reduces the intramyocardial tissue pressure and thus opposes the compression of the coronary vessels to increase the systolic flow.

The functioning of coronary collaterals cannot be derived from flowmeter tracings, since the overlap of the perfusion areas of two adjacent coronary arteries is unknown. Total occlusion of one vessel may increase the flow through the other, but it cannot be excluded that this phenomenon is caused by autoregulation of blood flow to the heavier loaded part of the heart, supplied by the non-occluded coronary artery (Shaw et al., 1962; Rubio and Berne, 1975).

1.5.4. Regional myocardial blood flow

The myocardial tissue pressure increases from epicardium to endocardium (Driscol et al., 1964; Kirk and Honig, 1964; Stein et al., 1980). Consequently, the oxygen supply of the endocardial layer is more impeded than in the epicardium (Rubio and Berne, 1975; Weber and Janicki, 1979). Under normal circumstances this is balanced by local perfusion, since the ratio between the blood flow per unit ventricular mass to the endocardium divided by that to the epicardium (endo/epi-ratio) is near to unity (Becker et al., 1971; Utley et al., 1974; Downey et al., 1975; Sanders et al., 1977; Schamhardt et al., 1979, 1980; Verdouw et al., 1980b). In contrast with the electromagnetic flowmeter, the microsphere technique (Domenech et al., 1969; Hales, 1974; Heymann et al., 1977; Schamhardt et al., 1979) permits the determination of intra-organ blood flow distribution and can thus be more helpful in the detection of local blood flow deficit (ischaemia; chapters 7,8 and 10).

The microsphere technique can be used with limited precision to

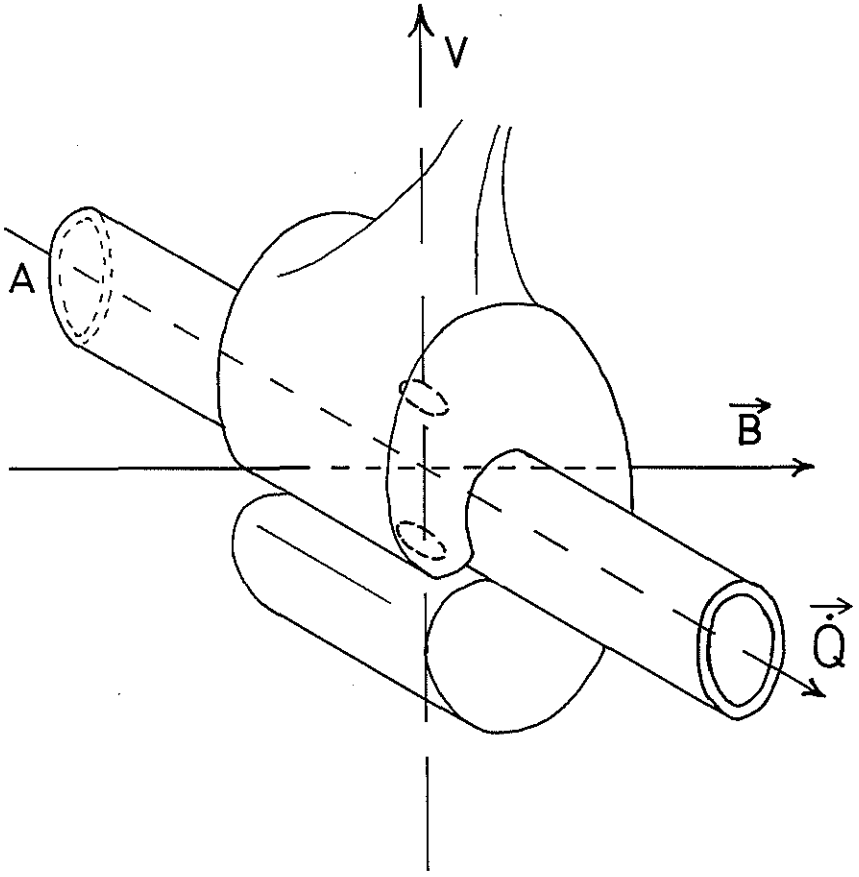

fig.1.2. Schematic diagram of the electromagnetic flow meter. The magnetic field $\vec{B}$ is generated by an electromagnet in the flow probe. The moving charges in the blood cause a potential difference V, that is proportional with $\vec{B}$ and the velocity ($\vec{v}$) of the charges. Multiplication of the surface area of the vessel A and the velocity $\vec{v}$ yields the flow $\vec{Q}$.

determine collateral blood flow (Flameng et al., 1975). The practical problems originate from the need to dissect the organ of interest in pieces in which the microsphere radioactivity can be detected in a gamma-scintillation counter. To quantify the collateral flow from one coronary arterial bed into another, the perfusion areas of both arteries must be separated carefully, which is, to say the least, extremely difficult.

1.6. Determinants of coronary blood flow

The myocardial tissue flow is determined by perfusion pressure (arterial minus central venous blood pressure) and local vascular resistance (Archie, 1975), while the blood viscosity acts as an additional resistance term that only plays a role during haemodilution or increased haematocrit. During unrestricted coronary inflow the local tissue perfusion is directly related to tissue oxygen demand, since the coronary blood flow is effectively autoregulated (Shaw et al., 1962; Moir and DeBra, 1967), and an increase in perfusion pressure is not followed by the same increment in coronary blood flow (Rubio and Berne, 1975; Weber and Janicki, 1979). The myocardial vascular resistance is not only regulated by tissue oxygen demand and supply (Berne et al., 1957), but is also affected by the myocardial tissue pressure during ventricular systole (Gregg, 1963). This extravascular restrictive force causes a sharp backflow spike in the phasic coronary blood flow pattern at the onset of systole (fig.1.1). Tachycardia and enhancement of ventricular contractile force change the throttling effect of tissue pressure (Snyder et al., 1975). Because the larger proportion of coronary perfusion occurs in diastole, the latter factor is of minor importance during normal myocardial perfusion. Moreover, ventricular pacing of the dog heart increases coronary flow more than oxygen demand (Weiss, 1979). The myocardial tissue pressure has been considered in the autoregulation of coronary blood flow (Driscol et al., 1964). An elevation of perfusion pressure results in an enhancement of the capillary pressure and, thus, in the intramyocardial tissue pressure, thereby increasing the capillary resistance to blood flow. However, when perfusion pressure is elevated after previous vasodilation induced by dipyridamole, no reduction in flow can be observed, although the tissue pressure increases to the same extent. Therefore, this mechanism of autoregulation is normally not active.

The regional distribution of coronary flow over the myocardium is only slightly affected by changes in the arterial blood pressure (Buckberg et al., 1972; Domenech, 1978). Increasing heart rate by atrial pacing causes slight or no effects on regional blood flow distribution from endocardium to epicardium in the dog and in the pig heart (Becker et al., 1976; Domenech and Goich, 1976; Bache and Cobb, 1977; Verdouw et al., 1980a). The endocardial vasodilatory reserve is well illustrated in exercising dogs (Ball and Bache, 1976; Barnard et al., 1977) and domestic pigs (Sanders et al., 1977), since the endo/epi-ratio of myocardial perfusion does not fall below its resting value of about 1.0 .

The close parallelism between cardiac activity and coronary blood flow is well established, but the identity of the factor that links coronary blood flow to the metabolic requirements of the heart is still unsettled, though adenosine is regarded as a probable candidate. Regardless of the means by which it has been altered, the myocardial oxygen consumption is felt to be the primary determinant of coronary blood flow.

1.7. Cardiac reflex pathways

The heart is innervated by both the sympathetic- and parasympathetic nervous system. The neurotransmittor-receptors of the sympathetic nervous system can be divided in alpha, $beta_1$ and $beta_2$ receptors. The dominant functional receptors in the heart are of the $beta_1$-type (Berne and Rubio, 1979), so a selective stimulation of the alpha-adrenergic system hardly affects the cardiac cells. This intervention mainly causes peripheral vasoconstriction by stimulation of the smooth musculature of the small arteries and arterioles. A simultaneous stimulation of the two types of beta-receptors (3.2.3) causes an augmentation of heart rate and myocardial contractility and peripheral vasodilation, while the resultant decrease in blood pressure is nearly balanced by an increase in cardiac output. The drugs used in a(nta)gonism of the sympathetic neurotransmitter receptors possess non-selective activities on all receptor types. Consequently, the observations after administration of these drugs are difficult to interpret, since they depend on the dose and the route of administration.

The neural control of the heart can explain several cardiac reflexes. For example, an augmented contractility elevates cardiac output, which, in turn, increases blood pressure. Via the baroreceptors this disturbance is transferred to the central nervous system. The resultant stimulation of the parasympathetic nerves reduces myocardial contractility and opposes the original disturbance. Under certain pathological conditions the sympathetic tone is relatively high, as reflected by a high heart rate and sometimes hypertension. Because of the comcomitant elevated myocardial oxygen demand this can be deleterious, especially in the presence of (partial) myocardial ischaemia. A blockade of the beta receptors in the heart is beneficial in these circumstances (see 3.3.1), since it may lead to a diminution of heart rate and contractility, thereby reducing myocardial oxygen demand.

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2. MYOCARDIAL FUNCTION DURING CORONARY FLOW REDUCTION

2.1. Introduction

A relatively slight reduction in coronary blood flow, caused by acute intervention or by chronic reduction of the lumen of a coronary artery, already rapidly leads to a disturbance in the integrity of electrophysiological, biochemical and mechanical function. This complex of events is called myocardial ischaemia. The lack of oxygen and substrates and the diminished removal of metabolites, poison the cells and cause a disturbance in the normal distribution of the intracellular ion concentrations. The heart attempts to shift its normal aerobic metabolism to anaerobic pathways, although the presence of the enzymes, needed for this step, is limited. The impaired metabolism is immediately followed by a decrease in local segmental wall function, reflected by a loss of systolic wall thickening. The global haemodynamics usually demonstrate a decrease in contractility, cardiac output and arterial blood pressure. A reduction of coronary blood flow also causes a redistribution of left ventricular blood flow so that the endocardium is relatively more deprived of blood than the epicardium. Reflex mechanisms try, and usually succeed, however, to minimize the effects of coronary flow reduction on peripheral tissue perfusion.

2.2. Electrophysiological changes and arrhythmias

The distribution of the ions over the cell membrane is a delicate equilibrium between passive diffusion and active transport. The latter is disturbed during coronary flow reduction. Therefore, the normal membrane potential can no longer be maintained. Moreover, the transport processes are dependent on the membrane potential itself, so a shift from the normal value augments the disruption of the membrane currents. Under certain conditions the disturbance of the ionic transport equilibrium results in spontaneous depolarization. The refractory state of the adjacent cells determine whether this extra focus leads to a premature excitation and ventricular contraction or not. The presence of an extra focus, myocardial ischaemia, haemodynamic depression and central nervous actions can even cause ventricular fibrillation (see 3.1; Hillis and Braunwald, 1977; Verdouw et al., 1978).

The ECG also reflects changes in myocardial (under-) perfusion. The depolarization of the ventricles takes more time and the amplitude of the R-wave decreases (Capone and Most, 1978), while the repolarization phase (ST-segment) shows, depending on the ECG lead chosen, an elevated or depressed voltage. The ST-segment voltages are used in the clinical setting to quantitate infarct size (Maroko et al., 1972), although the knowledge concerning the mechanism of ST-segment displacement is incomplete and poor correlations have been reported between ST-segment changes and infarct size or the severity of myocardial ischaemia (Vincent et al., 1977).

The relation between the degree of coronary flow reduction and the corresponding changes in the electrophysiological parameters is not

unique (Irvin and Cobb, 1977) and depends on the species under investigation: in dogs with their elaborate collateral circulation (Schaper, 1971) larger flow reductions, or even total occlusion of coronary arterial branches, can be withstood without serious lethal risks. In the pig, however, a reduction in the coronary flow below 40% of its base-line value introduces serious arrhythmias and a further reduction to 25% of base line causes ventricular fibrillation in about 30% of the animals within the first 30 min of ischaemia (Verdouw et al., 1978).

2.3. Haemodynamic changes

Acute ischaemia is associated with changes in the intracellular oxygen balance and pH. This results in abnormalities in the calcium transport over the cell membrane and within the cells (Katz, 1973), and causes an immediate loss of contractile function. The loss in contractility of the left ventricle, as reflected by a decrease in LVdP/dtmax, hampers the ejection of blood. Consequently, stroke volume and cardiac output decrease. Due to the diminished stroke volume both the left ventricular end-diastolic volume and pressure increase. This results in a further hampering of myocardial performance, since the increased end-diastolic volume elevates the required wall tension during ejection of blood (Laplace relationship). When the heart is no longer able to withstand this loading condition, one speaks of a "failing heart".

The reduction of cardiac output causes a decrease in mean arterial blood pressure. Depending on the degree and the duration of flow reduction a reflex tachycardia sometimes occurs in the in-situ preparation (Capone and Most, 1978; Verdouw et al., 1978; ten Cate et al., 1979; Kerber et al., 1979; Verdouw et al., 1979). The tachycardia is possibly a consequence of a central nervous reflex pathway (Peterson et al., 1973), but can also be mediated by the distension of the atrium, secondary to the reduced stroke volume (Zucker and Gilmore, 1974).

2.4. Myocardial wall thickness changes

The changes in myocardial segment length and regional myocardial wall thickness deliver qualitatively comparable information concerning the local myocardial function (Sasayama et al., 1976). Within seconds after reduction of the coronary blood flow below about 60% of base line, pronounced changes in the pattern of segmental myocardial wall shortening (Sasayama et al., 1976; Theroux et al., 1977) and wall thickening occur (ten Cate et al., 1979; Schamhardt et al., 1979; Verdouw et al., 1980). The ischaemic tissue no longer contributes to contraction, while frequently paradoxical systolic bulging takes place (Tennant and Wiggers, 1935; Althaus et al., 1977). The pattern of wall thickening further reveals a reduction in both EST and EDT and a loss of systolic wall thickening (fig.2.1). During systole, EST and EDT approach each other at a level which is even thinner or elongated compared with the base-line values (Sasayama et al., 1976; Verdouw et al., 1980). In diastole, EDT returns to its normal value and, being

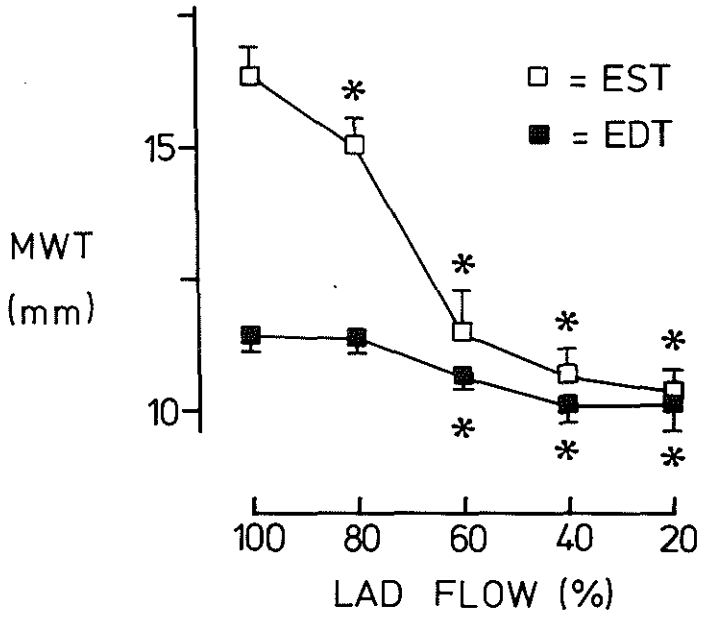

fig.2.1. The effect of coronary flow reduction on the end-systolic (EST) and end-diastolic (EDT) myocardial wall thickness (MWT). Data are presented as mean ± SEM (n=14). *, $p<0.05$ vs. base-line values (Student's paired t-test). Reproduced from: Verdouw et al., 1980.

thicker than in systole, it leads to diastolic "thickening". Whether this is a partially active contraction or a passive "recoil" remains obscure (see 11.5). The maximum wall thickness (maxT) is reached about 150 ms after closure of the aortic valves, in contrast to normally perfused myocardium, where maxT occurs within a few tens of milliseconds around closure of the valves (Verdouw et al., 1980).

The relation between coronary flow reduction and the pattern of myocardial wall thickening has been studied extensively. Depending on the species and the corresponding degree of myocardial collateralization, a nearly complete loss of systolic contractile function has been reported after flow reductions below 50% of its base-line value in dogs (Kerber et al., 1975; Wyatt et al., 1975; Waters et al., 1977) and below 60% of base line in pigs (Verdouw et al., 1980). Smaller reductions cause proportionally less extensive changes in the wall thickness pattern, while reductions below these levels result in no deterioration of function.

2.5. Myocardial oxygen balance

Reduction of coronary blood flow decreases the oxygen supply to the myocardial tissue. The heart attempts to meet its oxygen demand by augmentation of oxygen extraction, but this mechanism can hardly improve tissue oxygenation because of the already high oxygen extraction during normal coronary perfusion (1.4). During severe ischaemia the coronary venous oxygen saturation decreases to a fraction as low as 0.09 (unpublished data).

After initiation of ischaemia the concentration of lactic acid within the ischaemic cells rapidly increases (Liedtke et al., 1975, 1976), and lactate is released in the coronary venous blood (Opie, 1975; Brachfeld, 1976; de Jong et al., 1977; Verdouw et al., 1978; Schamhardt et al., 1980b). The intracellular lactate accumulation causes a sharp drop in intracellular pH. The low pH disturbs the calcium trigger mechanism for contraction and is, therefore, indirectly responsible for the decrease in contractility during ischaemia (Fabiato and Fabiato, 1979). The lack of oxygen impairs the oxydation of fatty acids, leads to the accumulation of intermediate products which augment tissue oxygen needs, intensifies ischaemia, depresses contractility, and probably precipitates arrhythmias (Hillis and Braunwald, 1977). In pigs under azaperone and metomidate anaesthesia, glucose becomes the principal source of energy for the ischaemic heart muscle cells (de Jong et al., 1977).

2.6. Myocardial perfusion

2.6.1. Total and phasic coronary blood flow

Reduction of the proximal lumen of a main coronary artery in the experimental animal is not proportionally followed by a reduction in flow. During progressive constriction of the LAD coronary artery in the pig (unpublished data) the first observation is an increase in the systolic fraction, together with a decrease in the diastolic fraction

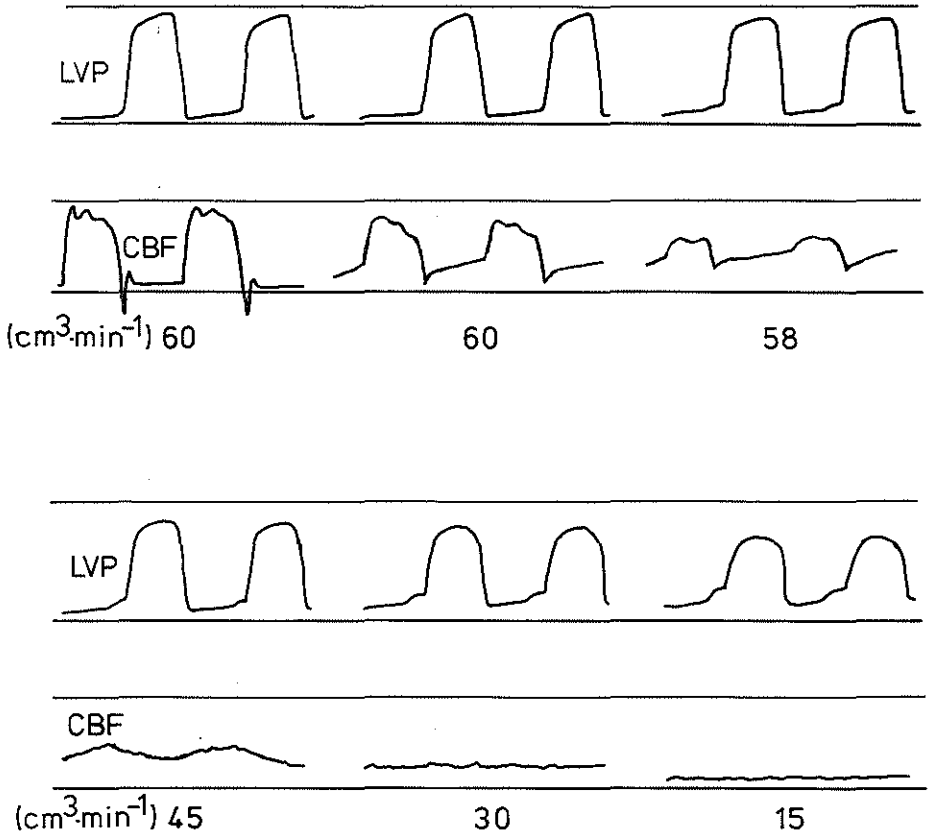

fig.2.2. Effect of coronary artery constriction on pulse profile and absolute value of coronary blood flow. Abbreviations: LVP, left ventricular pressure; CBF, coronary blood flow. Redrawn from original tracings, which have been slightly stylized for clarity.

of coronary blood flow, without significant changes in the averaged value, as illustrated in fig.2.2. A further reduction causes a continuation of the decrease in pulse profile and ultimately a nearly "direct-current" (DC) profile. Finally, the DC-flow amplitude decreases gradually to zero at complete occlusion. The relation between stenosis diameter and flow reduction is not linear: a stenosis diameter of 50% has no effect on the flow, while a stenosis of 5mm length that obstructs 92% of the vessel lumen results in a 50% flow reduction (Gould et al., 1975). A striking difference exists between these data and those from coronary arteriograms in man, where stenoses of more than 70% are already assumed to be "severe". The length and the shape of the stenosis -circular or partially filling the lumen of the coronary artery- affects the ability for dilation of the stenosis, the chance of induction of turbulence and, therefore, for its flow limiting capacity.

2.6.2. Regional myocardial blood flow

During unrestricted coronary inflow, the perfusion of the endocardium predominantly occurs in diastole, while the epicardium receives blood during the entire cardiac cycle (Archie, 1975a; 1975b; Hess and Bache, 1976). Furthermore, the endocardial impedence to blood flow is more pronounced than in the epicardium. During coronary flow reduction the diastolic fraction of coronary flow decreases. Therefore, a relatively slight reduction in coronary blood flow rapidly leads to underperfusion of the endocardium, while the total flow is hardly affected (Domenech et al., 1969; Becker et al., 1971; Bishop et al., 1976; Rivas et al., 1976; Fedor et al., 1978; Schamhardt et al., 1979, 1980a). This is especially true in the presence of an increased myocardial load, due to exercise, in dogs with a restricted coronary inflow (Ball and Bache, 1976). Coronary vasodilation following a period of myocardial ischaemia or the administration of vasodilating drugs does not result in an impaired endocardial perfusion, even at maximal vasodilation, since the endo/epi ratio during reactive hyperaemia after a 90 s total occlusion of a coronary artery or after the administration of adenosine or papaverine remain unaltered (Downey et al., 1975). The flow to the non-ischaemic left ventricle usually slightly increases. This probably reflects autoregulation since the healthy myocardium is subjected to augmented load due to non-participation of the affected segment in the expulsion of blood.

2.6.3. Collateral formation and perfusion

The influence of partial or total occlusion of a coronary artery on tissue perfusion depends largely on the species under investigation. In dogs, collateral flow restores upto 80% of normal flow within 24 to 96 hours of complete occlusion (Cox et al., 1975; Bishop et al., 1976), although the subendocardium remains slightly underperfused. Collateral formation occurs already within the first hour after onset of ischaemia (Marcus et al., 1976), but does not amount to more than 30% of the normal perfusion of the central ischaemic area. Complete

collateralization takes about 4 to 8 weeks (Flameng, et al., 1979). Collateral flow is then able to maintain normal myocardial perfusion (Becker and Pitt, 1971), but the functional capacity remains inadequate under stress conditions (Flameng et al., 1979).

Acute occlusion of a coronary artery in the domestic porcine heart leads to a nearly uniform decrease in perfusion of the ischaemic area to less than 1% of base line (Fedor et al., 1978). This finding confirms the absence of functional collaterals. It can, therefore, be stated that the contribution of collateral blood flow to the perfusion of myocardium distal to an acutely ligated or partially constricted coronary artery in the heart of domestic, Yorkshire pigs is negligible within the first hours after induction of ischaemia.

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3. INFLUENCES ON THE PERFORMANCE OF ISCHAEMIC MYOCARDIUM

3.1. Introduction

A number of the determinants of myocardial oxygen demand is shown in fig.3.1. Increases in heart rate, afterload, preload, contractility and in the duration of systole augment oxygen demand. Myocardial oxygen supply is delivered by the coronary blood flow. The latter, in turn, is determined by the arterial perfusion pressure, the ventricular tissue pressure, the duration of diastole, while the distribution of the blood flow within the myocardium also affects the relation between oxygen supply and demand. The effects of coronary flow reduction on the myocardial oxygen demand and supply equilibrium and its relation to local myocardial function have already been discussed in chapter 2.

In the treatment of myocardial ischaemia three objectives are pursued. Firstly, one tries to restore the myocardial oxygen demand and supply equilibrium by decreasing oxygen demand, or by increasing oxygen supply. Secondly, the haemodynamic consequences of myocardial ischaemia have to be improved with the goal to maintain tissue perfusion. Thirdly, one must aim to reduce the incidence of ischaemia induced dysrhythmias, which may lead to fatal ventricular fibrillation or, at least, to an impaired haemodynamic function (Hauswirth and Singh, 1979; Singh et al., 1980).

In the present chapter some interventions that are carried out to augment the oxygen supply to the ischaemic segment are briefly discussed. Thereafter, some examples of treatment to reduce the oxygen demand will be taken up. It must be noted that some of the interventions mentioned below may have a dualistic action. For example: increasing oxygen supply to myocardium may be outbalanced by higher energy needs of the myocardial tissue. Similarly, a decrease in myocardial oxygen demand by beta-adrenoceptor blockade is accompanied by a reduction in the myocardial oxygen supply.

3.2. INTERVENTIONS TO IMPROVE OXYGEN SUPPLY

3.2.1. Mechanical cardiac assist

The technique of the intraaortic balloon counterpulsation (IABP) is adopted in patients with an inadequate myocardial performance to lower the systolic and to elevate the diastolic aortic blood pressure and, concomitantly, to increase coronary blood flow, cardiac output and mean blood pressure. The working principle is as follows. A catheter tipped with a large rubber balloon is inserted into a femoral artery and pushed up to the aortic arch. The balloon is deflated during myocardial systole and ejection. Immediately after closure of the aortic valves the balloon is inflated with an inert gas (helium, argon). This causes the blood in the aorta to be pushed towards the periphery and back towards the cerebral and coronary circulation. Before the next ventricular ejection occurs, the balloon is deflated so that blood can now more easily enter the aorta since the immediate

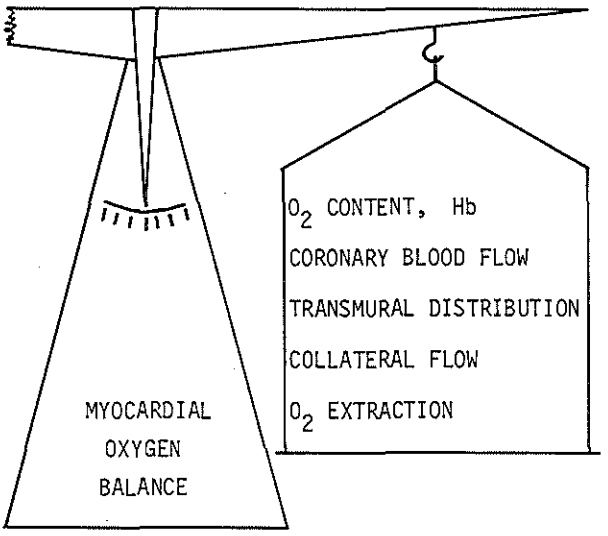

fig.3.1. Determinants of the myocardial oxygen supply.

deflation causes a reduction in momentary aortic pressure. The effects of IABP are threefold. Firstly, the myocardial load to pump blood into the circulation is reduced, since the IABP takes a part of the mechanical work for its account. This may cause a decrease in the myocardial oxygen demand. Secondly, as the ventricle is more rapidly emptied, it can operate on a lower afterload thus reducing wall stress and, in turn, myocardial oxygen demand. Thirdly, IABP may increase coronary blood flow due to the increased proximal aortic pressure, although autoregulation of coronary blood flow may occur, depending upon the existing level of the left ventricular work. It is, therefore, not surprising that both increases (Powell et al., 1970; Parmley et al., 1974), as well as, decreases (Leinbach et al., 1971; Shaw et al., 1974) in myocardial perfusion have been reported. The effects on the distribution of the myocardial blood flow during IABP, especially in the presence of regional myocardial ischaemia, are inconclusive. Depending on the technique of measurement, the size of the ischaemic zone, the species and the duration of IABP, both increases and decreases in the endo/epi ratio have been reported, while the flow to the ischaemic segment usually, but not consistently, increases (Gill et al., 1973; Swank et al., 1978). The function of the normally perfused, marginally and severely ischaemic segments have been studied in dogs using ultrasonic dimension gauges (Sasayama et al., 1979). IABP improves the segmental shortening of the normal and marginally ischaemic regions, but produced no change in the dyskinetic motion of the ischaemic segment. IABP seems to be of help to assist the mechanical function during the acute stage of myocardial infarction (Michels et al., 1980). Most studies reveal, however, that termination of IABP causes the immediate return of signs of ischaemia (Corday et al., 1972; Sasayama et al., 1979), so it seems only to be useful temporarily while other means to improve myocardial dysfunction must be used for the long-term restoration of function.

3.2.2. Pharmacological increase in perfusion pressure

The flow through a coronary artery is an equilibrium between driving pressure -the difference between arterial and central venous blood pressures- and the myocardial tissue vascular resistance. Increasing the arterial blood pressure (afterload) might result in an augmentation of myocardial perfusion. However, it has been shown, that within the range of upto 200 mmHg, the arterial blood pressure hardly affects the distribution of the coronary blood flow over the endocardium and the epicardium (Moir and DeBra, 1967; Walston et al., 1978), presumably because of autoregulation.

The presence of an obstruction that partially fills the lumen of a coronary artery may diminish the oxygen supply. The tissue in the perfusion area of the artery then becomes ischaemic and uses its autoregulatory reserve to maintain coronary flow. When the vasculature is maximally dilated, the flow becomes nearly proportional with perfusion pressure, while the myocardial wall tension only minimally modulates the flow (Sasayama et al., 1976; Verdouw et al., 1980a). Increasing the afterload may be, therefore, beneficial in

these circumstances. However, when the heart has to pump blood against an increased afterload, it performs more work. Hence, the myocardial energy-demand is higher which, if not successfully met, may jeopardize the myocardial tissue (Maroko et al., 1971; Hillis and Braunwald, 1977; Sasayama et al., 1980).

In conclusion, increasing the afterload only causes an increase in the perfusion of the myocardium when restrictions in the coronary inflow are present, e.g. when the coronary vascular resistance is minimal and the flow is pressure dependent. The net effect on the heart depends on the ability of the normally perfused zone to withstand the increased load and to meet its elevated oxygen demand.

3.2.3. Enhancement of myocardial contractility

Another way to augment the myocardial tissue perfusion is to elevate myocardial contractility, forcing the normally perfused region of the myocardium to compensate for the loss of function of the poorly perfused, ischaemic region. However, it has been shown that this kind of treatment may be deleterious for ischaemic myocardium, as it can enhance the degree of ischaemia (Schwartz et al., 1979) and extend infarct size (Maroko et al., 1971). Therefore, positive inotropic therapy, e.g. with beta-adrenergic agonists, is usually avoided in the presence of myocardial ischaemia. However, the oxygen debt of an inotropic intervention, which is sometimes associated with an elevated oxygen demand due to drug-induced tachycardia, is mitigated by a decline in intraventricular volume and wall stress which reduces oxygen demand (Weber and Janicki, 1977). Furthermore, the $beta_2$ receptor stimulation of the coronary vasculature in the normally perfused areas lowers their resistance to flow and causes an increase in coronary blood flow (Tuttle et al., 1977; Vatner and Baig, 1979). However, this may lead to a "steal" of blood by the normally perfused regions at the expense of the ischaemic tissue. The net effect of inotropic therapy is, therefore, difficult to predict and depends on the degree of ischaemia, the presence of intra-arterial collaterals and on the occurence of tachycardia.

Isoprenaline, which invariably causes increases in contractility, heart rate and transmural flow to non-ischaemic myocardium (Buckberg and Ross, 1973; Becker et al., 1975; Vatner and Baig, 1979), causes variable effects on the endo/epi blood flow ratio. The epicardial flow to ischaemic myocardium of anaesthetized dogs increases (Tuttle et al., 1977), while both the flow and the endo/epi ratio in the severely ischaemic zone in conscious dogs decreases (Vatner and Baig, 1979). The effects of isoprenaline on segmental wall function in the non-ischaemic control segment are parallel with those in flow, since the systolic wall shortening is enhanced (Theroux et al., 1974; Sasayama et al., 1976; Vatner and Baig, 1979). Although less extensively documented, the performance of the severely ischaemic dog myocardium has been reported to be temporarily improved (Theroux et al., 1974), but this beneficial action may be followed by a further deterioration of function after a longer period of treatment.

Other examples of positive inotropic drugs, which exert different

actions on arterial blood pressure and on heart rate, are dopamine (Goldberg, 1972) and dobutamine (Goldberg et al., 1977). The most pronounced difference between these agents and isoprenaline is, that dopamine and dobutamine cause a considerable increase in myocardial contractility with less extensive or absent drug-induced tachycardia. Dopamine and dobutamine cause a substantial increase in the blood flow to the normally perfused myocardium (Vatner and Baig, 1979). Administration of dobutamine in dogs (Tuttle et al., 1977) causes an increase in flow to the epicardium of ischaemic myocardium that equals the increase, induced by isoprenaline. However, the flow to the endocardium increases twice as much after dobutamine than after isoprenaline. Vatner and Baig (1979) report, after comparing dopamine, dobutamine and isoprenaline in equi-inotropic doses, that the segmental shortening of normal myocardium increases, but that the shortening of the severily ischaemic myocardium decreases only after isoprenaline. The transmural flow to and the endo/epi-ratio of the ischaemic segment decreases after isoprenaline, while both variables remain unaffected after the administration of dopamine and dobutamine. However, when the dose of the latter drugs is increased, causing tachycardia, the same undesirable effects like those of isoprenaline occur. Therefore, the administration of a positive inotropic drug can have a net beneficial effect on the perfusion and performance of both the normally perfused and the ischaemic myocardium, but only when the dose is chosen such that no appreciable tachycardia occurs (Tuttle et al., 1977; Vatner and Baig, 1979).

3.2.4. Collateral formation

The time course of, and the stimulants for, the formation of coronary collaterals have been discussed (Schaper, 1975; Scheel et al., 1976, 1977). After a chronic reduction of coronary inflow the number and the size of already present collaterals will increase, new collateral channels will be formed so that the collateral circulation becomes more significant in the protection of the tissue against necrosis after complete coronary occlusion. A potent stimulus for collateral formation is a gradual reduction of the lumen of a coronary artery for a period of several weeks, for example using an Ameroid constrictor (Schaper et al., 1976; Flameng et al., 1979). Physical stress, like excercise, has also been postulated to promote collateral growth (Neill and Oxendine, 1979). However, after exercising seven pigs on a treadmill for a period of six weeks a flow of less than 2% of base line was found distal to an acute ligation around the LAD (Scheffer et al., unpublished data from this laboratory). Therefore, the efficacy of exercise to increase collateral formation and function in the domestic porcine heart has not yet been proved.

3.3 INTERVENTIONS TO REDUCE MYOCARDIAL OXYGEN DEMAND

3.3.1. Reduction of heart rate

Beta adrenoceptor antagonists, like propranolol, are frequently used to reduce heart rate and to treat angina pectoris. The mechanism by which these agents reduce myocardial ischaemia remain, however, controversial. On the one hand, the beta-receptor antagonists reduce myocardial oxygen demand through negative inotropic and chronotropic actions. On the other hand, increases in oxygen supply caused by increases in blood flow to the ischaemic area, are probably an important factor (Tomoike et al., 1978). A concomitant improvement in the blood flow distribution in favour of the endocardium is frequently reported (Becker et al., 1971; Gross and Winbury, 1973; Warltier et al., 1976; Vatner et al., 1977). The blood flow to non-ischaemic tissue usually decreases parallel with myocardial oxygen demand, whereas the endo/epi ratio remains unaffected or even increases (Becker et al., 1975; Vatner et al., 1977; Buck et al., 1979).

The influence of beta adrenoceptor blockers on the contractile function of the (partially) ischaemic heart has been investigated thoroughly (Theroux et al., 1976; Vatner et al., 1977; Tomoike et al., 1978; Kumada et al., 1980). Generally, a reduction in segmental wall shortening has been observed in the non-ischaemic control segment, while the dysfunction of the severely ischaemic tissue improves. The beneficial effect is a consequence of the enhancement of perfusion of the ischaemic segment but especially of the reduction in the afterload.

Recently, alinidine, an N-allyl derivative of clonidine has been introduced. It causes an almost selective decrease in heart rate by direct influence on the sino-auricular node (Kobinger et al., 1979a; 1979b; Lillie et al., 1979; Tritthart and Windisch, 1979). Since alinidine represents a novel way of reducing heart rate and myocardial oxygen demand, we have endeavoured to study the effects of this agent on myocardial blood flow and function in the normal (chapter 9) and ischaemic (chapter 10) porcine heart.

3.3.2. Reduction of afterload

A reduction in the afterload can be realized by the administration of vasodilators, which act either via venodilation, arterial vasodilation, or both (see review by Mason, 1978). A typical example is nitroglycerin, which causes predominantly venodilation together with some arteriolar dilation. The drug has been used in the treatment of angina pectoris for more than a century. Nitroglycerin causes a reduction in left ventricular filling pressure, a decline in arterial blood pressure, frequently a reflex tachycardia and variable changes in cardiac output. Evidence has been presented for drug-induced improvements in the performance of areas in the myocardium showing ischaemic dysfunction (Theroux et al., 1974). The mechanism for this beneficial effect is not completely understood, but it has been attributed to increases in venous capacitance, reduction in afterload and favourable effects on the myocardial oxygen demand

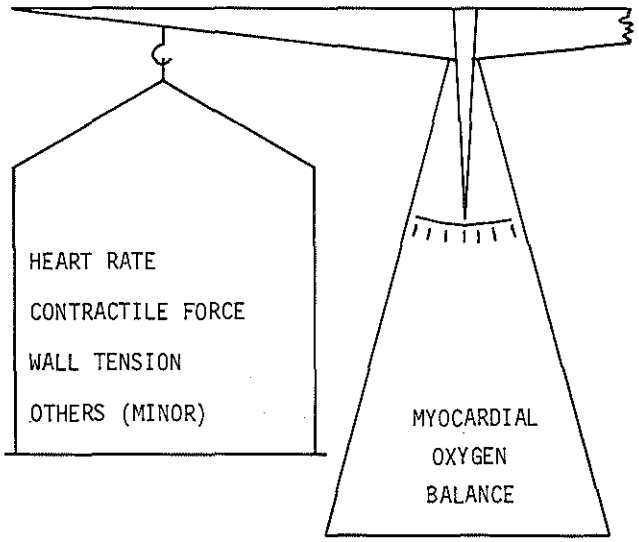

fig.3.2. Determinants of the myocardial oxygen demand.

and supply relationship and myocardial metabolism (Kugler, 1978). Direct effects of nitroglycerin on the coronary vasculature are less clearly established, but the possibility of a favourable redistribution of blood flow to the more vulnerable endocardium of the ischaemic region, probably caused by enhanced collateral formation (Capurro et al., 1977), has been proposed. However, in recent studies slight or no improvements have been observed in the myocardial blood flow distribution (Most et al., 1978; Nakamura et al., 1978; Kerber et al., 1979), and only marginally beneficial effects on the regional ischaemic function are noticed (Kerber et al., 1979; Komer et al., 1979; Ramanathan et al., 1979).

3.3.3. Calcium antagonists

Calcium antagonistic drugs, of which nifedipine is a typical example, has been shown to block or at least retard the transmembrane influx of calcium into myocardial cells (Lochner et al., 1975; Jatene and Lichtlen, 1976). Furthermore, they increase coronary blood flow and reduce myocardial contractility and systemic vascular resistance. These properties suggest that a favourable effect on the acutely ischaemic myocardium may be possible.

A study in the anaesthetised dog showed, that, when nifedipine is given in a dose of 40-60 microg.h^{-1}.$animal^{-1}$, starting 30 min after the onset of myocardial ischaemia, it causes significant increases in the (collateral) flow to and in the endo/epi blood flow ratio of the moderately ischaemic region. No improvements are observed in the blood flow to, and in the distribution within, the severely ischaemic area (Henry et al., 1978). After a bolus injection of 1 microg.kg^{-1} qualitatively the same observations have been made (Selwyn et al., 1979). The effects of nifedipine on regional myocardial function, assessed in the ischaemic dog heart (Perez et al., 1979), lead to a reduction in paradoxial systolic elongation in the severely ischaemic region and to a 50% improvement in the shortening of the marginally ischaemic segment.

In recent studies from this laboratory the effects of nifedipine on global haemodynamics and segmental myocardial perfusion and performance were investigated in the (ischaemic) porcine heart preparation after intravenous (i.v.) and intracoronary (i.c.) administration (Verdouw et al., 1980b; Verdouw et al., to be published). Nifedipine (3 microg.kg^{-1}, i.v.) caused a decrease by 20% in mean arterial blood pressure and only minor effects on cardiac output, LVdP/dtmax and coronary blood flow were observed. However, when administered i.c., the systemic haemodynamic effects were comparable, although coronary blood flow increases by 40% and systolic myocardial wall thickening disappeared. An i.v. infusion of 1 microg.kg^{-1}.min^{-1}, started 20 min after onset of coronary flow reduction to 25% of its base-line value, caused a 14% reduction in systemic vascular resistance. The other haemodynamic variables changed only marginally. Coronary blood flow to the normally perfused myocardium increased to 120% of base line. Moreover, the transmural flow to the central core of the ischaemic zone, which decreased to 11%

of its base-line value during coronary flow reduction, increased to 30% after nifedipine, favouring the endocardium, since the endo/epi-ratio increased from 0.4 to 0.6. The pattern of myocardial wall thickening closely followed that of regional perfusion. Finally, the incidence of ventricular ectopic activity, including ventricular fibrillation, was significantly reduced after nifedipine.

From these studies may be concluded, that treatment of acute myocardial ischaemia with a calcium antagonist may improve the blood flow to ischaemic myocardium as well as segmental function of this area through a protective action on cardiac cells.

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4. GENERAL ASPECTS OF MICROSPHERE TECHNIQUE

4.1. Introduction

The radioactive microsphere technique in its modern form, as introduced by Rudolph and Heymann (1967), is used to determine the distribution of cardiac output to different organs by the injection of carbonized plastic microspheres, labelled with gamma-emitter radionuclides. The principle of the method is that the microspheres after homogeneous mixing in the arterial blood, are entrapped in the organs and that the fraction of injected microspheres found in an organ is in proportion to the fraction of cardiac output distributed to that organ. If the flow to an organ and its microsphere content are known, the flow to any other organ not in series with other microvascular beds can be calculated. Even the latter can be determined as the sum of the flows to all organs wherefrom it receives blood. The use of microspheres with different isotope labels makes it possible to measure total-organ blood flow, intra-organ blood flow distribution and arteriovenous anastomotic blood flow after several interventions. In the present chapter the historical background, the theoretical and practical considerations and the precautions with regard to the use of the microsphere technique are reviewed and some adaptations to our experimental set-up are described, while chapter 5 considers the analysis of data by the required computer programs.

4.2. History

The historical development of the use of microspheres in studies of the circulation has been summarized by Wagner et al. (1969). The use of glass microspheres has been introduced in 1947 by Prinzmetal, Simkin, Bergman and Kruger, to detect vascular anastomoses in post mortem human hearts and in various organs in rabbits (Prinzmetal et al., 1948). After irradiation with a neutron beam the activated sodium isotopes in the glass spheres facilitates the detection of the spheres (Grim and Lindseth, 1958). However, glass spheres are considerably heavier than red blood cells (3 vs. 1.06 $g.cm^{-3}$) and sediment rapidly. More recently, lighter plastic microspheres with a density of about 1.3 $g.cm^{-3}$, labelled with different radionuclides, have been developed. The latest type are microspheres made of dextran. They have a density of 1.12 $g.cm^{-3}$ and must be labelled in the laboratory. However, they have not yet been as extensively investigated as the plastic microspheres. Both the plastic and the dextran microspheres are non-biodegradable, so they cannot be used in studies of the human circulation.

Macroaggregates of metabolizable human albumin, labelled with various isotopes have been used (Wagner et al., 1965), but unfortunately, they exhibit a wide variation of particle size. Although this problem has been minimized (Zolle et al., 1970), the

albumin microspheres have not found an extensive use in studies of the circulation.

4.3. THEORETICAL CONSIDERATIONS

4.3.1. Conditions in which the microsphere technique can be used

Depending on the size of the spheres, the number of available isotopes and the site of injection, the microsphere technique can be used in the following situations.

a) The distribution of cardiac output can be determined from the comparison of the number of spheres entrapped in the organs with the total number of spheres injected (Rudolph and Heymann, 1967; Domenech et al., 1969).

b) The blood flow in absolute terms is calculated from the microsphere content of an (artificial) organ with known blood flow. This can be realized by: 1) determination of cardiac output with another technique together with total dose of isotope injected; 2) determination of flow to one particular organ, for example using an electromagnetic flow meter, together with its microsphere content; 3) introduction of an "artificial organ" (Makowski et al., 1968), consisting of a blood withdrawal pump connected to a catheter, inserted into a peripheral artery. Both flow and microsphere content can then easily be determined (fig.4.1; see 4.4.3).

c) The intraorgan blood flow distribution is determined after the dissection of the organ of interest in fractions in which the microsphere content is measured.

d) The distribution of blood flow within an arterial bed can be mapped out by injecting microspheres into an artery. One can also determine the extent of arteriovenous shunting pertaining to that single bed (see g).

e) Determination of cardiac output from the microsphere content of a pulmonary arterial- or aortic blood sample after central venous- or left atrial microsphere injection (Hales, 1973a; see 5.3.7).

f) With a number of restrictions and precautions the microsphere content of the overlap area between two or more adjacent arterial beds (e.g. in the myocardium) can be used as an index of collateral blood flow.

g) To determine the systemic arteriovenous shunting (Lopez-Majano et al., 1969; AVA-flow). When microspheres with a diameter comparable with capillary size (below 15 micron) are used, a fraction of the arterially injected microspheres is shunted through the peripheral vasculature and travels into the venous circulation through arteriovenous anastomoses. These nonentrapped spheres, provided that their diameter is larger than about 8 micron, finally lodge in the microvasculature of the lungs (Ring et al., 1961; Kaihara et al., 1968; Warren and Ledingham, 1974). Therefore, the microsphere content of the lungs is a measure for peripheral AVA-flow.

h) The local AVA-flow can be determined by injection of microspheres into the artery that delivers the blood to the area of interest and from the withdrawal of a local venous blood sample or the

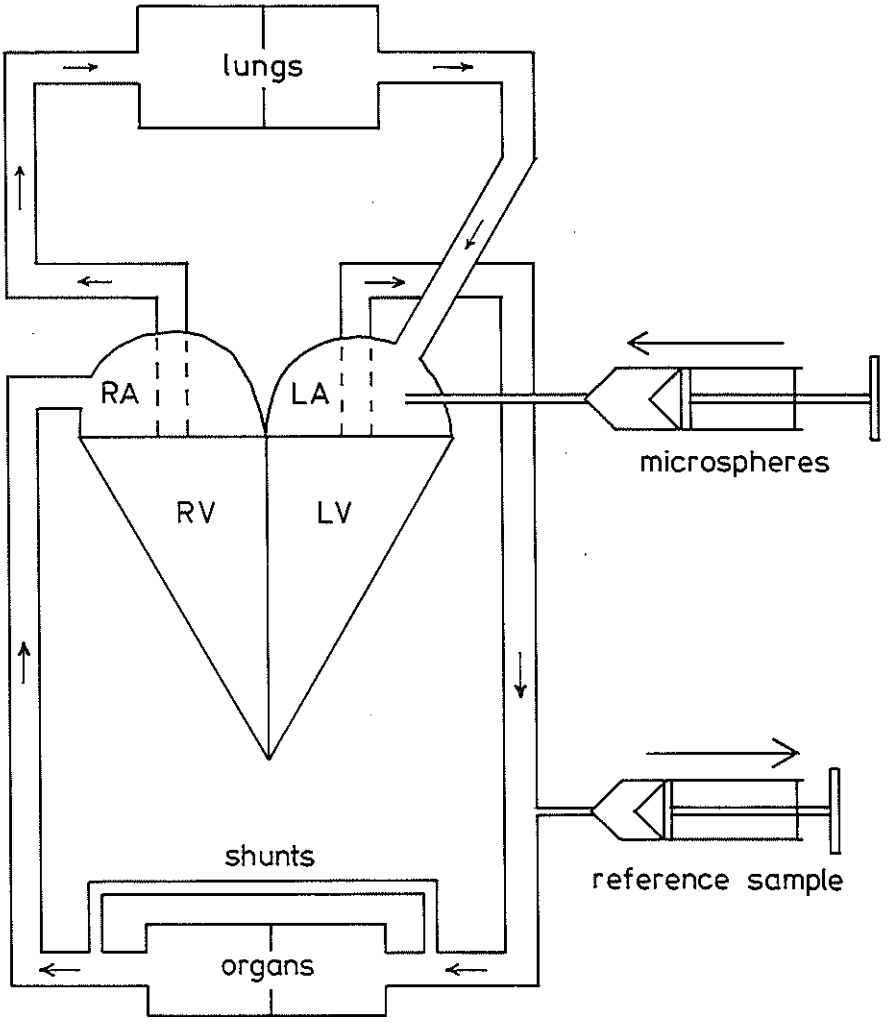

fig.4.1. Schematic diagram of microsphere injection, using an arterial reference sample for flow calibration. A fraction of the arterial blood is shunted through arteriovenous anastomoses (shunts) and bypass the organs. Shunted microspheres are finally entrapped in the lungs. Abbreviations: RA, LA, right and left atrium; RV, LV, right and left ventricle.

determination of the microsphere content of the lungs.

4.3.2. Choice of microsphere size

The smallest microspheres that are completely entrapped in the organ of interest during first circulation are considered to be of optimal size for determination of nutritional organ blood flow (Warren and Ledingham, 1974; Heymann et al., 1977). The larger spheres (over 25 micron) satisfy the criterion of entrapment for most organs (Forsyth et al., 1968). However, the sphere size affects the obtained blood flow values, since the simultaneous injection of three sizes of microspheres in dogs leads to the following observation: the ratio between endocardial- and epicardial perfusion of the left ventricle is considerably higher for 25 micron spheres than for 14 micron spheres (Domenech et al., 1969; Utley et al., 1974). At least two factors may explain this discrepancy:

1) particles migrate towards the axis of vessel with laminar flow and axial migration is greater with larger particles (Phibbs et al., 1967; Mason and Goldsmith, 1969; Phibbs and Dong, 1970). Microspheres of 7.5-10 micron diameter show the radial distribution which most closely approach that of erythrocytes (Phibbs and Dong, 1970).

2) Axial migration may result in skimming of the microsphere-poor blood into proximal branches and the delivery of microsphere-rich blood into terminal branches of an artery. These effects and the architecture of the coronary arterial system are probably responsible for the higher inner to outher ratio of the larger spheres. Microspheres smaller than 15 micron have acceptable streaming artefacts and approximate the true distribution of renal (Katz et al., 1971) and myocardial blood flow (Utley et al., 1974; Tripp et al., 1977a, 1977b). The optimal microsphere size in studies of the regional perfusion of the myocardium is, therefore, 15 micron (Marshall et al., 1976; Heymann et al., 1977; Fan et al., 1979).

4.3.3. Stability of microsphere entrapment

To measure actual blood flow with the microsphere technique, all microspheres must be entrapped and remain entrapped. Two possible sources of microsphere loss must be separated. A fraction of the microspheres passes via arteriovenous anastomoses and will not be entrapped at all. They are recovered in the venous blood draining the organ. However, some microspheres are temporarily entrapped and pass into the veins after a considerable delay. Another source of microsphere loss originates from tissue necrosis, especially after ligation of a coronary artery and the occurrence of myocardial infarction. This kind of microsphere loss is especially important in long-term survival studies after induction of regional ischaemia or coronary ligation.

Acute shunting of 9 micron spheres through the myocardial vasculature of the dog ranges from 1 to 13% within two to 30 min after microsphere injection, but the commonly reported value is about 5% (Marshall et al., 1976; Tripp et al., 1977a; Consigny et al., 1979; Crystal et al., 1979; Fan et al., 1979). Even an elevation of

perfusion pressure to 200 mmHg results in a microsphere loss of only 6% from non-infarcted myocardium (Crystal et al., 1979).

The shunting of microspheres depends largely on microsphere size, since the above mentioned data are reduced to 1 to 7% when using 15 micron spheres (Marshall et al., 1976), and usually to no more than 2% (Fortuin et al., 1971; Tripp et al., 1977a; Fan et al., 1979). The shunting of 25 micron spheres is absent (Crystal et al., 1979), even at increased perfusion pressure.

The loss of 7-10 micron and 15 micron spheres from necrotic myocardium in the perfusion area of an acutely ligated coronary artery has been described recently. Capurro et al. (1979) report a loss of about 30% from the endocardium after one day. Thereafter, no further loss occurs, since this figure is also about 30% after two and eight days. The infarcted epicardium does not lose its microspheres within two days, but a comparable amount is lost after a few days. Levken et al. (1980) show, that 11 to 60% (averaged 26%) of the 15 micron spheres are lost from the necrotic myocardium within 10 hours. About 20% of 7-10 micron spheres disappear from necrotic myocardial tissue (Jugdutt et al., 1979), and the degree of loss is closely correlated with the degree of necrosis. They state, that 40% hereof is an "apparent loss" due to tissue oedema (which causes a reduction in the flow per gram of wet weight of tissue), and the remaining 60% is actual physical loss. No spheres escape from non-ischaemic or non-necrotic tissue in the occluded area within six hours after onset of occlusion.

We confirmed in a number of experiments that no shunting of 15 micron spheres occurred over the normal myocardium of the domestic pig heart (unpublished data). The duration of our experiments never exceeded two hours after injection of the first batch of microspheres. Therefore, the loss of microspheres from the heart cannot have affected reported data on regional myocardial blood flow.

4.3.4. Required number of microspheres

Even if microspheres are well mixed with blood and distributed in proportion to regional blood flow, the number of spheres per unit volume will not be identical in two samples, equal in size and flow. This is due to the statistical variation in the microsphere distribution. The variability of distribution of spheres to any one organ, if the experiment is done many times under identical conditions, approximates a Poisson distribution with X being the mean number of spheres reaching that organ and $\sqrt{X}$ the corresponding standard deviation. The calculation of X to achieve a percentage error of 10% at the 95% confidence limit follows from:

$$1.96 \times \sqrt{X} = 0.1 \times X$$

so X=384 (Buckberg et al., 1971). For practical purposes the number of 400 spheres in the smallest area of interest allows enough statistical precision.

The radioactive decay causes a number of desintegrations which is

also distributed in time according to the Poisson statistic. To obtain the summed uncertainty, each of the errors of the microsphere distribution and of the radioactive decay have to be squared, after which the square root of the sum yields the final error. When the counting uncertainty is smaller than 3%, its contribution to the summed error is smaller than 10% and can, therefore, be neglected. The required number of counts (C) can thus be calculated analogously from:

$$1.96x\sqrt{C}=0.03xC$$

yielding C=4268. When the counting time is chosen long enough to achieve at least 5000 counts from at least 400 microspheres, entrapped in the smallest tissue sample, the statistical error in the blood flow value approximates 10%.

The required number of microspheres (TD) to be injected into the systemic circulation of the animal can be calculated when the fraction of cardiac output (CO) perfusing the smallest tissue sample with flow $\dot{Q}$tis is known, since the total dose is:

$$TD=COx400/\dot{Q}tis.$$

The upper limit of this figure follows from the maximum number of capillaries that can be obstructed without deleterious effects on the circulation, see next paragraph.

4.3.5. Effects on the myocardial circulation

The microsphere-induced embolization of the microcirculation depends on the size of the microspheres: Larger microspheres plug a larger vessel and obstruct a greater portion of the circulation. The degree of obstruction can be calculated from the number of capillaries in the heart and the required number of spheres in the sample. The heart has about $6x10^6$ capillaries per gram of tissue (Yipintsoi et al., 1973). Assuming that the smallest sample of interest has a weight of about 0.1 gram and contains 400 microspheres, the total number of embolized capillaries becomes is far below 1%.

From an elegant study Hales and Cliff (1977) describe the behaviour of 15 micron microspheres at the microcirculatory level in the tissue of the rabbit ear within a surgically established perspex chamber by direct microscopic vision techniques. They report that microspheres reach their final location within a few seconds, although for one sphere a time of 58 min has been noted. The microspheres lodge in the end-arterioles, the nutrient vessels or even in mid-stream in an arteriole. Whenever entrapped, the microspheres remain entrapped by the tissue. The obstruction of the local microcirculation is negligible, since collateral circulation is established through previously non-patent channels within 30 s.

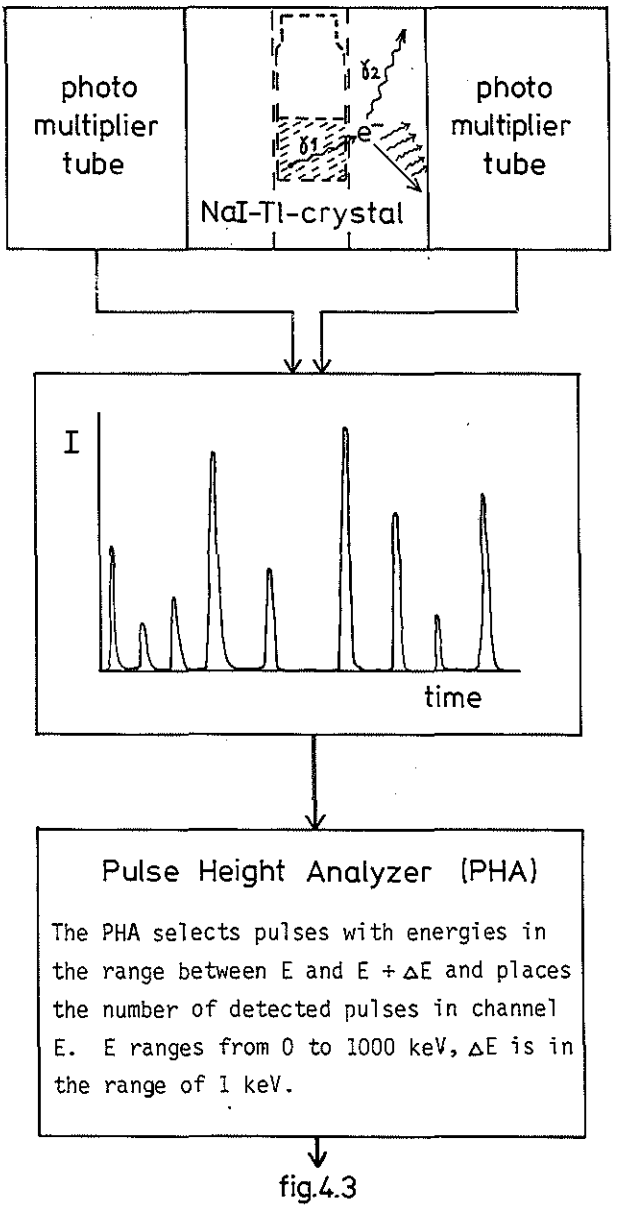

fig.4.2. Schematic diagram of gamma detection. The intensity (I) of the group of scintillations in the NaI-Tl-crystal is in proportion with the gamma energy.

4.3.6. Gamma radioactivity and detection

The nuclei of some isotopes are in an excited state, which can decay under the emission of gamma radiation. A gamma quantum represents an energy package of electromagnetic radiation in the range from 10 keV upto several MeV. The fraction of the decay that results in gamma emission is called abundance. When more than one gamma quantum is emitted in the same decay, the abundance can be greater than 100%. The data on isotopes frequently used in microsphere labelling are listed in table 5.2. The time after which one half of the active nuclei has been decayed is denoted as half-life. The interaction between gamma radiation and matter is weak. Therefore, the detection of gamma radiation is relatively difficult. The usual way to detect gamma radioactivity from microspheres is to convert the gamma energy into kinetic energy of an electron in a Tl-activated NaI-crystal (fig.4.2). This transfer is mainly governed by two processes. Firstly, at gamma energies below 100 keV the total gamma energy is transmitted to the electron by the "photo effect". At increasing gamma energy the photo effect becomes overshadowed by the second type of interaction, the "Compton- scattering", in which only a fraction of the gamma energy, depending on the collision angle between gamma and electron, is transferred. At a photopeak energy of 513 keV (^{85}Sr) the Compton scattering is responsible for about 53% of the energy transfer, while only 47% is found in the photo peak (fig.4.3).

The primary high energy electron in the NaI-crystal loses its energy via collisions with other electrons in the crystal under the emission of visible light. Using a photo multiplier tube these scintillations are converted into an electric current pulse. The size of this pulse is proportional with the gamma energy (fig.4.2). A plot of the detected number of desintegrations as function of the energy is shown in fig.4.3 for the single gamma emitter ^{85}Sr. The large fraction of photopeak counts is clearly shown.

A radioisotope has its own characteristic energy spectrum. When isotopes are mixed in one sample, their energy spectra add, resulting in a complex sum of Compton scatter contributions with the photopeaks of the isotopes superimposed thereon. One of the problems in gamma spectrometry using the microsphere method is to recover the contributions of the single isotopes out of the complex spectrum.

The interaction of gamma radiation and matter is inversely related to the gamma energy. The usual NaI-crystal dimensions of three inch in diameter and three inch height absorb 95% of the ^{125}I-radiation, but only about 58% of the energy of ^{85}Sr. To obtain comparable count rates for all isotopes or to determine the radioactivity in a sample in Curies, this must be taken into account.

4.3.7. Detection efficiency versus sample volume

The ideal sample in which radioactivity has to be detected, is small and centered in the heart of the crystal, so that there is maximum chance of interaction between the gamma radiation and the crystal material (Katz and Blantz, 1972; Heymann et al., 1977). This conflicts with the usual requirement to determine the microsphere

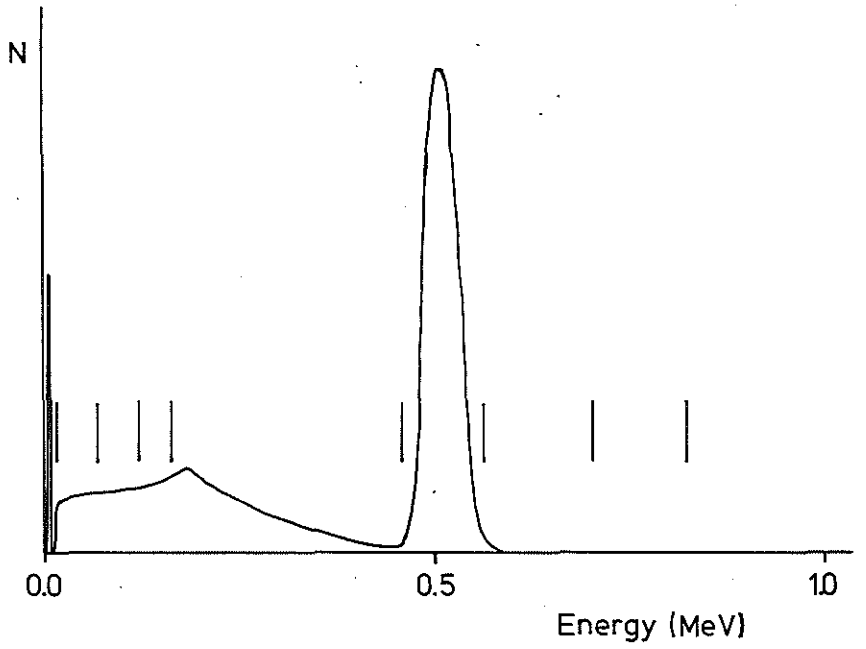

fig.4.3. Energy spectrum of ^{85}Sr. The vertical lines mark the window settings corresponding with ^{125}I, ^{141}Ce, ^{85}Sr and ^{95}Nb, respectively. Table 5.2 presents further data concerning these isotopes. The peak at about 10 keV is caused by radiation, scattered within the crystal and by the lead shielding surrounding the crystal. The total number of counts in the photopeak and those in the Compton-scattering approximate each other, since the photopeak fraction is 0.47 (table 5.2).

content of relatively large tissues (e.g. the liver or the lungs in rabbits or pigs). Furthermore, the microspheres are more or less homogeneously distributed throughout the tissue, so the entire sample volume must be counted with nearly optimal efficiency. Our Packard 5986 gamma counter (see 5.6.) accepted vials of 2.5 cm diameter which could be filled to about 2.5 cm height. With the bottom of the vial located 1.5 to 2 cm from the bottom of the crystal, a counting efficiency over 97% was realized for both samples with all radioactivity concentrated at the bottom of the vial (blood samples) and for samples that contained about 10 g homogeneously radioactive kidney tissue (fig.4.4). It is extremely important not to fill the vials above this height, since the decrease in counting efficiency at increasing sample volume is very sharp (Katz and Blantz, 1972; own unpublished data).

4.4. PRACTICAL USE OF THE MICROSPHERE TECHNIQUE

4.4.1. Checks prior to injection

Immediately after arrival of a batch of microspheres the shape and the size of the spheres were checked using a calibrated eypiece microscope. 50 to 100 beads were measured to determine the diameter distribution. The latter has been proposed in an earlier report (Reneman et al., 1975) as a possible source of error in the method, but the microsphere batches now have a much better size distribution. Improvements in the production method are probably responsible herefor. The absence of serious clumping of microspheres and of bizarre shaped spheres was verified by global optical checking. The correct labelling was deduced from the energy spectrum. Leaching of the isotope labels was determined by counting the fluid in which the microspheres were suspended (10% dextran). A 5, 10 or 20 times dilution of the batch in 10% dextran was made for convenience of injection. One drop of tween 80 per 10 ml of microsphere suspension was added to prevent clumping of the spheres. The number of counts per microsphere was calculated from the radioactivity of a predetermined number of spheres.

4.4.2. Injection of microspheres

Prior to injection the microsphere suspension was ultrasonicated for at least 1 min to break possible bridges between microspheres. The spheres were then dispered by moving an air bubble through the injection syringe. The microspheres were injected into the left atrium. Many investigators point to the importance of complete mixing of microspheres in the blood, especially in studies of the myocardial perfusion (Kaihara et al., 1968; Hoffbrand and Forsyth, 1969; Buckberg et al., 1971; Warren and Ledingham, 1974). As a validity check on microsphere mixing the blood flow to both kidneys was measured routinely. We never observed differences of more than 2%. A more sensitive measure is the comparison of the blood flows to small, adjacent pieces of the most critical organ, the heart. Notifying the differences that may be expected from the statistical uncertainty

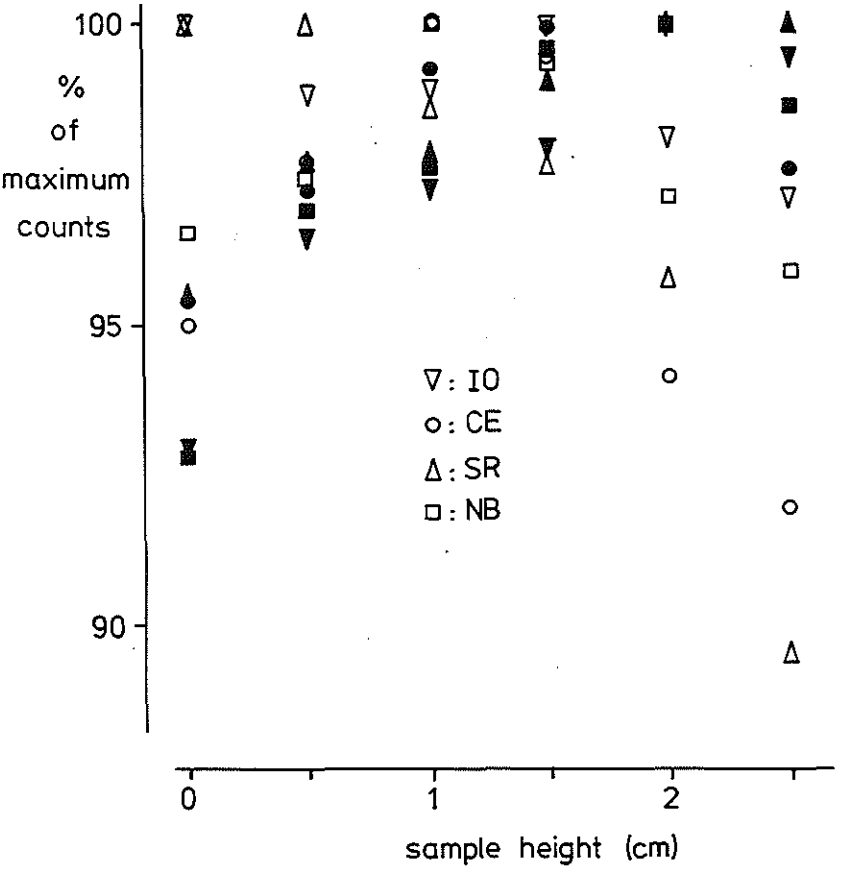

fig.4.4. Percentage of maximum count rate as function of the height of the sample, measured from the bottom of the scintillation crystal. Closed symbols have been used for samples with all activity located at the bottom of the vial, open symbols for samples with their activity in the entirety of the sample volume of about 10 cm^3. The optimal setting is between 1 and 2 cm. Abbreviations: see legend of fig.4.5.

sources, the observed differences of less than 4% guaranteed that a good mixing was established throughout all our experiments.

4.4.3. Arterial reference sample method

Absolute blood flows can be determined using an "artificial organ" with known blood flow, consisting of a catheter inserted into a peripheral artery, connected with a withdrawal pump (Makowski et al., 1968; Domenech et al., 1969; Bartrum et al., 1974). The microspheres in the syringe on the pump are "completely entrapped in first circulation", while the flow rate can be easily controlled. To establish a steady flow through the catheter, the pump was started a few seconds before injection of the microspheres. The withdrawal of blood was continued to cover the time between injection and passage of the last microsphere along the sampling site. The time necessary to entrap all the passing microspheres is reported to be about one min (Buckberg, 1975; Heymann et al., 1977). We confirmed, that within 30 s more than 90% of the microspheres had entered the syringe. For safety, 60 to 75 s was used routinely. The arterial catheter was located in the abdominal aorta.

The sample flow rate does not affect the results of blood flow calculations when the blood sample contains more than 400 microspheres (Buckberg et al., 1971). Because of the vital importance of the reference sample for the calculation of absolute blood flow values, we usually drew a sample that contained over 3000 spheres. The flow rate of the pump was set to approximate 0.5 to 1% of cardiac output, as has been recommended (Hales, 1973a) for sampling from a central artery.

When used with these precautions the relatively easy reference sample technique leads to accurate flow values, with a high reproducibility in large, as well as in small (rat) laboratory animals (Makowski et al., 1967; Domenech et al., 1969; Hales, 1973a; Bartrum et al., 1974; Malik et al., 1976).

4.4.4. Sample preparation and gamma counting

After termination of the experiment the organs of interest were dissected from the body and put in counting vials. The heart was stored for at least 24 hours in 4% formalin to facilitate sectioning. The dissection of the heart was carried out according to a protocol, that depended on the type of the experiment. A detailed description is given in chapters 8 and 10 (Schamhardt et al., 1979). Care was taken to assure that tissue was packed as close to the bottom of the vials as possible (see 4.3.7.). With these precautions the laborious carbonization of tissue (Heymann et al., 1977) was not necessary.

4.4.5. Calculation of blood flow

The procedure which we developed for the decomposition of the complex spectra was as follows. Firstly, energy windows were located around the photo peaks of the used isotopes. Although the energy of the gamma radiation is sharply defined, the detection process in the NaI-crystal broadens the photo peaks at higher energies. The boundaries were chosen as illustrated in fig.4.3. Secondly, pure

	IO	CE	SR	NB
IO	0.9999	0.0000	0.0000	0.0000
CE	0.2385	0.7613	0.0001	0.0001
SR	0.1214	0.0974	0.7805	0.0006
NB	0.1088	0.0920	0.1104	0.6888

fig.4.5. Overlap matrix giving count rates of various isotopes in other energy windows (see 5.3.4. and fig.5.10.). Abbreviations: IO, Iodine-125; CE, Cerium-141; SR, Strontium-85; NB, Niobium-95.

$$C_{nNB} = \frac{C_{rNB}}{0.6888}$$

$$C_{nSR} = \frac{C_{rSR} - 0.1104 \times C_{nNB}}{0.7805}$$

$$C_{nCE} = \frac{C_{rCE} - 0.0920 \times C_{nNB} - 0.0974 \times C_{nSR}}{0.7613}$$

$$C_{nIO} = \frac{C_{rIO} - 0.1088 \times C_{nNB} - 0.1214 \times C_{nSR} - 0.2385 \times C_{nCE}}{0.9999}$$

fig.4.6. Illustration of the "stripping"-procedure. After subtraction of the background radioactivity, the count rate C_r of a certain isotope is obtained. The net counts C_n are determined using the above formulae. Abbreviations: see fig.4.5.

isotopes were counted and the contribution of the isotopes in their own and in the other windows was determined. Since the shape of the energy spectrum is not affected by the activity of the samples, the overlap fractions only depend on the window settings. After subtraction of the "background" counts, obtained from counting empty vials, the net counts of the highest energy isotope were determined by dividing the rough counts and the fraction of counts in its own window (fig.4.5 and 4.6). Using the overlap fractions, the counts in the lower energy windows could be calculated and subtracted from the corresponding rough counts in these windows. By subsequent "stripping" from higher to lower energy windows, the net counts for all isotopes were determined.

The formulas used in the calculation of tissue blood flows from radioactivity data and the necessary computer programs have been described in detail in chapter 5. The following procedures were carried out. The counts belonging to a certain isotope were derived from the complex spectrum with the "stripping" method (Heymann et al., 1977). The counts of the vials belonging to one organ were added to obtain total counts pertaining to that organ. Organ counts were summed to achieve counts for organ groups. Finally, the flow as percent cardiac output, absolute blood flow, flow per gram of tissue, vascular resistance and resistance times gram of tissue were calculated. The programs were also attended to calculate organ blood flow ratios, systemic and local arteriovenous shunt flows.

4.4.6. Estimation of total error in blood flow values

The numerous handlings with microspheres prior to and during injection, the arterial reference sample technique, and the calculation of organ blood flow from tissue radioactivity introduce some arbitrary errors, that add in the final result as:

$$(\Delta \dot{Q})^2 = \sum_i \left(\left(\frac{\partial \dot{Q}}{\partial A_i} \right) \Delta A_i \right)^2$$

where $\dot{Q}$ is the blood flow, Δ denotes absolute error, ∂ the partial derivative of $\dot{Q}$ to one of its determinants A_i and $\sum$ the summation over all components (Squires, 1968). Assuming that the microsphere distribution and the radioactive decay process follow the Poisson statistic, the total error in blood flow values due to these factors can be calculated in a straight forward manner, although rather time consuming. The blood flow value to an organ containing at least 400 microspheres, which delivered over 5000 counts, could be determined with an uncertainty of about 14% at the 95% confidence level. The error decreased rapidly with increasing number of microspheres.

4.4.7. Repeatability of microsphere blood flow measurements

A simple method to obtain information on the reliability of the microsphere blood flow measurements is to inject in one animal simultaneously a number of differently labeled microspheres of identical size. This technique leads to blood flow values that are identical within 10% for all tissues (Neutze et al., 1968; Buckberg

et al., 1971) containing more than 400 microspheres, but other investigators report, that no differences exceed 4% (Kaihara et al., 1968; Hales, 1973a). Correlations between the measurements higher than 0.99 with a root mean squar error of about 0.1 $cm^3.min^{-1}.g^{-1}$ have been reported (Hales, 1973a, 1974), even using microspheres from different manufacturers (3M, NEN) (Hales et al., 1979). The correlation between the simultaneously measured blood flows per gram of tissue to the left and the right kidneys is a special type of reliability test, since these flows may be expected to be nearly identical. The correlation is, therefore, often used as an internal check on the homogenous mixing of microspheres in the blood. Correlation coefficients from 0.97 to 0.99 ($p<0.01$) have been reported (Neutze et al., 1968; Bartrum et al., 1974; Verdouw et al., 1980).

The reference sample technique has been shown to be reproducible within about 5% (Makowski et al., 1967; Bartrum et al., 1974). The overall repeatability, which includes the physiological differences between two microsphere injections one hour apart, is within 10% (Neutze et al., 1968). Measurements in the anaesthetized pig preparation after four doses of microspheres within 8 min showed flow values that differed no more than 6% (unpublished data).

4.4.8. Comparison with other techniques

The microsphere technique has been compared with several other independent methods of blood flow measurement. The calculated cardiac output from microsphere injections and arterial reference sampling differs by no more than 10% from that, obtained from a calibrated roller pump in a right heart bypass (Archie et al., 1973), or obtained from dye dillution in various species (Hoffbrand and Forsyth, 1969; Archie et al., 1973; Bartrum et al., 1974). The comparison of blood flow values obtained using the microsphere technique or gathered from diffusable radioisotope clearance give comparable results (Mendell and Hollenberg, 1971; Yipintsoi et al., 1973; Becker et al., 1974; Prokop et al., 1974; Cannon, 1975; Tripp et al., 1977b; Knoebel et al., 1978). Total renal blood flow as measured with an electromagnetic flow meter and with microspheres yield identical flow values within 6% (Buckberg et al., 1971) or a correlation of 0.88 ($n=22$) between them (Katz et al., 1971). The differences between coronary blood flow measurements using these techniques are 13% (Utley et al., 1974), while the collection of the coronary venous effluent results in differences less than 10% in 66% of the cases and less than 20% in 90% of them (Buckberg et al., 1971; Domenech et al., 1969; Utley et al., 1974).

4.4.9. Arteriovenous anastomotic blood flow

As mentioned in 4.3.1. g) and h) the microsphere method can also be used for the determination of arteriovenous shunting. The most direct method to measure the proportion of microspheres that bypass a specific organ is to compare the microsphere content of the total venous effluent with those entrapped in the organ (Grim and Lindseth, 1958; Domenech et al., 1969; Buckberg et al., 1971; Katz et al.,

1971). Secondly, the shunted microspheres can be entrapped by an organ in series with the organ under investigation (liver for studies of the mesenteric organs (Delaney, 1969), or the lungs for the detection of nonentrapment in the peripheral microcirculation (Kaihara et al., 1968; Neutze et al., 1968; Bartrum et al., 1974; Warren and Ledingham, 1974; Johnston and Saxena, 1978; Spierings and Saxena, 1979). A third method involves the withdrawal of a blood sample from the vein draining the organ and to compare the microsphere content in that sample with the total number of spheres injected or lodged in the organ (Kaihara et al., 1968; Archie et al., 1973; Hales, 1973a,b; 1974).

A crucial difference between the microsphere technique and the electromagnetic flow meter is that the latter measures total blood flow within an artery (nutritional plus non- nutritional), while the former measures, depending upon the size of the spheres, predominantly nutritional flow. The shunting through the rabbit ear as measured with 15 micron spheres is as high as 80% (Warren and Ledingham, 1974), while the shunting of microspheres larger than 9 micron through the myocardial vasculature is negligible (Fortuin et al., 1971; Marshall et al., 1976; Tripp et al., 1977a; Capurro et al., 1979; Crystal et al., 1979; Fan et al., 1979; own unpublished data).

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COMPUTER PROGRAMS FOR THE RADIOACTIVE MICROSPHERE TECHNIQUE: DETERMINATION OF REGIONAL BLOOD FLOWS AND OTHER HAEMODYNAMIC VARIABLES IN DIFFERENT EXPERIMENTAL CIRCUMSTANCES

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NORTH-HOLLAND PUBLISHING COMPANY – AMSTERDAM

COMPUTER PROGRAMS FOR THE RADIOACTIVE MICROSPHERE TECHNIQUE: DETERMINATION OF REGIONAL BLOOD FLOWS AND OTHER HAEMODYNAMIC VARIABLES IN DIFFERENT EXPERIMENTAL CIRCUMSTANCES

Summary. Computer programs in FORTRAN IV have been designed to calculate regional blood flow values from injections of gamma radiation emitting radioactive microspheres. The first program PRSO constitutes a file containing the names of organs, organ groups and systemic haemodynamic variables and data about the isotopes in use. The second program PRSI transfers the data of a particular experiment concerning protocol, systemic haemodynamic variables, organ weights, number of vials belonging to each organ and the radioactivity from each vial into a data file that is subsequently analyzed by the third program PRSII. One of 5 data conditions which later directs calculations in PRSII is determined by PRSI. PRSII deducts background radioactivity, decomposes the spectrum from the mixture of upto 6 nuclides using the "stripping" method, and corrects for isotope decay during the counting period. Depending upon the data condition, PRSII calculates one or more of the following variables: (i) cardiac output (CO); (ii) the distribution of CO; (iii) regional blood flow values and tissue vascular resistances; (iv) the distribution of blood flow within an arterial bed; (v) arteriovenous anastomotic blood flow; (vi) some specified systemic haemodynamic variables and (vii) blood flow ratios. The systemic and regional haemodynamic data belonging to experiments are saved for later statistical analyses (e.g. mean and standard error of the mean of the variables, chnges from the base line, comparison between two groups of experiments etc.) by other programs. PRSO, PRSI and PRSII can be operated by investigators with little or no special training in computer use.

5.1. INTRODUCTION

Ever since the introduction of the radioactive microsphere method in its modern form (Rudolph and Heymann, 1967; Wagner et al., 1969), its use for the determination of regional blood flow in physiological and pharmacological investigations has been steadily growing (for references, see: Hales, 1974; 3M bibliography, 1976; Heymann et al., 1977). Various validity studies have been performed (Hoffbrand and Forsyth, 1969; Buckberg et al., 1971) and the technique has been adapted for the determination of (i) cardiac output (CO) (Johnston, 1976; Forsyth and Saxena, 1978); (ii) the distribution of CO (Kaihara et al., 1968; Mendell and Hollenberg, 1971); (iii) regional

blood flows (Johnston and Owen, 1975; Saxena et al., 1978); (iv) the distribution of blood flow within a tissue (e.g. the myocardium, (Buckberg, 1975; Schamhardt et al., 1979)) or in an arterial bed (e.g. the carotid (Lopez-Majano et al., 1971; Spierings and Saxena, 1979)) and (v) total peripheral or regional arteriovenous anastomotic (AVA) blood flow (Hales et al., 1978; Johnston and Saxena, 1978; Saxena, 1978; Spierings and Saxena, 1979). The main advantage of the radioactive microsphere technique is that simultaneous measurements in a large number of tissues or organs can be made without extensive surgery or the use of anaesthetics (Forsyth et al., 1968; Saxena et al., 1978). Amongst the disadvantages could be mentioned the relatively high costs of the method, the limited number of measurements that can be made in a single experiment and the difficulties in not only the separation of the complex spectrum of nuclide mixture but also in the handling of a large amount of data generated from one experiment.

The problems in the spectral analysis of the radioactivity (Bentley, 1963; Schosser et al., 1979) and in the handling of the haemodynamic data make the use of sophisticated computer facilities obligatory. In the course of more than 10 years that the microsphere technique is being used by us, we have developed a number of computer programs for the analysis of the microsphere-data. In contrast to a previously described programs (Lydic et al., 1977; Schosser et al., 1979) the present ones are sufficiently flexible so as to deal with most experimental circumstances, denoted in this paper as data conditions (see 5.5.3.7). Depending on the data condition, i.e. whether the total amount of radioactivity injected is known or has to be derived by counting the body organs or from a reference blood sample (Heles, 1974; Heymann et al., 1977), the program calculates regional blood flow as percent of cardiac output or arterial blood flow, absolute and normalized (per unit weight) flows, tissue vascular resistance and (peripheral) AVA-flow of organs and organ groups using different equations. In addition, both primary (measured) and some specified secondary (derived) haemodynamic variables associated with the microsphere data can be simultaneously processed. Both the original data and the obtained results, per experiment, are saved. When needed, the results of a group of experiments can be analyzed statistically using other programs compatible with the output of the present programs.

The purpose of this paper is to describe in detail the three programs needed for the determination of regional blood flow and the processing of haemodynamic variables. The programs are written in FORTRAN IV. They are run completely conversationally so that people who are not familiar with computer handling can use the programs with little help.

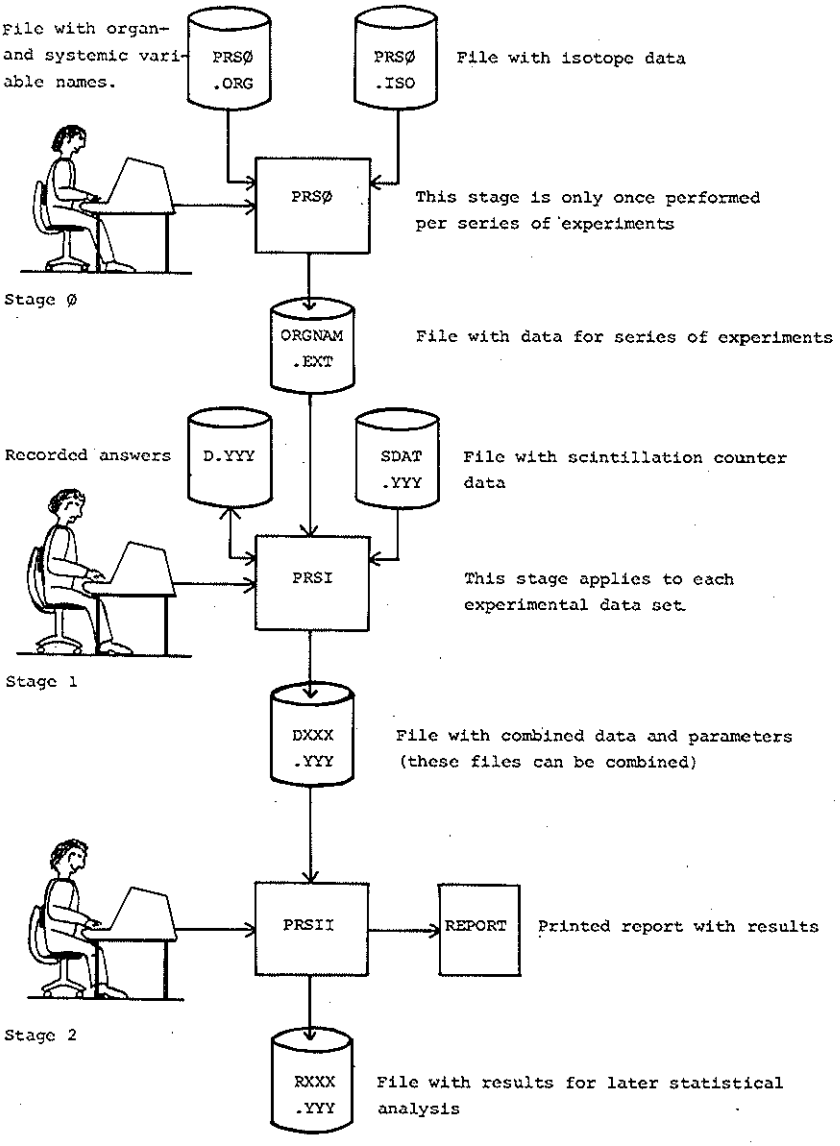

fig.5.1. Processing stages using PRSO, PRSI and PRSII. The file D.YYY can either be prepared off-line or be created by reading data from the terminal. Several files DXXX.YYY can be combined and analyzed in one run of PRSII.

5.2. PRINCIPLES OF THE MICROSPHERE METHOD AND PROGRAM DESIGN

5.2.1 Microsphere method

The principles, the validation tests and other relevant details pertaining to the radioactive microsphere technique have been described elsewhere (Rudolph and Heymann, 1967; Hoffbrand and Forsyth, 1969; Wagner et al., 1969; Buckberg et al., 1971; Hales, 1974; 3M Bibliography, 1976). The microspheres (available in several diameters and labelled with different gamma-emitting radionuclides) are injected into the left side of the heart or into an artery. Since the spheres are entrapped in the capillaries and the arterioles, the ratio of the tissue radioactivity (Itis) and the injected radioactivity (Itot) represents the fraction of blood flow delivered to that tissue. The measurement of Itot is not obligatory if an arterial blood sample is withdrawn during the microsphere injection (flow rate $\dot{Q}r$), since the tissue blood flow ($\dot{Q}tis$) can be calculated directly from:

$$\dot{Q}tis=(Itis/Ir)x\dot{Q}r$$

where Ir is the radioactivity in the blood sample. However, if in addition to the withdrawal of an arterial blood sample Itot is known or computed by counting the radioactivity in the whole body, the microsphere method can be used to determine cardiac output. Moreover, comparison of the microsphere content of venous blood with those trapped in the organs delivers direct information concerning the degree of shunting since, depending on the size of the microspheres, a fraction of the injected spheres escapes through arteriovenous anastomoses. With the above possibilities in mind the present programs have been written.

5.2.2. Computer programs

The laborious handling of microsphere data has been subdivided into three parts, as illustrated in fig.5.1. Before processing any experimental data a file ORGNAM.EXT (EXT is specified by the user) is created by PRSO. This file contains the names of the organs, organ groups and systemic haemodynamic variables and isotope data. Secondly, PRSI is used to read data of a particular experiment, including certain special codes as explained later. Various checks are performed and all data are written on the disk. Finally, PRSII performs the analysis of the data on radioactivity and calculates tissue blood flows and some specified systemic haemodynamic variables.

Most calculations that can generate error conditions (e.g. floating zero divide) are tested for and have been protected. PRSO and PRSI test the format of the input data and PRSI determines the consistency of the experimental protocol, counts the number of entered organ-, group- and systemic variable names and warns in case of errors in the scintillation counter output.

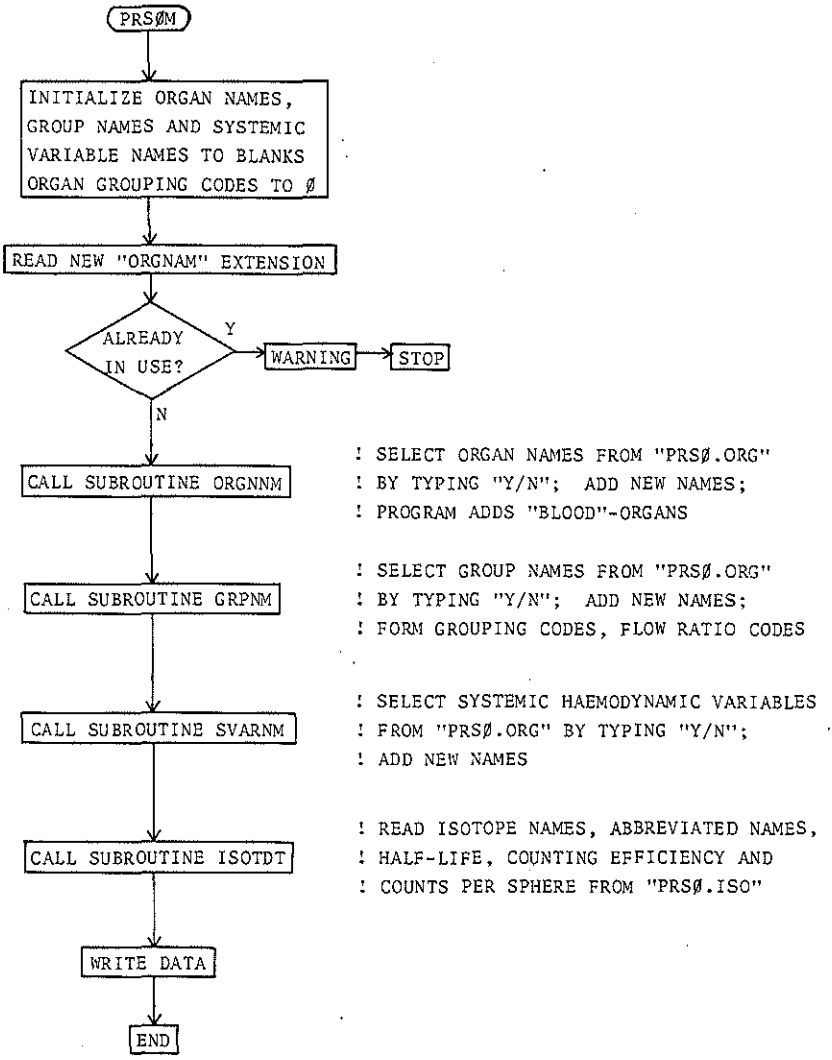

fig.5.2. Flow chart of PRSO.

5.2.3. Program requirements/limitations

The following are the requisites/limitations of PRSO, PRSI and PRSII: (i) a maximum of 60 organs including maximally 25 organ groups can be processed; (ii) between the names of organs and organ groups must be those of arterial and venous blood samples, alternating. Only one arterial and venous blood sample per isotope is permitted here. Any number of arterial or venous samples can, however, be inserted as pseudo-organs. (iii) any individual organ or organ group can be assigned to a maximum of four organ groups; (iv) a maximum of 60 systemic haemodynamic variables, including ratios of the blood flows (e.g. endo/epi ratios in the myocardium) is permitted; (v) a maximum of 300 vials per experiment is allowed and (vi) a maximum of 6 microsphere labels can be used in an experiment.

5.3. PROGRAM PRSO FOR PRODUCING ORGAN NAME FILE "ORGNAM.EXT"

Whenever a new series of experiments is to be initiated, the program PRSO (fig.5.2) has to be used to create a new file ORGNAM.EXT. The main unit of the program, PRSOM, requests the user to specify a new extension (.EXT) for the file. The SUBROUTINES ORGNNM, GRPNM and SVARNM, respectively, read the names of organs, the names of organ groups and the names of systemic haemodynamic variables from a standard file PRSO.ORG. The user selects or rejects these names by typing Y(ES) or N(O) on the terminal after each name. Subsequent to the selection of each group of names from PRSO.ORG one can enter additional names to the list. Instructions for organ grouping, containing the number of the group to which organs will be assigned, and those for the calculation of ratios of blood flows ($ml.min^{-1}.100g^{-1}$) are also generated by GRPNM. Table 5.1 illustrates an example of the use of these instructions.

In both PRSI and PRSII it is not necessary for the variables to be in any fixed sequence, except that the last "organs" must be arterial and venous blood samples which are added to the list automatically by PRSO. For ease of recognition of some variable names by PRSII it is imperative that their names be written in a predetermined format (e.g. mean arterial blood pressure (MBP)).

Following the selection of the various names and codes, SUBROUTINE ISOTDT reads data concerning isotopes (see table 5.2) from another standard file PRSO.ISO. The isotope data consist of their complete and abbreviated names, half-life, efficiency for gamma-counting, counts per minute per microsphere (cpm/sphere) and the date on which cpm/sphere was determined. Finally, the user types the name of the device (e.g. SY0:) and the name of the data file (output of PRSI) on the terminal. All of this information is written in the file ORGNAM.EXT. Whenever a new batch of microspheres arrives, the cpm/sphere should be determined and appropriate changes must be made in PRSO.ISO and ORGNAM.EXT.

Table 5.1. An example of a file ORGNAM.EXT to illustrate the use of organ grouping codes and flow ratio codes.

	organ name	grouping codes 1	2	3	4	ratios 5	6
1	LEFT ATRIUM	1	5	0	0	0	0
2	RIGHT ATRIUM	1	5	0	0	0	0
3	LUNGS	0	0	0	0	0	0
4	LV ISCH END1	2	6	0	0	1	0
5	LV ISCH END2	2	0	0	0	0	0
6	LV ISCH EPI1	3	0	0	0	0	0
7	LV ISCH EPI2	3	7	0	0	0	1
	9999	0	0	0	0	0	0
1	ATRIA	0	0	0	0	0	0
2	LV ISCH ENDO	4	0	0	0	2	0
3	LV ISCH EPI	4	0	0	0	0	2
4	LV ISCH	5	0	0	0	0	0
5	HEART	0	0	0	0	0	0
6	LV END1	0	0	0	0	3	0
7	LV EPI2	0	0	0	0	0	3
	9999	0	0	0	0	0	0
1	HR (B/MIN)						
2	MBP (MM HG)						
3	CO (ML/MIN)						
	9999						

Left and right atrium together form the group ATRIA; they are also assigned to group 5: HEART. Organs 4 and 5 constitute group 2: LV ISCH ENDO. Organs 6 and 7 constitute group 3: LV ISCH EPI. Groups 2 and 3 are added to form group 4: LV ISCH. Alternatively, a 4 could be noted behind organs 4,5,6 and 7. Flow ratios are noted as: divide organ 4 flow by organ 7 flow; group 2 flow by group 3 flow, and group 6 flow by group 7 flow. The string "9999" denotes the end of the respective names.

5.4. PROGRAM PRSI TO CREATE DATA FILE

5.4.1. General structure

PRSI (fig.5.3) has been subdivided into a main unit (PRSIM) and 9 SUBROUTINES (INIT, RDORGN, EXPINF, ISOTSP, PRTCOL, RDSVAR, WRTOUT, RDORDT AND RDSCDT) and it is used to form a data file for a given experiment. The program obtains its input from three sources - the file ORGNAM.EXT, a file D.YYY, where YYY is the experiment name and the file SDAT.YYY, containing the scintillation counter data. The file D.YYY contains all other data concerning a particular experiment.

5.4.2. Input for PRSI

After initializing variables (SUBROUTINE INIT), RDORGN reads the names of the organs, organ groups, and primary (measured) and secondary (to be derived from the primary) systemic haemodynamic variables, isotope data and the name of the data-file to be formed from ORGNAM.EXT.

The SUBROUTINES EXPINF, PRTCOL, RDSVAR and RDORDT are employed to derive from D.YYY input containing all information specific to a particular experiment as detailed in fig.5.4. It consists of: (a) the extension (.EXT) of the file ORGNAM.EXT to be used for this experiment; (b) date of experimentation from which CPS for each isotope on that day is determined in PRSII0; (c) experiment name, special remarks, weight of the animal, number of background and experimental vials and a code about the type of measurement of cardiac output or aortic flow; (d) window adjustments in the scintillation counter; (e) the site of injection and the isotope labels of the different microspheres used; (f) interventions before each microsphere injection, total dose in counts per minute of the isotopes, arterial and venous blood withdrawal rates and information regarding the calculation of one of the five data conditions: see 5.5.3.7; (g) data for each of the primary (measured) systemic variables measured at each microsphere injection; (h) the number of vials, weight and aliquot weight for each of the organs and (i) the number of vials for each of the arterial and venous blood samples.

All these input data can either be entered via the terminal or can be prepared off-line using paper tape or the TEXT-EDITOR. In order to minimize mistakes, a standard protocol (fig.5.4) has been designed. However, as a result, one has to answer to some superfluous questions; for example: arterial withdrawal rate when no blood sample was withdrawn. In all such cases, and also when a particular variable was not determined in a certain experiment, the input should be given as "0" (zero).

The final source of input to PRSI provides scintillation counter data. The paper tape output of the counter (see section 5.6), which contains data concerning the vial number, the counting time and the counts accumulated in the different windows, is first read into a file SDAT.YYY on the disk. The experimental vials are preceeded by at least one empty vial to determine the background count rate and by vials containing as many pure isotopes as counting windows used.

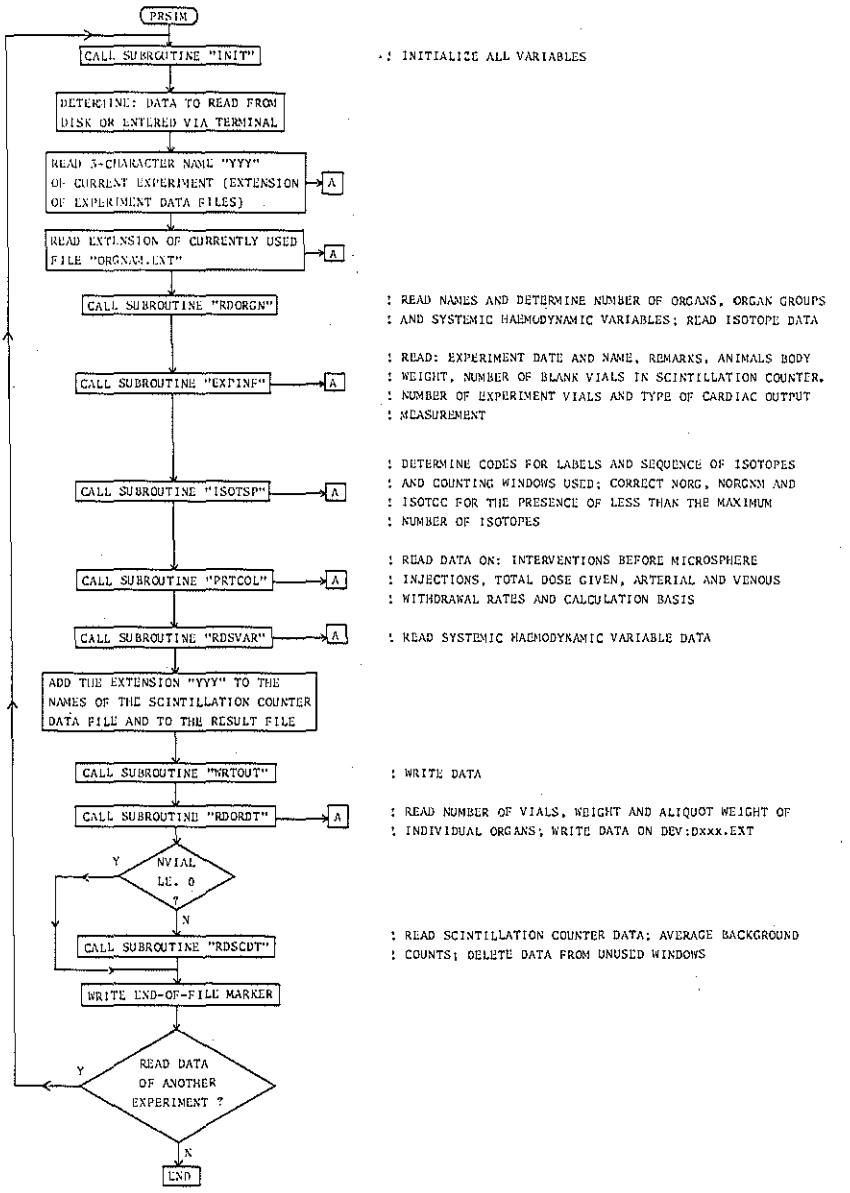

fig.5.3. Flow chart of PRSI. A denotes that data are saved in the file D.YYY when entered from the terminal, while the same file is used for read when information is already present on the disk.

Table 5.2. Data of frequently used microsphere labels.

Isotope	half life days	photo peak keV	recommended window keV	photopeak fraction	abundance %	counting efficiency %
Iodine-125 (IO)	60	27-35	18- 72	0.99	147	1.383
Cerium-141 (CE)	32.5	145	127-167	0.68	48	0.320
Chromium-51 (CR)	27.8	320	268-362	0.69	9	0.067
Tin-113 (SN)	115	393	363-428	0.23	64	0.099
Ruthenium-103 (RU)	39	497	463-537	0.42	88	0.222
Strontium-85 (SR)	64	513	468-568	0.47	100	0.273
Niobium-95 (NB)	35	765	706-826	0.37	100	0.181
Scandium-46 (SC)	84	889	827-952	0.22	100	0.102

A maximum of six isotopes of which the window settings do not overlap, can be chosen from this list. The counting efficiency is defined as the fraction of detected photopeak counts resulting from one radioactive desintegration. The 2-character names between brackets are used in the programs.

```
EXTENSION DATA FILE (EXP.NAME):          D01
EXTENSION "ORGNAM":                      DFE
DATE OF EXPERIMENT:                      20 06 80
EXPERIMENT NAME:                         D01
REMARKS:                                 IO (2ND MICR) GIVEN IV.
WEIGHT OF ANIMAL:                        3
NO. OF BACKGROUND VIALS:                 4
NO. OF EXPERIMENT VIALS:                 122
CARDIAC OUTPUT:                          N  (A  OR  C)        *1*
NO. OF COUNTING WINDOWS:                 4
1ST WINDOW:                              IO
2ND WINDOW:                              CE
3RD WINDOW:                              SR
4TH WINDOW:                              NB
MICROSPHERES INJECTED INTO:              LEFT ATRIUM
NO. OF ISOTOPES USED:                    4
1ST LABEL:                               SR
2ND LABEL:                               IO
3RD LABEL:                               NB
4TH LABEL:                               CE
1ST INTERVENTION:                        BASE LINE
2ND INTERVENTION:                        FEN 25 UG/KG
3RD INTERVENTION:                        FEN 50 UG/KG
4TH INTERVENTION:                        FEN  0 UG/KG
TOTAL DOSE PER ISOTOPE (CPM):            0 0 0 0
ART. WITHDRAWAL RATE (ML/MIN):           4.55 4.55 4.55 4.55
VEN. WITHDRAWAL RATE (ML/MIN):           0 0 0 0
CALCULATIONS BASED ON:                   T  (OR  A)           *2*
HR (B/MIN):                              300 0 258 252
SBP (MMHG):                              115 0 115 135
.
.
ATRIA          NO. VIALS, WT, AL. WT.:   1.2.20 0
RT VENTRICLE   NO. VIALS, WT, AL. WT.:   1 1.48 0
.
.
LUNGS          NO. VIALS, WT, AL. WT.:   3 15.3 0
.
.
ART BLOOD A    NO. VIALS:                4
VEN BLOOD A    NO. VIALS:                0
.
.
VEN BLOOD D    NO. VIALS:                0
```

fig.5.4. Typical example of the protocol sheet, enumerating the questions put by PRSI and the corresponding answers to be given by the user. The protocol sheet and the sequence of the questions have a rigorous format in order to avoid reading errors. Abbreviations: IO, 125-I; CE, 141-Ce; SR, 85-Sr; NB, 95-Nb; WT, weight; AL.WT, aliquot weight. *1*, can be answered as C (cardiac output), A (aortic blood flow) or N (not measured); *2*, can be answered as A (arterial blood sample) or T (total dose of radioactivity). Data not measured are entered as "0" (zero).

These standards are used for the determination of spectral overlap. SUBROUTINE RDSCDT reads data from SDAT.YYY, checks for errors in the input and selects only counts accumulating in those windows which correspond to the isotopes actually used. This facilitates the time-shared use of the gamma-counter with others, who may be using additional isotopes.

5.4.3. Output of PRSI

The SUBROUTINES WRTOUT, RDORDT and RDSCDT are used for output of all data concerning a given experiment into a data file DEV:DXXX.YYY, where DEV and XXX have been specified for the whole series of experiments in the file ORGNAM.EXT and YYY is the experiment name.

A part of the output of PRSI is shown in fig.5.5. The string 1 2 3 4 0 0 after MICROSPHERE SEQUENCE denotes that the injection sequence of microspheres was sequential and had not been changed. If this string is changed with the help of the TEXT-EDITOR, one can shift the results obtained with an isotope into any other column as explained in 5.5.3.9. The next two lines show the remarks and interventions. The isotope data (name, abbreviated name, half-life, counting efficiency, counts per sphere per minute and the date on which they were measured) are specified by PRSI in the ascending order of energy. MICROSPHERE INJECTION SEQUENCE denotes in this example that the third isotope (SR) was given first, the 1st isotope (IO) as second, etc. The selected data condition (see 5.5.3.7) was number 3, while cardiac output (ICO=0) was not measured. The first organ (ATRIA) is assigned to group 1 (HEART). The atria were put in 1 vial and weighed 2.20 gram. The scintillation counter data contain the averaged counts per 10 min of empty vials in vial 296. Vials 297 to 300 are standards containing pure IO, CE, SR and NB, respectively. Vial 1 contains the atria. The file is closed with the end-of-experiment marker "9999".

5.4.4. Error messages from PRSI

The program prints on the terminal error warnings when: (i) answers on the program generated questions are given incorrectly; (ii) the number of organs, groups or systemic variables exceeds the array dimensions; (iii) missing vials are found in the scintillation counter data; (iv) any vial is counted twice; (v) a fault is recognized in the scintillation counter data; (vi) end-of-file is encountered in the scintillation counter data before the required number of vials has been read, and (vii) the total number of vials attributed to the experiment does not correspond with the sum of the vials attributed to the individual organs.

```
DFEN.D01  EXPERIMENT DATE: 20- 6-80  NO OF MICROSPHERE LABELS:  4
MICROSPHERE SEQUENCE: 1 2 3 4 0 0
IO (2ND MICR) GIVEN IV.
BASE LINE    FEN 25 UG/KG  FEN 50 UG/KG  FEN  0UG/KG
WEIGHT  OF THE ANIMAL:   3.00 KG
NO OF ORGANS:  36 NO OF GROUPS:  6   NO OF SYST. VARIABLES:  14+  8
NO OF VIALS: 122
IODINE       IO   60.0    1.383    16.400
CERIUM       CE   32.0    0.320     6.380
STRONTIUM    SR   64.0    0.272     2.430
NIOBIUM      NB   35.0    0.181     3.210
ISOTOPE CPS WERE COUNTED ON: 14- 3-80
MICROSPHERE INJECTION SEQUENCE: 3 1 4 2
IDATA= 3    ICO= 0   MICROSPH. INJECTED INTO: LEFT ATRIUM
    TOTAL DOSE              0.          0.          0.          0.
    WRA                  4.550       0.000       4.550       4.550
    WRV                  0.000       0.000       0.000       0.000
 1. HR (B/MIN)         300.000       0.000     258.000     252.000
 2. SBP (MM HG)        115.000       0.000     115.000     135.000
  .
  .
21. NCO (ML/MIN)         0.000       0.000       0.000       0.000
22. NRES (U)             0.000       0.000       0.000       0.000
 1 ATRIA          1  0  0  0  0  0  1          2.20        0.00
 2 RT VENTRICLE   1  0  0  0  0  0  1          1.48        0.00
 .
 .
 7 LUNGS          0  0  0  0  0  0  3         15.30        0.00
 .
 .
29 ART BLOOD A    0  0  0  0  0  0  4          0.00        0.00
30 VEN BLOOD A    0  0  0  0  0  0  0          0.00        0.00
 .
 .
36 VEN BLOOD D    0  0  0  0  0  0  0          0.00        0.00
 1 HEART          0  0  0  0  0  0
 .
 .
 6 TOTAL BRAIN    0  0  0  0  0  0
296 10.0        368.        200.        244.         99.
297 10.0     936557.        223.        259.        106.
298 10.0     192194.     612470.        326.        151.
299 10.0      88279.      70745.     565338.        531.
300 10.0      41007.      34561.      41479.     257474.
  1 10.0      25207.      20378.      20953.       9851.
  2 10.0      18304.      38983.      33439.      16858.
  3 10.0      21108.      45138.      39508.      20829.
  4 10.0      17825.      38051.      33579.      16298.
  .
  .
121 10.0        327.        306.        230.        134.
122 10.0        278.        217.        256.        113.
9999
```

fig.5.5. Part of the output of PRSI showing data file DFEN.D01 for the experiment D01. See 5.4.3 for further details.

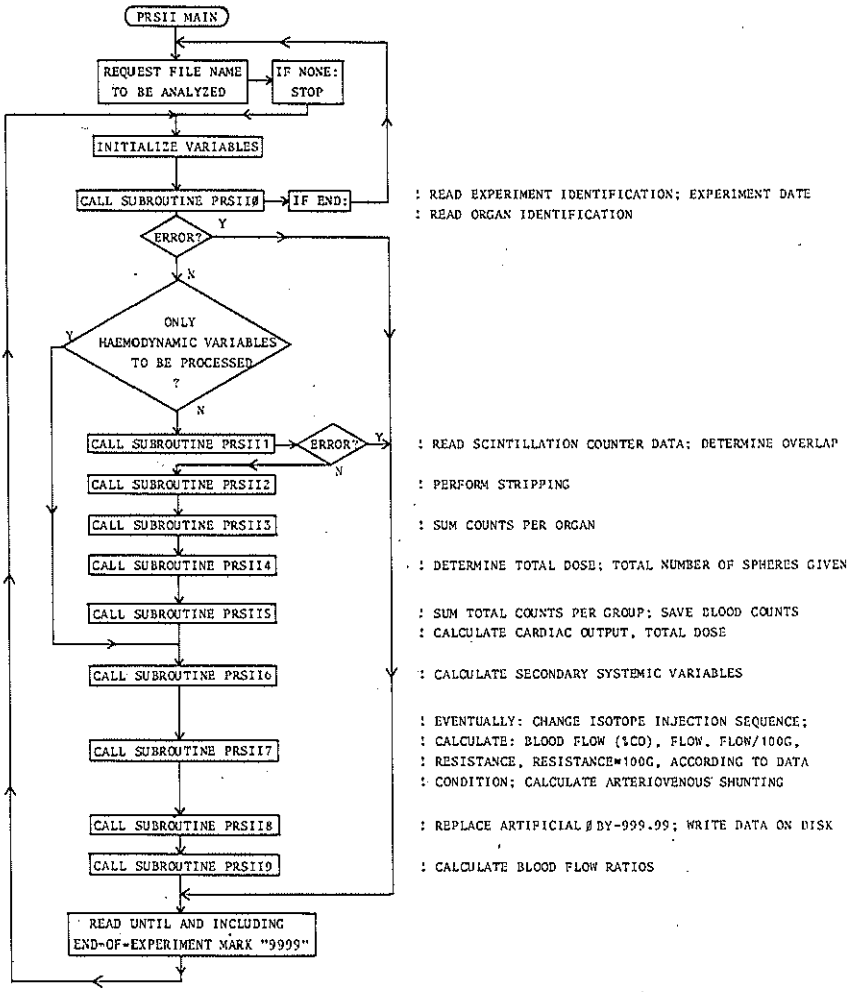

fig.5.6. The flow chart of the main program PRSIIM. The function of the SUBROUTINES is denoted after the exclamation marks. When only haemodynamic variables have to be processed the reading of scintillation counter data and the calculation of blood flows is skipped. If an ERROR condition is encountered the analysis of the current experiment is suspended and the marker "9999" is located. Subsequently, the analysis of the next experiment starts.

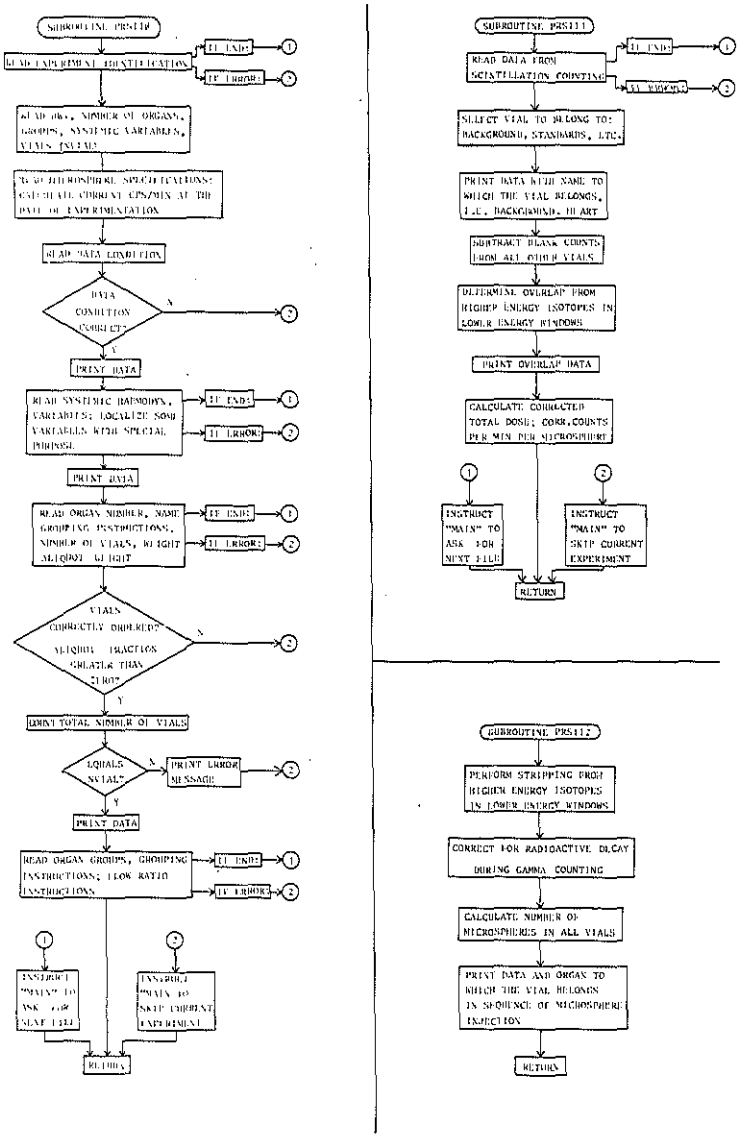

fig.5.7. Flow charts of PRSII0, PRSII1 and PRSII2. The flow charts of PRSII0 and PRSII1 show that both ERROR and END-OF-FILE conditions are tested in the same read instruction. BWT denotes body weight. The SUBROUTINES PRSII1 and PRSII2 read scintillation counter data and decompose the isotope spectra by the "stripping" method.

5.5. PROGRAM PRSII FOR CALCULATING REGIONAL BLOOD FLOW VALUES

5.5.1. General considerations

The program PRSII contains a main unit PRSIIM which sequentially calls 10 SUBROUTINES, named PRSII0, PRSII1 to PRSII9, and its purpose is to calculate the blood flow values to organs and organ groups as specified by PRSI. If any of the blood flow values cannot be calculated or if any of the systemic haemodynamic values is not measured, such variables shall be assigned a dummy value -999.99 for exclusion in the statistical programs to be used later. If ascending aorta blood flow (AOF) has been filled in, an attempt will be made to locate HEART (or LEFT VENTRICLE) as an organ and its flow is added to AOF to calculate cardiac output. Some of the specified systemic haemodynamic variable data (e.g. cardiac output per kg body weight) are calculated automatically. The results are listed on the line printer and are saved on the disk.

5.5.2. Input for PRSII

The output of PRSI constitutes the input for PRSII. Any data file made by PRSI can be analyzed by PRSII by typing its name on the terminal. If several experiments are to be analyzed sequentially, the data files belonging to these experiments are combined into a new file and its name is entered on the terminal. The end-of-experiment marker "9999" separates the various experiments.

5.5.3. Flow charts and description of PRSII

The essential features of PRSII are shown in figs.5.6-5.9.

5.5.3.1. MAIN (fig.5.6)

Upon starting the program, MAIN asks for the name of the data file (containing the data of one or more experiments) to be analyzed. All variables are initialized and the subroutines are called. If an error condition is encountered, the analysis of the on-going experiment is suspended and the end-of-experiment marker is located. Upon finding the marker, the control of the program goes back to the initial segment, the variables are initialized and the analysis of the next experiment begins. After succesful analysis of an experiment the end-of-experiment marker in the specified file is again located and the analysis of the next experiment begins until all experiments in that file have been analyzed. Thereupon the program control goes back to the very begin to request for the next data file. The program stops if "RETURN" is typed upon such request.

5.5.3.2. SUBROUTINE PRSII0 (fig.5.7)

SUBROUTINE PRSII0 reads data as specified in the output of PRSI (see 5.4.3). The counts per minute per microsphere on the day of the experiment is calculated. If aliquot weight was filled in as zero (e.g. when the whole organ was counted) aliquot fraction is made 1; otherwise aliquot fraction is calculated by dividing aliquot weight by

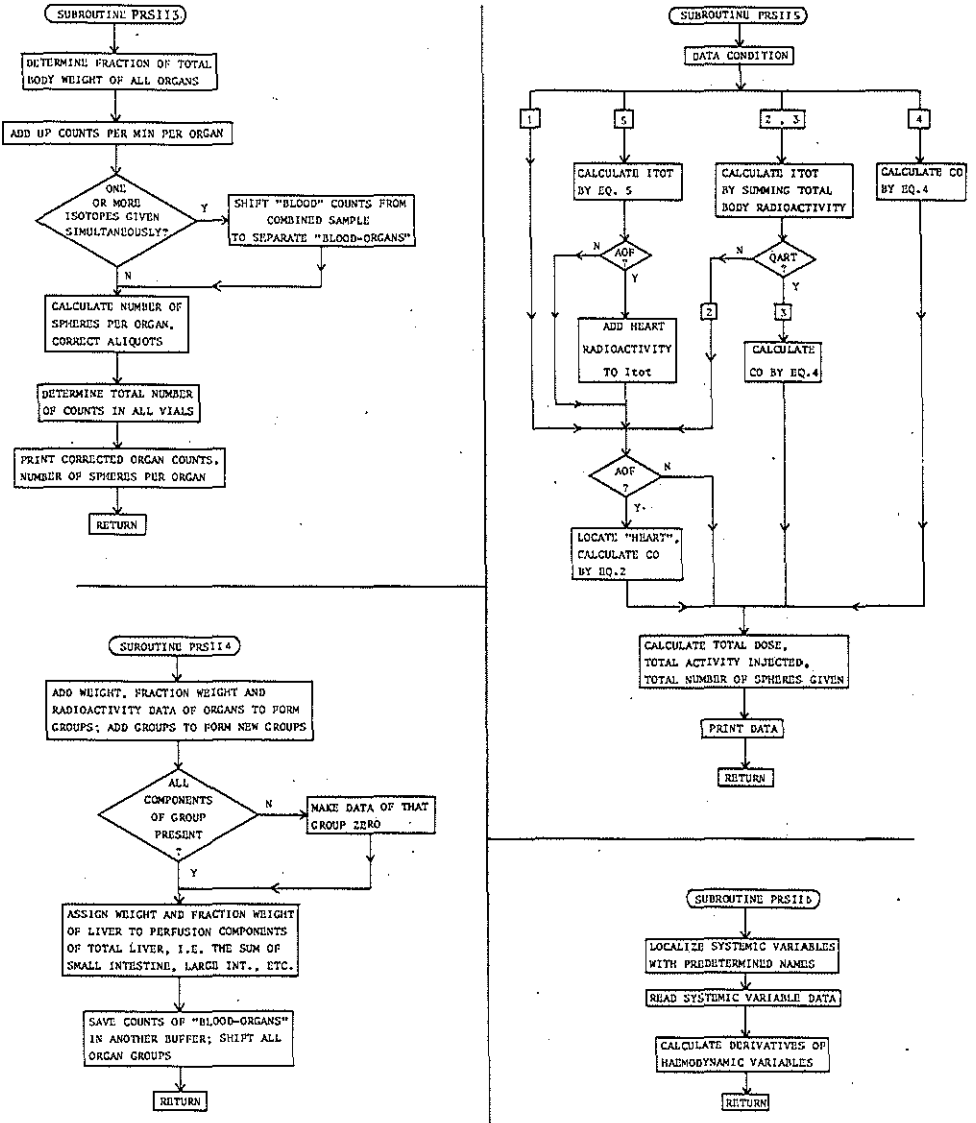

fig.5.8. This figure shows the flow charts of PRSII3, PRSII4, PRSII5 and PRSII6. When two or more isotopes are given simultaneously, there are no corresponding "blood" vials, though the reference sample flow rate is greater than zero. The counts belonging to the different isotopes are then shifted to the regular place. PRSII4 is used to form organ groups from data of the individual organs, as illustrated in table 5.1. The flow chart of PRSII5 shows the calculation pathways in the different data conditions; compare with 5.5.3.7! PRSII6 calculates the secondary (derived) haemodynamic variable data after localization of the necessary primary (measured) variables. Abbreviations: Itot, total dose of microspheres injected; QART, arterial reference sample flow rate; AOF, aortic flow; CO, cardiac output.

the organ weight. Although already performed in PRSI, the vials belonging to each organ are summed up and if the sum does not equal the total number of experimental vials, an error message is generated.

5.5.3.3. SUBROUTINE PRSII1 (fig.5.7)

The scintillation counter data, consisting of accumulated counts in the different windows and the counting time is read and converted into counts per minute (cpm) per vial in each window. The background radioactivity obtained from counting empty vials is deducted from the cpm in all other vials. Subsequently, the overlap from higher energy isotopes in the lower energy windows is calculated. A typical example is shown in fig.5.10a, marked B. The corrected total dose and the corrected counts per microsphere per minute are calculated by dividing the total dose and cpm/sphere by the fraction of counts in its own window.

5.5.3.4. SUBROUTINE PRSII2 (fig.5.7)

This subroutine performs the "stripping" procedure (Heymann et al., 1977) to deduct the net counts per isotope from the mixed spectrum. Furthermore, a correction for the decay of the isotopes during counting is carried out. Since the chosen isotopes have a relatively long half-life (table 5.2), the correction is not absolutely essential. However, if the counting time per vial is long (e.g. 20 min) or an experiment contains a large number of vials, we felt that the correction for isotope decay would improve the reliability of the data. The correction according to equation 1 has been incorporated in PRSII2:

$$cpm(t=0)=cpm(t)\times EXP((\ln 2/T_{1/2})\times t) \qquad (1)$$

where t=0 is the time at which the first experimental vial was counted and $T_{1/2}$ is the half-life of the involved isotope (see table 5.2). Lastly, the counts per vial per isotope are ordered in the sequence of isotope injection, using microsphere injection sequence codes. The number of microspheres per vial is calculated by dividing the cpm per vial by the corrected counts per microsphere of the respective isotopes.

5.5.3.5. SUBROUTINE PRSII3 (fig.5.8)

This subroutine calculates the fraction of total body weight per organ and adds up the cpm and number of spheres due to the different isotopes from vials belonging to the same organ. Thereafter, the total cpm/organ per isotope is obtained by dividing the sum cpm by aliquot fraction. If the isotopes were given simultaneously and an arterial sample was withdrawn, the radioactivity data are assigned to the respective blood organs.

5.5.3.6. SUBROUTINE PRSII4 (fig.5.8)

The number of vials, weight, fraction weight and radioactivity of the specified organs are added to form organ groups; see example in table 5.1. In case TOTAL LIVER (portal blood flow) is present as organ group, the LIVER weight will represent its weight and not the total of the weights of the components constituting TOTAL LIVER flow. If any of the components of a group is missing, the data of that group are made zero. Lastly, the arterial and venous blood sample counts are saved in separate buffers and arterial and venous blood are deleted from the list of organ names.

5.5.3.7. SUBROUTINE PRSII5 (fig.5.8)

This subroutine calculates the total dose (Itot) and the cardiac output depending on the data condition.

Data condition No 1: The essential aspect of this data condition is that Itot is already known from the difference of the radioactivity in the syringe before and after the injection of microspheres. Arterial blood sample has not been withdrawn but cardiac output (CO) or ascending aorta flow (AOF) may have been determined by some other technique. If AOF was filled in, CO will be calculated as:

$$CO = AOF/(1-Iheart/Itot) \qquad (2)$$

where Iheart is the radioactivity in the heart.

Data condition No 2 or 3: In these data conditions Itot is calculated by summing the radioactivity of the whole body:

$$Itot=\Sigma Itis+Iart+Iven \qquad (3)$$

where ΣItis is the sum of the tissue radioactivities and Iart and Iven are the radioactivities in the arterial and venous blood samples, respectively. Data condition 2 is specified when an arterial blood sample has not been withdrawn but CO or AOF may have been measured. If AOF (and not CO) has been filled in, CO will be calculated by equation 2. If both AOF and CO are unknown and an arterial blood sample has been drawn (data condition 3), CO will be calculated from equation 4:

$$CO=(Itot\times\dot{Q}art)/Iart \qquad (4)$$

where $\dot{Q}$art is the rate of withdrawal of arterial blood.

Data condition No 4: The feature of this data condition is that, as in data condition 1, Itot is known from the syringe difference. However, in stead of CO (or AOF) being known, arterial blood samples have been taken. Accordingly, CO will be calculated from equation 4.

Data condition No 5: It signifies that arterial blood sample is withdrawn but Itot has not been determined beforehand. If CO (or AOF)

is known, Itot would be calculated as:

$$Itot=(CO \text{ (or } AOF)xIart)/\dot{Q}art \quad (5)$$

In case AOF is used in the above equation the radioactivity in the heart will be added to Itot. In addition, CO will be calculated from AOF using equation 2.

Before returning the control to MAIN, the total dose in cpm and in microcuries, the summed radioactivity in all experimental vials of the particular experiment, and the total number of spheres given are calculated. The calculation of the radioactivity in microcuries, performed using the photopeak fraction of the isotopes, their counting efficiency and abundance (table 5.2), are carried out to determine radioactive waste.

5.5.3.8 SUBROUTINE PRSII6 (fig.5.8)

The purpose of this subroutine is to calculate a number of secondary (derived) haemodynamic variables from the primary variables that have been measured. In order to identify the sequence in which the data of these variables appear in the output of PRSI, PRSII6 reads the names of the variables from the standard organ file PRSO.ORG and compares them with those already read from ORGNAM.EXT. The secondary variables include cardiac output/kg, stroke volume, total systemic vascular resistance, differences in arteriovenous blood gases, etc. A full list has been included in the listing of this SUBROUTINE. Should a need arise to calculate variables other than those included so far, both PRSO.ORG and SUBROUTINE PRSII6 can be adapted easily.

5.5.3.9. SUBROUTINE PRSII7 (fig.5.9)

If the required instruction has been given (see MICROSPHERE SEQUENCE in fig.5.5), the top part of this subroutine changes the sequence of the microsphere data, the corresponding haemodynamic variables and the tissue radioactivities. For example, if the values of MICROSPHERE SEQUENCE have been filled in as 4 3 1 0 5 2, the fourth isotope will become 1st, the 3rd isotope 2nd, the 1st isotope 3rd, the data of the 4th isotope will be made zero, the 5th isotope will remain 5th and the 2nd isotope will be shifted to the last. Such changes may be needed for statistical analyses where the results obtained with a certain dose of a drug must be brought in the same column.

The subroutine then calculates the regional blood flow values and A-V shunting. The percent cardiac output delivered to the tissues (%COtis) is calculated as:

$$\%COtis=(Itis/Itot)x100\% \quad (6)$$

In data conditions 1,2 or 3 the tissue blood flow is calculated from:

$$\dot{Q}tis=(Itis/Itot)xCO \quad (7)$$

or in data conditions 4 or 5 from:

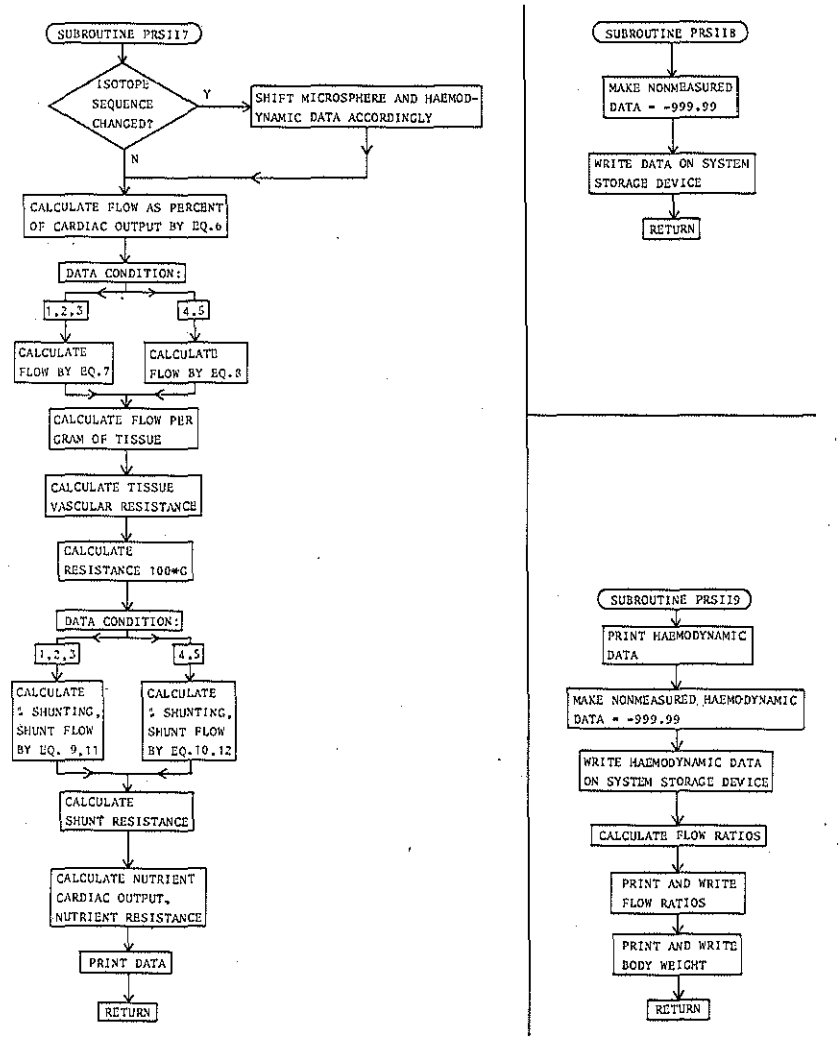

fig.5.9. Flow charts of PRSII7, PRSII8 and PRSII9. The top part of PRSII7 uses the numbers associated with MICROSPHERE SEQUENCE in the data file to eventually rearrange data columns. Thereafter, blood flows and tissue vascular resistances are calculated according to the equations prescribed by the data condition. Finally, the shunting variables are obtained. Before writing the data on the disk all variables which have not been measured or calculated are assigned a dummy value -999.99 . Finally, the animals body weight is printed and written.

$$\dot{Q}tis=(ItisxQart)/Iart \quad (8)$$

The tissue blood flow per gram of tissue is derived by dividing flow by tissue weight; the tissue vascular resistance by dividing driving pressure by flow.

The arteriovenous shunt fraction (%AVA) can be calculated from radioactivity in arterial and venous blood samples from the pulmonary artery, or from a representative local vein in data conditions 1,2 or 3 using:

$$\%AVA=(Iven/Itot)x(CO/\dot{Q}ven)x100\% \quad (9)$$

where $\dot{Q}ven$ denotes the venous withdrawal rate, and in data condition 4 or 5 by:

$$\%AVA=(Iven/Iart)x(\dot{Q}art/\dot{Q}ven)x100\% \quad (10)$$

An index of local tissue AVA shunt flow ($\dot{Q}AVA$) can be determined by multiplication of the shunt fraction and the venous withdrawal rate, yielding:

$$\dot{Q}AVA=(Iven/Itot)xCO \quad (11)$$

in data conditions 1,2 or 3, and:

$$\dot{Q}AVA=(Iven/Iart)x\dot{Q}art \quad (12)$$

in data conditions 4 or 5.

The microsphere content of the lungs (Ilung) can be used as a fair index of total peripheral AVA flow since the bronchial arterial contribution into Ilung is comparatively rather small (Johnston and Saxena, 1978; Saxena, 1978; Saxena et al., 1978). The nutrient fraction of CO is then calculated by subtracting $\dot{Q}lung$ from CO. PRSII7 prints the regional blood flow data on the line printer.

5.5.3.10. SUBROUTINE PRSII8 (fig.5.9)

This subroutine is used to output data on the disk. The experiment name appears as extension on the result file name. However, before this is done all values that could not be calculated are assigned a dummy value of -999.99 which is ignored while performing statistical analyses.

5.5.3.11. SUBROUTINE PRSII9 (fig.5.9)

The values of the systemic haemodynamic variables are printed. If some of these variables were not calculated or recorded in the current experiment they are converted into -999.99 . Thereafter, the flow ratios are calculated (for an example, see table 5.1). These ratios are then printed and written on the output file. The organ numbers of which the blood flow ratio has been calculated is given in

PROGRAM "PRSII" EXECUTED: 09-JUL-80 AT 14:06:48

****

RESULTS BELONGING TO : DO1

****

EXPERIMENT CONDUCTED ON: 20- 6-80

IO(2ND MICR) GIVEN I.V.

A

DATA CONDITION NO: 3

CO AND AOF UNKNOWN

NVIAL=122 NORG= 36 NOGP= 6

		BP (MM HG)	AOF OR CO (ML/MIN)	CPM/ SPHERE	ART.WITH. (ML/MIN)	VEN.WITH. (ML/MIN)	TOTAL DOSE (CPM)	INTERVENTION
1	STRONTIUM :	100.	0.000	0.86	4.550	0.000	0.	BASELINE
2	IODINE :	0.	0.000	5.41	0.000	0.000	0.	PEN 25UG/KG
3	NIOBIUM :	105.	0.000	0.48	4.550	0.000	0.	PEN 50UG/KG
4	CERIUM :	120.	0.000	0.80	4.550	0.000	0.	PEN 0UG/KG

DO1 NAMES OF ORGANS AND GROUPS, GROUPING CODES, NUMBER OF VIALS,
DO1 WEIGHT AND ALIQUOT WEIGHT, SEE OUTPUT OF "PRSI"

DO1 SCINTILLATION COUNTER OUTPUT, SEE OUTPUT OF "PRSI"

FRACTION OF CNTS IN LOWER ENERGY WINDOWS

B

WINDOW:	IO	CE	SR	NB
ISOTOPE				
IO	0.9999	0.0000	0.0000	0.0000
CE	0.2385	0.7613	0.0001	0.0001
SR	0.1214	0.0974	0.7805	0.0006
NB	0.1088	0.0920	0.1104	0.6888

CORRECTED COUNTS PER VIAL IN ONE MINUTE AND NUMBER OF SPHERES PER VIAL

C

	ORGAN	VIAL NO.	STRONTIUM		IODINE		NIOBIUM		CERIUM	
				(SPHERES)		(SPHERES)		(SPHERES)		(SPHERES)
DO1	ATRIA	1	2456.	(2231.)	1516.	(280.)	1416.	(2034.)	2165.	(2067.)
DO1	RT VENTRICLE	2	3912.	(3554.)	28.	(5.)	2433.	(3495.)	4301.	(4106.)
DO1	LV EPICARD	3	4608.	(4187.)	7.	(1.)	3010.	(4324.)	4952.	(4727.)
DO1	LV MESOCARD	4	3942.	(3581.)	14.	(3.)	2352.	(3379.)	4186.	(3996.)
DO1	....									
DO1	....									

COUNTS IN ONE MINUTE AND NUMBER OF SPHERES GIVEN
PER ORGAN IN THE ORDER OF ISOTOPE GIVEN

D

			STRONTIUM		IODINE		NIOBIUM		CERIUM	
DO1	ATRIA	1	2456.	(2231.)	1516.	(280.)	1416.	(2034.)	2165.	(2067.)
DO1	RT VENTRICLE	2	3912.	(3554.)	28.	(5.)	2433.	(3495.)	4301.	(4106.)
DO1	LV EPICARD	3	4608.	(4187.)	7.	(1.)	3010.	(4324.)	4952.	(4727.)
DO1	LV MESOCARD	4	3942.	(3581.)	14.	(3.)	2352.	(3379.)	4186.	(3996.)
DO1	....									
DO1	....									

fig.5.10a. A part of the line-printer output of PRSII is shown. The general experiment information is given on the front page (A). In order to locate errors conveniently all important data (original scintillation counter output, overlap matrix, corrected counts per vial and corrected counts per organ) are printed (B, C and D).

E

NAME	ORGANS	NO	JT	WEIGHT	PR. WT.	PERCENT OF CARDIAC OUTPUT			
						SR	IO	NB	CE
D01	ATRIA	1	1	2.20	0.0007	0.61	0.12	0.87	0.77
D01	RT VENTRICLE	2	1	1.48	0.0005	0.98	0.00	1.49	1.54
D01	LV EPICARD	3	1	1.40	0.0005	1.15	0.00	1.85	1.77
D01	....								
D01	LUNGS	7	1	15.30	0.0051	5.35	99.72	22.54	6.72
D01	....								
D01	HEART	29	1	8.70	0.0029	5.78	0.13	8.75	8.82
D01	....								

F

NAME	ORGANS	NO.	JT	BLOOD FLOW ML/MIN			
				SR	IO	NB	CE
D01	ATRIA	1	2	3.22	0.00	3.18	2.89
D01	RT VENTRICLE	2	2	5.12	0.00	5.46	5.73
D01	LV EPICARD	3	2	6.03	0.00	6.76	6.60
D01	....						
D01	LUNGS	7	2	28.02	0.00	82.42	25.06
D01	....						
D01	HEART	29	2	30.27	0.00	32.00	32.89
D01	....						

G

NAME	ORGANS	NO	JT	BLOOD FLOW ML/MIN/100G			
				SR	IO	NB	CE
D01	ATRIA	1	3	146.14	0.00	144.54	131.22
D01	RT VENTRICLE	2	3	346.05	0.00	369.25	387.40
D01	LV EPICARD	3	3	430.96	0.00	482.88	471.49
D01	....						
D01	LUNGS	7	3	183.12	0.00	538.71	163.77
D01	....						
D01	HEART	29	3	347.88	0.00	367.86	378.04
D01	....						

H

NAME	ORGANS	NO.	JT	RESISTANCE			
				SR	IO	NB	CE
D01	ATRIA	1	4	31.10	0.00	33.02	41.57
D01	RT VENTRICLE	2	4	19.53	0.00	19.21	20.93
D01	LV EPICARD	3	4	16.57	0.00	15.53	18.18
D01	....						
D01	LUNGS	7	4	3.57	0.00	1.27	4.79
D01	....						
D01	HEART	29	4	3.30	0.00	3.28	3.65
D01	....						

I

NAME	ORGANS	NO	JT	RESISTANCE/100G			
				SR	IO	NB	CE
D01	ATRIA	1	5	0.68	0.00	0.73	0.91
D01	RT VENTRICLE	2	5	0.29	0.00	0.28	0.31
D01	LV EPICARD	3	5	0.23	0.00	0.22	0.25
D01	....						
D01	LUNGS	7	5	0.55	0.00	0.19	0.73
D01	....						
D01	HEART	29	5	0.29	0.00	0.29	0.32
D01	....						

J

SYSTEMIC HAEMODYNAMIC VARIABLES AND ENDO/EPI RATIOS.

		SR	IO	NB	CE
D01	1. HR (B/MIN)	300.000	0.000	258.000	252.000
D01	2. SBP (MM HG)	115.000	0.000	115.000	135.000
D01	.				
D01	.				
D01	21. MCO (ML/MIN)	495.438	0.000	283.238	347.670
D01	22. NRES (U)	201.842	0.000	370.713	345.155
D01	23. RATIO(3/ 5)	0.976	0.000	1.007	1.006
D01	24. BODY WEIGHT	3.000			

fig.5.10b. The haemodynamic variables including tissue blood flow as percent cardiac output (E), absolute flow (F) and flow per 100 gram of tissue (G) and tissue vascular resistances (H and I) are both printed and written on the disk. The final information concerning systemic haemodynamic variables and the blood flow ratios is closed by the animals body weight.

parentheses.

5.5.4. Output of PRSII (fig.5.10a, 5.10b)

As already stated, the output of PRSII goes to two devices: line printer and disk. The output on the line printer consists of detailed information regarding (i) experiment name; (ii) isotope names, sequence, interventions; (iii) special remarks (marked A in fig.5.10a); (iv) the names of organs, groups, grouping codes and the number of vials, weight and aliquot weight of the organs (B); (v) scintillation counter output, consisting of counts accumulated in the different windows and the counting time (B); (vi) fraction of counts in lower energy windows (B); (vii) corrected counts per minute and number of microspheres per vial in the order of isotope given (C); (viii) corrected counts per minute and number of spheres per organ (D); (ix) total dose and number of spheres injected; (x) weight, fraction weight, percent of cardiac output (E), flow (F), flow per 100 gram of tissue (G), tissue vascular resistance (H), resistance per 100 gram of tissue (I); (xi) systemic haemodynamic variables (J); (xii) flow ratios, and (xiii) body weight (J). The output to the disk contains only the latter parts (x-xiii) of the above output.

5.5.5. Error messages from PRSII

Since PRSII uses as input the data files formed with the help of PRSI -where a large number of possible error conditions are already checked- it is not likely that an error should result during a run of PRSII, unless certain changes have been incorrectly made via the TEXT EDITOR in the data file. The program generates error messages in the following situations: (i) some essential organ names, organ group names or systemic haemodynamic variables are not specified; (ii) the number of vials attributed to the experiment does not correspond with the sum of vials attributed to the individual organs and (iii) vials are not in sequence.

5.6. HARDWARE REQUIREMENTS

A gamma scintillation counter (Packard Instruments, model 5986, Downers Grove, Ill., U.S.A.), equipped with a multichannel pulse height analyser was used for the determination of microsphere radioactivity. The computer programs have been run using PDP11/10 (RT11-system) or PDP11/70 (RSX11M-system) configurations, connected with console, disk, paper-tape reader and line-printer. However, any computer system which is able to compile and execute programs written in FORTRAN IV and is connected to suitable peripheral devices, after minor modifications, can be used.

5.7. SOFTWARE SPECIFICATIONS

The programs PRS0, PRSI and PRSII have been written in FORTRAN IV. SUBROUTINE RDSCDT can easily be made compatible with other gamma-counters. The programs consume only a few seconds central processor time and an experiment can be analyzed, including the line printer output, within a few minutes. The line printer output is designed for a printer having 132 characters per line.

5.8. PROGRAMS FOR STATISTICAL ANALYSIS

The output of PRSII is compatible with the input of certain statistical programs, which will be described later. It will suffice here to mention the different calculations that can be performed for a group of experiments. These are: (i) mean, SEM and SD of base-line values; (ii) mean, SEM and SD of the variables and their changes from the base line, together with a Student's paired t-test and a Wilcoxon's rank test; (iii) significance of differences between the variables of two groups using the Mann-Whitney U-test; (iv) correlation between any two variables in a group of experiments using parametric (linear) or non-parametric (Spearman-Rank) tests and (v) X-Y-plot diagram of any two variables.

5.9. DISCUSSION

5.9.1. Decomposition of gamma spectra

The count rates belonging to the individual nuclides can be derived from the mixture of nuclides by using either the "stripping" (Heymann et al., 1977) or the "matrix" (Schosser et al., 1979) method. When isotopes are considered having overlap only in lower energy windows, the two ways of decomposing the nuclide-mixture are essentially identical. The "matrix"- (Schosser et al., 1977) and the "stripping" methods (Heymann et al., 1977) differ only when the nuclide spectra overlap into energy ranges both higher and lower than their own photopeak, since the "stripping" method considers only the overlap into the lower energy range. Consequently, the "matrix" method can indeed increase the choice of isotopes to be used in the same experiment by including those which overlap into higher energy range (e.g. ^{169}Yb); this may be an advantage if one whishes to obtain more frequent measurements of "high flow" organs such as the kidneys. However, it must be emphasized that the limitation on the number of isotopes that can be used in one experiment, especially when one includes "low flow" organs as well, is not primarily due to the lack of the availability of suitable isotopes with distinct photo-peak energies and only lower energy scatter but, much earlier, it is due to problems associated with deterioration of the biological preparation, blood loss and microvascular embolism. For this reason we could use the "stripping" method and have limited the program to the use of 6

isotopes. Should one desire, on can relatively easily replace the "matrix" method for the "stripping" method and enlarge the arrays for the use of more isotopes per experiment. We feel, however, that there would be no substantial advantage for most users who use a maximum of 4 isotopes in any given experiment (see Hales, 1974; 3M bibliography, 1976; Heymann et al., 1977).

5.9.2. Determination of the total radioactivity injected

Precise determination of Itot by subtracting the radioactivity in the syringe before and after microsphere injection (Schosser et al., 1979) is, in our opinion, subject to error since large amounts of radioactivity cannot be easily counted due to dead time of the detection equipment. Special precautions are generally needed which make the method more prone to error. Alternatively, Itot can be calculated if arterial blood is withdrawn and cardiac output measured by another technique; see equation 5 and Schamhardt et al. (1979); Verdouw et al. (1980). However, it is our view that summing the radioactivity in the total body to obtain Itot is less erroneous but more laborious (Forsyth and Saxena, 1978; Saxena et al., 1978).

5.9.3. Arteriovenous anastomotic (AVA) blood flow

It has been shown that AVAs usually have a diameter of 25 micron and more (Daniel and Prichard, 1956; Sherman, 1963), so that microspheres of smaller size can pass through them to appear into the venous circulation. These spheres are finally trapped in the microvasculature of the lungs (Kaihara et al., 1968; Hoffbrand and Forsyth, 1969). In case the specific tissue through which the microsperes are being shunted is known, the total AVA-blood flow from that tissue ($\dot{Q}avt$) can be calculated as:

$$\dot{Q}avt=\dot{Q}tisx(\%AVA/(100-\%AVA)) \qquad (13)$$

Furthermore, the substitution of equations (7) and (9) or (8) and (10) into (13) yields, for data conditions 1,2 or 3:

$$\dot{Q}avt=(IvenxItisxCO^2)/(Itotx(Itotx\dot{Q}ven-IvenxCO))$$

and for 4 or 5:

$$\dot{Q}avt=(IvenxItisx\dot{Q}art^2)/(Iartx(Iartx\dot{Q}ven-Ivenx\dot{Q}art))$$

In order to convert the tissue blood flow as measured with microspheres ($\dot{Q}tis$) into total flow as could be measured with, for example an electromagnetic flow meter ($\dot{Q}emf$), $\dot{Q}tis$ can be corrected for shunting using the formula:

$$\dot{Q}emf=\dot{Q}tisx(100/(100-\%AVA)) \qquad (14)$$

The above formula is only valid when $\dot{Q}tis$ and %AVA belong to the same tissue. The practice of adding shunt flow (averaged over the total

body) to local tissue blood flow ($\dot{Q}tis$) (Schosser et al., 1979) in an attempt to calculate total blood flow ($\dot{Q}emf$) must, therefore, be strongly discouraged. It is well known (Warren and Ledingham, 1974; Johnston and Saxena, 1978; Saxena, 1978; Saxena et al., 1978) that the degree of AVA-shunting varies enormously from tissue to tissue and, hence, calculation of $\dot{Q}emf$ based on average shunt flow is wrong.

5.10. AVAILABILITY OF PRSI AND PRSII

PRSO, PRSI and PRSII can be made available on floppy-disc, casette or on magtape on a payment of $1500.- . Brief instructions concerning the use of these programs will accompany the listings. The programs will be supported for a period of two years from the date of this publication. Most statistical programs, having been written for the use of maximally 4 different isotopes only, are then available on request free of charge. Requests may be sent to Pramod R. Saxena, M.D., Professor in Pharmacology, Faculty of Medicine, Erasmus University, 3000 DR Rotterdam, The Netherlands (Tel. 010-635388/99).

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Reprint

ARZNEIMITTEL-FORSCHUNG
DRUG RESEARCH

Arzneim.-Forsch. (Drug Res.) in press
Editio Cantor / D-7960 Aulendorf (W.-Germany)

EFFECTS OF OXYFEDRINE ON GLOBAL HAEMODYNAMICS AND MYOCARDIAL LACTATE BALANCE IN ACUTE ISCHAEMIA OF THE PORCINE HEART PREPARATION

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EFFECTS OF OXYFEDRINE ON GLOBAL HAEMODYNAMICS AND MYOCARDIAL LACTATE BALANCE IN ACUTE ISCHAEMIA OF THE PORCINE HEART PREPARATION

Summary. The haemodynamic and myocardial metabolic effects of the beta-adrenergic cardiac stimulant oxyfedrine were studied in young Yorkshire swine. Eleven pigs received a dose of 0.2 $mg.kg^{-1}$ before reduction of the flow through the left anterior descending coronary artery (LAD) to 25% of its base-line value, while another ten animals served as controls. Before LAD-flow reduction oxyfedrine caused an increase by 25% in heart rate (HR), while the maximum rate of left ventricular pressure development (LVdP/dtmax) nearly doubled. The left ventricular ejection time decreased 15% more than expected from the increase in HR alone. The relative increase in diastolic time resulted in an increase in LAD-flow by 30% which exceeded the increased myocardial oxygen demand. The other haemodynamic and myocardial metabolic parameters did not change significantly. The decrease in overall haemodynamic performance during coronary flow reduction tended to be less in oxyfedrine-treated than in untreated groups, although the decreases in mean arterial blood pressure, LVdP/dtmax and cardiac output (20-40%) were not statistically significant between the groups. The lactate uptake during normal myocardial perfusion changed into lactate release during ischaemia and was not modified by oxyfedrine. After 60 minutes of reperfusion the haemodynamic parameters of treated and untreated animals remained depressed and did not differ between the groups. Lactate release returned to uptake within five minutes of reperfusion. Before, during and after coronary flow reduction the arterio-coronary venous difference in lactate concentration correlated well ($r=0.91$, $p<0.001$, $n=92$) with the arterio-coronary venous pH-difference.

It is concluded that oxyfedrine improved the overall myocardial performance during normal myocardial perfusion and ischaemia, without increasing the lactate release of the ischaemic tissue.

6.1. INTRODUCTION

The anaerobic reserve of the heart is very limited (Rubio and Berne, 1975; Weber and Janicki, 1979). Increases in the oxygen demand are compensated to some extent by higher oxygen extraction, but it is primarily the increase in coronary blood flow which is called upon to meet the oxygen needs of the myocardium. It is thus apparent that stenosis of a coronary artery, paticularly in the presence of high myocardial oxygen demand, causes an imbalance in the myocardial oxygen demand and supply relationship. The resultant myocardial

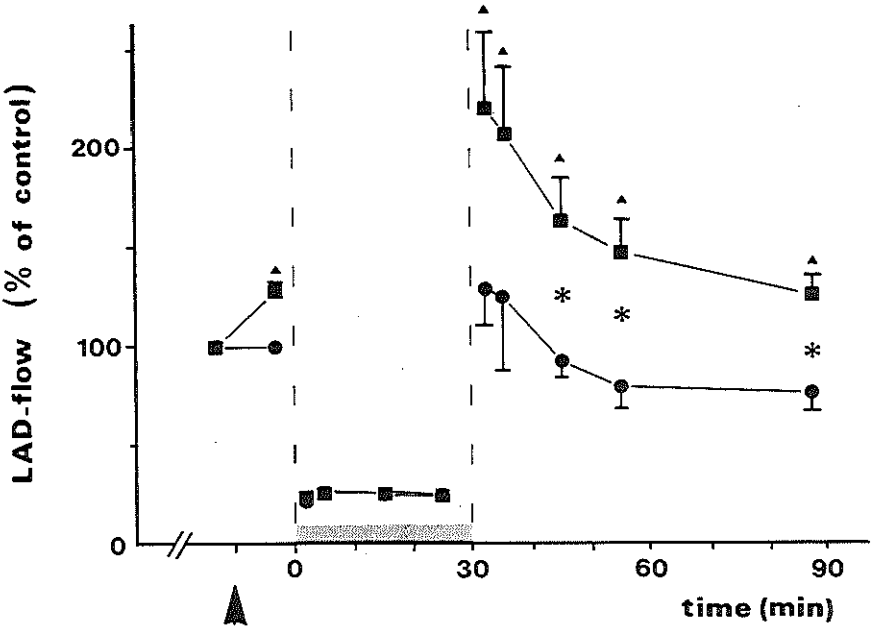

fig.6.1. Flow through the left anterior descending coronary artery (LAD) in controls (n=10, ●) and oxyfedrine treated (0.2 mg.kg^{-1}) pigs (n=11, ■) during base line, partial occlusion (t=0-30 min) and reperfusion (t=30-90 min). During occlusion the flow was kept at 25 % of the base-line values for both groups of animals. The reactive hyperaemia was larger ($p<0.05$) in the treated animals. Values are given as percentage of the base-line data, listed in table 6.1. ✻, $p<0.05$ vs. control group; ▲, $p<0.05$ vs. base-line value.

ischaemia is characterized by certain biochemical changes, e.g. a reduction in the coronary venous oxygen content, a lowering of coronary venous blood pH and a release of lactate from the ischaemic tissue. Furthermore, the contribution of the ischaemic tissue to the contractile force usually diminishes and pump failure can ensue. However, the use of beta-adrenergic stimulants, like isoprenaline, during myocardial ischaemia is controversial and may even be deleterious. Although these agents improve myocardial pump function and may relax coronary arterioles, the concomitant increase in myocardial oxygen demand generally overshadows the increase in oxygen supply (Maroko et al., 1971; Kerber et al., 1974; Vatner et al., 1974; Vatner and Baig, 1978; Vatner and Baig, 1979). In case of the beta-adrenergic stimulant oxyfedrine (ildamen®), it seems that the the enhancement in myocardial oxygen consumption, due primarily to an increase in myocardial contractility (Kuehn et al., 1972; Moore and Parratt, 1972; Raberger et al., 1972; Szekeres et al., 1972; Parratt and Marshall, 1977), but sometimes also in heart rate (Kuehn et al., 1972; Ledingham et al., 1972; Moore and Parratt, 1972; Raberger et al., 1972; Szekeres et al., 1972), is amply met by an elevation in myocardial perfusion (Ledingham et al., 1972; Parratt and Ledingham, 1972; Raberger et al., 1972; Timogiannakis et al., 1978), since the coronary venous oxygen content in the normal myocardium increases (Moore and Parratt, 1972; Kugler et al., 1975). The question arises whether such an effect of the drug is also observed in the presence of myocardial ischaemia where the coronary arteries of the affected segment are probably already maximally dilated due to intrinsic metabolic regulatory mechanisms. Therefore, the present study was undertaken in pigs to investigate the effects of oxyfedrine on the ischaemic myocardial function on the one hand and on the biochemical marker of ischaemia -myocardial lactate release- on the other hand.

Usually, ischaemia has been induced in dogs by complete ligation of a coronary artery but the gross anatomical and physiological differences between the human and the dog heart (Schaper, 1971) make the interpretation in terms of clinical relevance rather difficult. It is for this reason that we have employed pigs which, like humans, do not have an elaborate collateral circulation (Schaper, 1971; Fedor et al., 1978).

6.2. MATERIALS AND METHODS

6.2.1. Experimental preparation

Studies were performed on young Yorkshire pigs (19 to 31 kg). The experimental preparation has been described in detail elsewhere (Verdouw et al., 1978). Briefly, the animals were anaesthetized with azaperone and metomidate (2 and 8 $mg.kg^{-1}.h^{-1}$, respectively) and pancuronium bromide (4 $mg.kg^{-1}$) was infused to achieve neuromuscular blockade. After intubation artificial ventilation was started with a mixture of $N_2O:O_2=2:1$. Respiration was adjusted or sodium

bicarbonate was infused to keep arterial blood gases within the normal range ($7.35<pH<7.45$; $2.5<pCO_2$ (kPa)<3.5; $13<pO_2$ (kPa)<19; arterial oxygen saturation >96 %). A 7F Millar catheter was positioned in the left ventricular cavity for the measurement of pressure (LVP). Mean arterial blood pressure (MBP) was measured via an 8F standard Cournand catheter positioned in the descending aorta. The pulmonary artery was catheterized via the left femoral vein with a triple-lumen balloon-tipped 6F catheter for the determination of cardiac output (CO) by thermodilution. The injection lumen was at the level of the right atrium and was also used for the measurement of the right atrial pressure (RAP). The systemic vascular resistance was calculated as:

$$SVR=(MBP-RAP)/CO \ .$$

The chest was opened via a midsternal split and the heart was exposed. A segment of the left anterior descending coronary artery (LAD) was dissected free from the surrounding tissue between its origin and its first branch. A precalibrated electromagnetic flow probe (Skalar, Delft, The Netherlands) and a J-shaped screw clamp were placed around the vessel. The accompanying coronary vein was catheterized and arterial (a) and coronary venous (cv) blood samples were taken for the determination of blood gases and oxygen saturation. Lactate was measured in 1.5 cm^3 deproteinized blood (Apstein et al., 1970).

6.2.2. Experimental protocol

After completion of the surgical procedure the animals were allowed to stabilize for at least 30 min. Base-line values were then determined and the animals were divided into two groups. Ten animals served as controls and 11 animals were treated with 0.2 $mg.kg^{-1}$ oxyfedrine ten minutes before the flow through the LAD was reduced by tightening the screw on the clamp. In order to obtain the same oxygen-supply during ischaemia in both treated and untreated animals, the flow was reduced to 25 % of its base-line value (fig.6.1) in both groups of animals. Except for the cardiac output the other haemodynamic variables were monitored continuously. The measurement of blood gases, pH and lactate were carried out at base line, seven min after administration of oxyfedrine or saline and at 2, 5, 15, and 25 min of flow reduction. After 30 min the screw on the clamp was released and the LAD-area was reperfused for 60 min. The variables were measured again at 2, 5, 15, 25, and 60 min of reperfusion.

6.2.3. Statistical analysis

The base-line values of all variables have been shown in absolute terms. The effect of the administration of oxyfedrine or saline and of coronary flow reduction and reperfusion are presented, unless otherwise stated, as percentage change from the base-line values. The unpaired Student's t-test (Snedecor and Cochran, 1967) was used to compare the oxyfedrine- and ischaemia induced changes in parameter values between the two groups of animals, while the paired t-test was

Table 6.1. Base-line values of haemodynamic and metabolic parameters.

parameter	units	saline treated n=10	oxyfedrine treated 0.2 $mg \cdot kg^{-1}$ n=11
HR	$b \cdot min^{-1}$	106 ± 6	113 ± 6
MBP	kPa	11.1 ± 0.6	10.8 ± 0.5
LVdP/dtmax	$kPa \cdot s^{-1}$	345 ± 28	380 ± 50
CO	$dm^3 \cdot min^{-1}$	3.05 ± 0.21	3.38 ± 0.22
SVR	$kPa \cdot dm^{-3} \cdot min$	3.55 ± 0.25	3.15 ± 0.23
CBF	$cm^3 \cdot min^{-1}$	39 ± 3	39 ± 3
CVR	$kPa \cdot cm^{-3} \cdot min$	0.277 ± 0.014	0.278 ± 0.017
cv O_2-sat		0.35 ± 0.03	0.312 ± 0.022
a-[lact]	$mmol \cdot dm^{-3}$	3.4 ± 0.4	2.7 ± 0.3
(a-cv)[lact]	$mmol \cdot dm^{-3}$	0.63 ± 0.13	0.65 ± 0.15
(a-cv)pH		0.072 ± 0.011	0.050 ± 0.007

Abbreviations: HR, heart rate; MBP, mean arterial blood pressure; LVdP/dtmax, maximum rate of rise of left ventricular pressure; CO, cardiac output; SVR, systemic vascular resistance; CBF, coronary blood flow; CVR, coronary vascular resistance; cv, coronary venous; O_2-sat, oxygen saturation; a, arterial; [lact], lactate concentration; pH, negative logarithm of proton concentration.

employed for comparisons within a group. A value of $p<0.05$ was considered statistically significant. All values are given as mean±SEM.

6.3. RESULTS

6.3.1. Effects of oxyfedrine during normal perfusion of the myocardium

The base-line values of the haemodynamic and myocardial metabolic parameters are listed in table 6.1. Administration of oxyfedrine increased myocardial perfusion by 30 % ($p<0.001$; fig.6.1), due to a similar decrease in the diastolic coronary vascular resistance. The drug also caused marked changes in many determinants of cardiac output: heart rate increased by 25 % ($p<0.05$), LVdP/dtmax nearly doubled ($p<0.001$), and the left ventricular end-diastolic pressure decreased by 0.24±0.07 kPa (38 %; $p<0.01$). The effects on cardiac output itself were a non-significant increase by 8 %. Neither the systemic vascular resistance nor the mean arterial blood pressure altered after administration of the drug (fig.6.2).

The increase in heart rate from 113±6 upto 136±4 min^{-1} (24±8 %) was accompanied by a decrease in the duration of systole (DS) from 276±11 to 204±5 ms (-25.1±2.2 %; $p<0.001$). Atrial pacing from 80 to 180 min^{-1} in another series of seven pigs (unpublished results) yielded the following linear equation:

$$DS=-1.31*HR+416 \text{ ms} \quad (r=0.97; n=36)$$

The decrease in DS (72±6 ms) after oxyfedrine was 40±4 ms (15 %, $p<0.001$) more than what is predicted by the above equation.

The increase in the myocardial oxygen demand after the administration of oxyfedrine was amply met by an increase in supply, since the coronary venous (cv) oxygen saturation increased by 11±5 % ($p<0.05$). The arterial (a) lactate concentration ([lact]) increased from 2.7±0.3 to 3.4±0.3 $mmol.dm^{-3}$ ($p<0.05$).

6.3.2. Changes during coronary flow reduction

6.3.2.1. Arrhythmias

Reduction of the LAD-flow to 25 % of its base-line value led to ventricular fibrillation in five animals: two in the untreated and three in the treated group. The incidence of ventricular fibrillation corresponds closely with that reported earlier (Verdouw et al., 1978). During reperfusion five animals fibrillated: three in the untreated and two in the treated group. No relation could be observed between the haemodynamic status or the lactate release of fibrillating and surviving animals. Fibrillating animals were included in the study until the incidence of ventricular fibrillation.

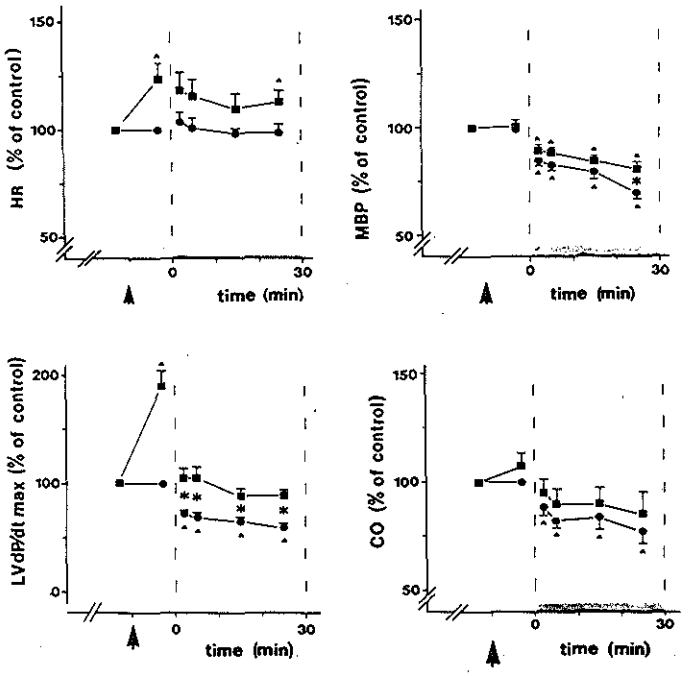

fig.6.2. Effects of pretreatment with 0.2 mg.kg^{-1} oxyfedrine on cardiovascular function during LAD-flow reduction to 25 % of its pretreatment value (fig.6.1.). Comparison with data of the control (●) pigs demonstrate, that oxyfedrine (■) resulted in an increased heart rate (HR), a smaller decrease in mean arterial blood pressure (MBP), higher LVdP/dtmax values and a smaller decrease in cardiac output (CO). ✻, $p<0.05$ vs. control group; ▲, $p<0.05$ vs. base-line values. Base-line values have been listed in table 6.1.

6.3.2.2. Haemodynamics

In the untreated animals, the reduction of the LAD-flow did not affect heart rate but decreased LVdP/dtmax to 60 % of its base-line value. This resulted in a decrease in cardiac output to 78 % (fig.6.2). Since systemic vascular resistance did not change, a similar decrease in mean arterial blood pressure occured.

In treated animals a slightly different pattern was observed. Heart rate decreased gradually, but remained above its base-line value. The decline in mean arterial blood pressure to 80 % of its base-line value was less, compared with those in untreated animals. LVdP/dtmax, although doubled seven min after oxyfedrine administration, returned to its base-line value within two min of LAD-flow reduction and remained consistently larger than in the untreated animals. Cardiac output decreased to 86 % which was a slightly smaller reduction than in the untreated animals (fig.6.2).

6.3.2.3. Regional myocardial metabolism

During coronary flow reduction in untreated pigs, the myocardial oxygen supply to the LAD-perfused area decreased to about 25 % of base line. The oxygen extraction of this area became nearly maximal since the cv-oxygen saturation decreased from 31 to about 20 %. Within two min after the LAD-flow was reduced, the myocardium started to release lactate, reaching a peak after five min. Although the arteriovenous difference in lactate concentration in the treated animals tended to be more negative than in the controls, levels of significance were not reached (fig.6.3). In both untreated and oxyfedrine treated animals an excellent correlation coefficient of $r=0.91$ ($p<0.001$, $n=92$) was found between (a-cv)pH and (a-cv)[lact] (fig.6.4).

6.3.3. Haemodynamics and metabolism during reperfusion

Immediately after the screw on the clamp was released, a reactive hyperaemia was observed (fig.6.1). In untreated pigs the LAD-flow gradually declined to 75 % of base line after 60 min of reperfusion. The diastolic coronary vascular resistance returned to its base-line value. In the oxyfedrine-treated animals, peak reactive hyperaemia was larger than in untreated animals ($p<0.05$; fig.6.1), but the pattern of decline in flow was similar. After 60 min of reperfusion, the LAD-flow was slightly higher than its base-line value, while the diastolic coronary vascular resistance decreased to 60 % of its base-line value. In the untreated animals, heart rate, mean arterial blood pressure, LVdP/dtmax and cardiac output did not improve during reperfusion, while in treated animals only a tendency to a less depressed cardiovascular performance could be noticed.

Myocardial lactate balance of the ischaemic zone returned to base-line values within five min of reperfusion in the untreated and in the treated animals. The coronary venous oxygen saturation increased to 60 % in untreated and to 70 % in oxyfedrine treated animals immediately after onset of reperfusion, and were still elevated after 60 min of reperfusion (45 and 49 %, respectively).

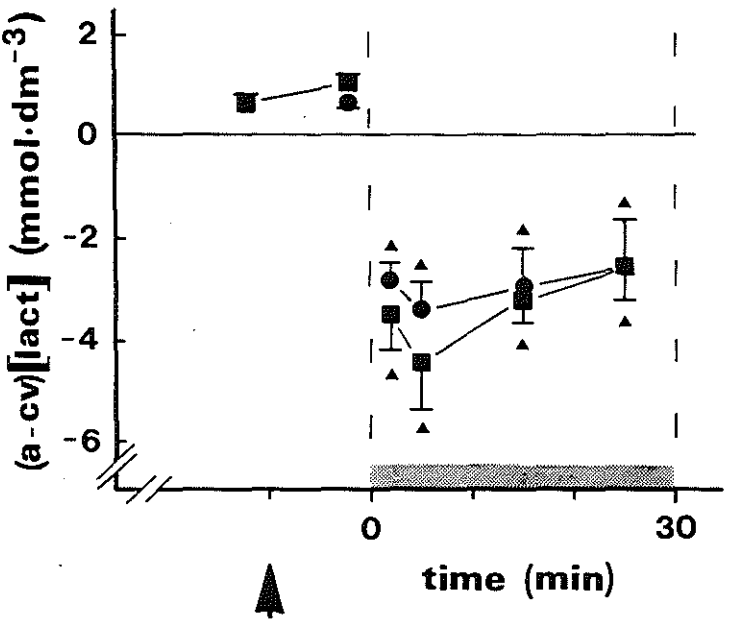

fig.6.3. Effect of pretreatment with 0.2 $mg.kg^{-1}$ oxyfedrine on myocardial lactate balance before and after oxyfedrine administration and during LAD-flow reduction (shaded bar). ●, control, ■, treated animals. ▲, $p<0.05$ vs. base-line values, listed in table 6.1.

6.4. DISCUSSION

The rationale for the use of a beta-sympathomimetic drug during myocardial ischaemia results from the positive inotropic effect which may improve the deteriorated haemodynamic function and, consequently, the perfusion of the ischaemic tissue. Oxyfedrine not only increased myocardial contractility but also elevated heart rate. Both variables augment the myocardial oxygen demand which was, however, amply compensated for by an increase in coronary blood flow. This increase in coronary blood flow in the normally-perfused heart was apparently out of proportion to the augmentation of myocardial oxygen demand since the coronary venous oxygen content was increased after administration of the drug. The elevation in the myocardial perfusion may, in part, be due to increases in the diastole/systole ratio. However, during ischaemia the systolic and diastolic fractions of the flow do not differ to a great extent and, as a result, this mechanism is then not likely to be very effective.

The augmentation of the coronary blood flow after the administration of oxyfedrine not only occurs in the normal heart, but has also been reported during ischaemia (Parratt and Ledingham, 1972; Raberger et al., 1972; Szekeres et al., 1972) and in patients undergoing an atrial pacing stress test (Kugler et al., 1975). In our model of myocardial ischaemia, the flow through the LAD was not allowed to change from 25 % of its base-line value. Nevertheless, beneficial effects on global haemodynamic function were observed: the decreases in cardiac output, mean arterial blood pressure, and LVdP/dtmax, occurring in untreated pigs, though not prevented, were less in treated animals. The increase in LVdP/dtmax after oxyfedrine administration was not likely to be due to an elevation of heart rate, since atrial pacing over the same range does not affect this index significantly (Verdouw et al., 1980).

The similar reductions (i.e. to 25 % of the base line) of the LAD-flow in both treated and untreated groups in the face of increased myocardial oxygen demand after oxyfedrine apparently leads to a greater oxygen deficit in the ischaemic myocardium of the treated animals. Consequently, a slightly larger lactate release during ischaemia in the oxyfedrine treated animals was observed but the differences in (a-cv)[lact] were not significant between the two groups (fig.6.3).

Several investigators have shown that reperfusion after complete coronary occlusions of longer than 5 min did not result in complete recovary from the depressed myocardial function during ischaemia (Heyndrickx et al., 1975; Althaus et al., 1977; Capone and Most, 1978). Similar observations were made after the LAD-flow was reduced to 50% of base line (Ramanathan et al., 1978; Verdouw et al., 1979). In our study, the size of the ischaemic area (about 40% of the left ventricle), the duration of the period of flow reduction (30 min) and the absence of a substantial contribution of collateral flow to the perfusion of the ischaemic area (Schaper, 1971; Fedor et al., 1978) may be responsable for this observation.

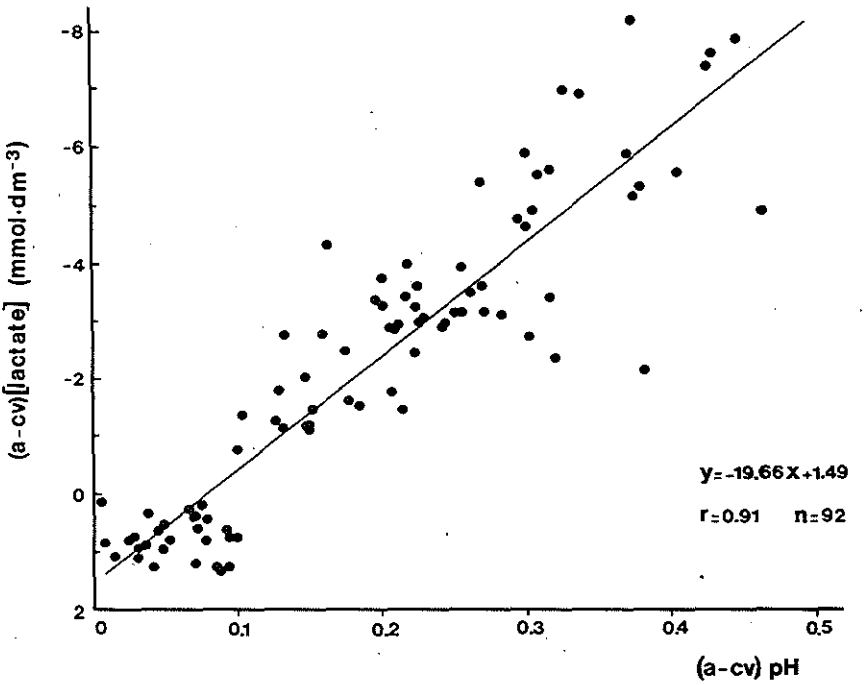

fig.6.4. Relation between differences in arterio-coronary venous pH ((a-cv)pH) and lactate ((a-cv)[lactate]) before and during LAD-flow reduction.

It is concluded that oxyfedrine can be useful in the treatment of pump failure resulting from acute myocardial ischaemia, since the drug increases contractility, does not increase mean arterial blood pressure, and prevents a further reduction of the cardiac output in a preparation, which was subjected to severe ischaemia. The administration of oxyfedrine did not result in a more anaerobic myocardial metabolism.

6.5. Acknowledgement

The authors gratefully acknowledge the technical assistance of Miss A.M. Rutteman, R.H. van Bremen and W.A.P. Breeman. Chemiewerk Homburg A.G. (Frankfurt a.M., W.Germany) supplied the oxyfedrine (Ildamen ®).

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REGIONAL MYOCARDIAL BLOOD FLOW AND SEGMENTAL WALL FUNCTION AFTER OXYFEDRINE ADMINISTRATION IN THE ISCHAEMIC PORCINE HEART

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Reprinted from Cardiovascular Research
Volume XIV, *No.* 8, pages 451-457, *August* 1980

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BRITISH MEDICAL ASSOCIATION
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REGIONAL MYOCARDIAL BLOOD FLOW AND SEGMENTAL WALL FUNCTION AFTER OXYFEDRINE ADMINISTRATION IN THE ISCHAEMIC PORCINE HEART

Summary. The effects of the cardiostimulant oxyfedrine on segmental myocardial wall function and regional myocardial blood flow were investigated in normally perfused and ischaemic regions in the porcine heart. Myocardial ischaemia was induced by reducing the left anterior descending coronary artery flow to approximately 40% of its base-line value. Six animals served as controls, while oxyfedrine (0.15 $mg.kg^{-1}$) was administered to 11 animals after 10 min of flow reduction. The induction of ischaemia caused the maximum rate of rise of left ventricular pressure to decrease by 30%, mean arterial blood pressure and cardiac output to decrease by 10%, while heart rate remained unchanged. Oxyfedrine increased the maximum rate of left ventricular pressure development to 25% above its base-line value, the mean arterial blood pressure to pre-occlusion values, and heart rate by 20%. Flow reduction resulted in a decrease in the flow to the central core of the ischaemic area from 1.27±0.19 to 0.67±0.15 $cm^3.min^{-1}.g^{-1}$ and decreased the endo/epi-ratio from 0.96±0.03 to 0.65±0.13 ($p<0.001$). Oxyfedrine increased the flow to the ischaemic area to 1.01±0.21 $cm^3.min^{-1}.g^{-1}$ ($p<0.05$), but did not affect the endo/epi-ratio. The flow to the normal perfused region increased from 1.30±0.16 to 1.41±0.13 $cm^3.min^{-1}.g^{-1}$. Without pronounced effects on the end-diastolic myocardial wall thickness (EDT), the mean velocity of systolic wall thickening, which declined to virtually zero during flow reduction, increased to 40% of the base-line value after drug administration. The systolic thickening itself returned to 18±5% of EDT; the values at the base line and during flow reduction being 49±5 and 5.2±2.3% of EDT, respectively. The combined effect on the end-systolic thickness was a return to 78% of the base-line value. It is concluded that oxyfedrine increases the blood flow to both the normal and the ischaemic myocardium, the velocity of systolic myocardial wall thickening and improves the ischaemic myocardial performance.

7.1. INTRODUCTION

Oxyfedrine is a beta-adrenergic stimulant, which produces marked increases in myocardial contractility (Kuehn et al., 1972; Moore and Parratt, 1972; Raberger et al., 1972; Szekeres et al., 1972; Parratt and Marshall, 1977) and some increase in heart rate in both animals and man (Kuehn et al., 1972; Moore and Parratt, 1972;

Parratt and Ledingham, 1972; Raberger et al., 1972; Szekeres et al., 1972; Rettig, 1977). These changes elevate myocardial oxygen demand which, however, is amply met by increases in coronary blood flow (Parratt and Ledingham, 1972; Raberger et al., 1972; Szekeres et al., 1972) to an extent that the coronary venous oxygen content increases (Moore and Parratt, 1972; Parratt and Ledingham, 1972). This oxygen sparing effect may, in part, result from a reduction in the intramyocardial tension, caused by a decrease in the left ventricular end-diastolic volume and pressure (Moore and Parratt, 1972; Kugler et al., 1975). The effects of oxyfedrine on the flow to the ischaemic myocardium are, however, inconclusive. Some investigators have reported an increase in the total flow to the ischaemic area (Ledingham et al., 1972; Parratt and Ledingham, 1972; Szekeres et al., 1972; Parratt and Marshall, 1977), while others found that only the flow to the border zone increased, but that to the central core decreased (Timogiannakis et al., 1978), possibly due to the "steal-phenomenon" (Vatner and Baig, 1979). In view of the increased myocardial oxygen demand it remains questionable, whether an improvement in overall haemodynamic performance with beta-adrenergic stimulants (such as oxyfedrine) is also accompanied by an improvement in the function of the ischaemic segment of the myocardium. The latter can be determined from the shortening of the ventricular wall (Wyatt et al., 1975; Waters et al., 1977), or, alternatively, from the changes in the segmental wall thickness (Sasayama et al., 1976; Gaasch et al., 1977; Schamhardt et al., 1979; Verdouw et al., 1980). The present study was undertaken, therefore, to investigate the effects of oxyfedrine on the regional myocardial blood flow together with the wall thickness variables in the ischaemic segment of the porcine heart (Verdouw et al., 1979a).

7.2. MATERIALS AND METHODS

7.2.1. General

Young Yorkshire pigs (25 to 33 kg) were surgically prepared as described in detail elsewhere (Verdouw et al., 1979a; Verdouw et al., 1980). Briefly, they were anaesthetized with azaperone and metomidate (2 and 8 $mg.kg^{-1}.h^{-1}$, respectively) and 4 $mg.h^{-1}$ pancuronium bromide was infused to achieve neuromuscular blockade. After intubation, artificial ventilation was started with a mixture of $N_2O:O_2=2:1$. Ventilation was adjusted or sodium bicarbonate was infused to keep arterial blood gases within the normal range ($7.35<pH<7.45$; $2.5<pCO_2$ [kPa]<3.5; $13<pO_2$ [kPa]<19). The left ventricle was catheterized with a 7F Millar, the thoracic aorta with a 6F Millar and the pulmonary artery via the left femoral vein with a triple-lumen balloon-tipped thermodilution catheter for pressure and cardiac output measurements. A standard 8F catheter was passed via the right femoral artery into the abdominal aorta and an 8F double-lumen and an 8F single-lumen catheter via the external and internal jugular veins into the vena cava inferior for the infusion of anaestetics, drugs and

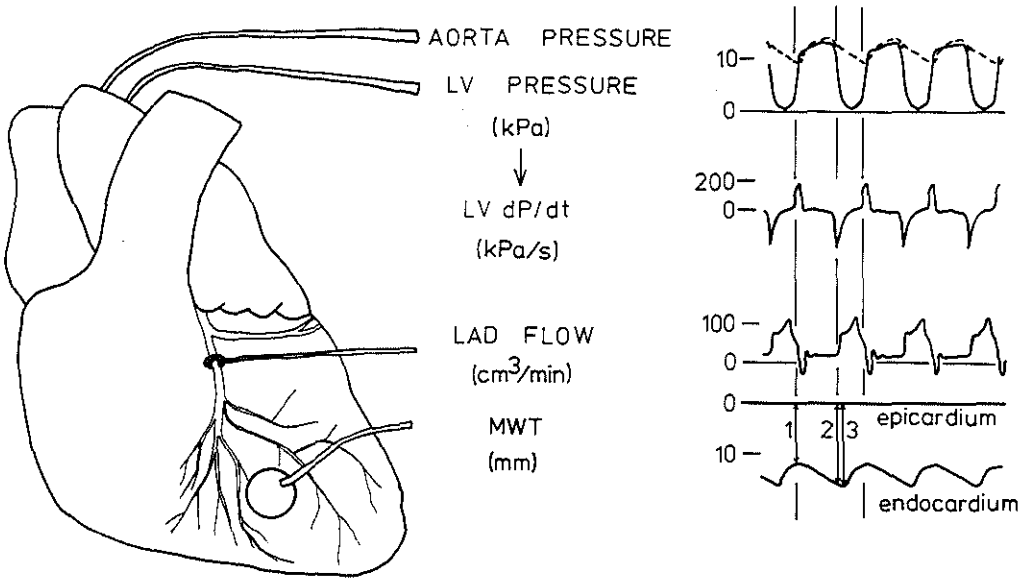

fig.7.1. Illustration of the methods employed to measure systemic haemodynamics and segmental myocardial wall thickness. The left ventricular and aortic pressures were obtained from catheter tip mounted micromanometers; LVdP/dtmax from analogous differentiation of LVP. The coronary flow was measured with a precalibrated perivascular electromagnetic flowprobe. The ultrasound crystal for measuring the local myocardial wall thickness was sutured on the epicardium in the territory supplied by the LAD. 1, 2 and 3 denote the end-diastolic, end-systolic and maximal wall thicknesses. The left atrium was catheterized for the injection of microspheres. 100 mmHg= 13.3 kPa.

fluid. The heart was exposed via a midsternal split. A 1 mm o.d. catheter was inserted into the left atrial appendage for the injection of microspheres. A segment of the left anterior descending coronary artery (LAD) was dissected free from the surrounding tissue and a precalibrated electromagnetic flow probe (Skalar, Delft, The Netherlands) and a J-shaped screw clamp were placed around the vessel.

7.2.2. Myocardial wall thickness

Myocardial wall thickness tracings were obtained from the area of the heart that was later made ischaemic. To this end, a miniature ultrasound crystal (5 MHz Krautkramer-Branson, Lewistown, Pa., USA) was sutured on the epicardial surface in the region supplied by the LAD. Registration and analysis of the wall thickness tracings were carried out as described elsewhere (Verdouw et al., 1980). The local thickness of the myocardial wall at end-diastole (EDT), at end-systole (EST; end-systole was defined to occur at the incisura of the ascending aorta pressure tracing), and its maximum value (maxT) were determined (fig.7.1). The systolic wall thickening (swt) was calculated as:

$$swt=100x(EST-EDT)/EDT$$

The mean velocity of systolic thickening was calculated as swt/100, divided by the duration of systole (s^{-1}). After LAD-flow reduction, wall thickening in the ischaemic myocardium occured during the diastole (dwt) and it was calculated as:

$$dwt=100x(maxT-EST)/EDT$$

7.2.3. Regional myocardial blood flow

Regional myocardial blood flow was measured with the radioactive microsphere technique (Heymann et al., 1977; Schamhardt et al., 1979) in 11 animals (6 treated with saline and 5 treated with oxyfedrine). Starting just before the injection of approximately $2x10^6$ microspheres (15 micron diameter, 3M Co), labelled with ^{125}I, ^{141}Ce or ^{85}Sr, a reference arterial blood sample was withdrawn (rate 23 $cm^3.min^{-1}$) over a period of about one min. The amount of blood withdrawn was replaced by polygeline (Haemaccel®). After the termination of the experiment the LAD-area was visualized by an injection of 1.5 cm^3 of methylene blue dye. The heart was exised, washed and fixed for at least 24 h in 4% formalin. The LAD-area was cut out and transmurally divided into four layers of equal thickness. In the posterior wall a segment equal in size to the LAD-area was used as control and prepared identically. The radioactivity in the different tissues was measured with a Packard model 5986 gamma counting system. Blood flows were calculated as described in detail elsewhere (Schamhardt et al., 1979). The endo/epi-flow ratio was calculated by dividing the weight-normalized flows to the outermost layers of the ventricle.

Table 7.1. Systemic haemodynamic variables at the base line (A), after 10 min of LAD-flow reduction (B), and the effects of saline (C) or oxyfedrine, 0.15 $mg \cdot kg^{-1}$ (D) on these variables.

Haemodynamic variable	Units	base-line value (A) n=17	at 10 min of ischaemia (B) n=17	Percentage changes from (B) at 25 min of ischaemia	
				saline-treated (C) n=6	oxyfedrine-treated (D) n=11
Heart rate	$beats \cdot min^{-1}$	94 ± 4	98 ± 5	2 ± 4	18 ± 6 ab
Mean arterial blood pressure	kPa	11.5 ± 0.3	10.05 ± 0.29 a	-1 ± 3 a	13 ± 7
Cardiac output (d)	$dm^3 \cdot min^{-1}$	3.40 ± 0.26	2.99 ± 0.25 a	-13 ± 4 ab	1 ± 8
Stroke volume (d)	cm^3	35.9 ± 2.0	30.2 ± 2.2 a	-13 ± 5 a	8.8 ± 2.7 ab
Systemic vascular resistance (d)	$kPa \cdot min \cdot dm^{-3}$	3.64 ± 0.26	3.7 ± 0.3	13 ± 3 b	11 ± 10 a
LV dP/dt max	$kPa \cdot s^{-1}$	401 ± 27	270 ± 17 a	-3 ± 8 a	86 ± 20 bc
LV end-diastolic pressure	kPa	0.93 ± 0.11	1.38 ± 0.16 a	-0.11 ± 0.05 e	-0.51 ± 0.14 be
Duration of systole	ms	330 ± 15	330 ± 17	-1 ± 4	-23 ± 4 abc
Diastolic time per minute	%	49.9 ± 1.4	48.1 ± 1.4	-1 ± 4	9 ± 3 b

LV, left ventricle; dP/dt max, maximum rate of rise of LV pressure; a, different from base-line value (A), $p<0.05$; b, different from (B), $p<0.05$; c, different from saline treated group (C), $p<0.05$; d, n=5 oxyfedrine-treated animals and, thus, n=11 in (A) and (B); e, absolute changes.

7.2.4. Experimental protocol

After completion of the surgical procedure the animals were allowed to stabilise for at least 30 min. The left ventricular end-diastolic pressure was monitored and polygeline was administered if nesessary to supplement fluid needs. The base-line measurements of the haemodynamic and myocardial wall thickness variables were obtained and LAD-flow was reduced to approximately 40% of its base-line value by tightening the screw on the J-clamp. Occasionally, the screw on the clamp had to be readjusted to annul any changes in the blood flow occuring in the first 5 min of ischaemia (Gould et al., 1975). Since these variables achieved a steady state within 10 min of the onset of ischaemia and remained constant for at least a further period of 30 min (Verdouw et al., 1979b) haemodynamic and wall thickness variables were measured again after 10 and 25 min of ischaemia. In one group of animals (n=11) a dose of 0.15 $mg.kg^{-1}$ oxyfedrine was administered just after the second set of measurements (at 10 min of ischaemia) while another group (n=6) received saline at the same time. The choice of the dose of oxyfedrine used in this study was based on preliminary experiments in which it was noticed that 0.15 $mg.kg^{-1}$ oxyfedrine had prominent inotropic and coronary dilator effects without causing much tachycardia. Higher doses caused unacceptable tachycardia, while lower doses did not have much effect on myocardial contractility.

7.2.5. Data presentation and analysis

The values of the various variables obtained at the base line and after reduction of the LAD-flow have been shown in absolute terms. The effect of oxyfedrine and saline administration are presented, unless otherwise stated, as the percentage change from the values at 10 min of ischaemia, i.e. just before treatment. The unpaired Student's t-test (Snedecor and Cochran, 1967) was employed for comparison of changes in the various variables between oxyfedrine and saline treated animals, whereas the paired Student's t-test was used to determine the significance of changes induced by ischaemia and by oxyfedrine within the groups. $p<0.05$ was considered statistically significant. All data are presented as mean$\pm$SEM.

7.3. RESULTS

7.3.1. Systemic haemodynamics

The effects of LAD-flow reduction and oxyfedrine administration on systemic haemodynamic variables are listed in table 7.1. Ten min after reduction of the LAD-flow LVdP/dtmax had decreased by 30%, mean arterial blood pressure and cardiac output both by 10%, while heart rate, the duration of systole and the diastolic time per minute had not changed. An increase in the left ventricular end-diastolic pressure by 0.45 kPa was observed. Except for an additional 10% decrease in cardiac output, no further changes occured in the saline-treated animals during the following 15 min of ischaemia.

Table 7.2. Regional myocardial blood flow at base line (A), after LAD-flow reduction (B), and the effects of saline (C) or oxyfedrine, 0.15 $mg \cdot kg^{-1}$ (D) on these variables.

Regional blood flow	Units	base-line value (A) n=11	values at 10 min of ischaemia (B) n=11	Percentage change from (B) at 25 min of ischaemia	
				saline-treated (C) n=6	oxyfedrine-treated (D) n=5
Total ischaemia area					
LAD-flow [1]	$cm^3 \cdot min^{-1}$	49 ± 7	17.5 ± 1.7	8 ± 8 a	66 ± 30 a
Blood flow	$cm^3 \cdot min^{-1} \cdot g^{-1}$	1.35 ± 0.17	0.75 ± 0.07 a	-9 ± 9 a	54 ± 17 bc
Endo/epi-ratio		1.09 ± 0.04	0.68 ± 0.07 a	-3 ± 11 a	22 ± 15 a
Central core ischaemic area [2]					
Blood flow	$cm^3 \cdot min^{-1} \cdot g^{-1}$	1.35 ± 0.23	0.65 ± 0.19 a	-	60 ± 28 a
Endo/epi-ratio		0.96 ± 0.07	0.56 ± 0.11 a	-	7 ± 14 a
Border zone ischaemic area [2]					
Blood flow	$cm^3 \cdot min^{-1} \cdot g^{-1}$	1.27 ± 0.19	0.67 ± 0.15 a	-	47 ± 20
Endo/epi-ratio		0.96 ± 0.03	0.65 ± 0.13 a	-	13 ± 12
Normal zone					
Blood flow	$cm^3 \cdot min^{-1} \cdot g^{-1}$	1.30 ± 0.16	1.41 ± 0.13	-7 ± 9	29 ± 13 ac
Endo/epi-ratio		1.28 ± 0.06	1.18 ± 0.05	-3 ± 6	-3 ± 3

1, LAD-flow was measured with an electromagnetic flow meter; 2, ischaemic zone was separated into central core and border zone only in 4 oxyfedrine-treated animals; a, different from base-line value (A), $p<0.05$; b, different from (B), $p<0.05$; c, different from saline-treated group (C), $p<0.05$

Administration of oxyfedrine during flow reduction caused an 18% increase in heart rate and a return of mean arterial blood pressure and of left ventricular end-diastolic pressure towards base-line values, while the further decrease in the cardiac output was prevented. However, the most pronounced changes were the increase of LVdP/dtmax to 25% above base line, and the decrease in the duration of systole by 23% ($p<0.001$; table 7.1).

7.3.2. Regional myocardial blood flow

The data pertaining to regional myocardial blood flow are listed in table 7.2. As expected, the reduction of the LAD-flow caused a significant decrease in the blood flow to, and in the endo/epi-ratio of, the ischaemic zone. The endo/epi-ratio in the central core of the ischaemic area was reduced by 40% ($p<0.001$) after 10 min of flow reduction, whereas this figure was 33% in the border zone. The flow to the control area in the posterior wall did not alter. In saline-treated animals this pattern of flow distribution was apparently stable during the entire period of ischaemia, since the values measured at 25 min did not differ from those obtained at 10 min of ischaemia. The reduction of the LAD-flow did not cause any appreciable changes in the blood flow to, or in the endo/epi-ratio of, the normal zone.

Administration of oxyfedrine after 10 min of flow reduction resulted in a significant increase in the blood flow to the total ischaemic zone but the changes in the endo/epi-ratio did not achieve the level of significance. The drug also increased the transmural flow to the central core of the ischaemic zone by 60% and to the border zone by 47% (table 7.2). The flow to the control area increased by 29%, which was significantly different from the change in the saline-treated animals. However, the endo/epi-flow ratios in these regions did not change after oxyfedrine.

7.3.3. Myocardial wall thickness

Administration of oxyfedrine (0.15 $mg.kg^{-1}$) in seven pigs with a normal coronary circulation resulted in slight increases in the end-diastolic wall thickness (EDT; $7.3\pm2.1\%$; $p<0.05$) and in the end-systolic wall thickness (EST; $6.9\pm2.2\%$; $p<0.05$). The mean velocity of systolic wall thickening increased by $44\pm5\%$ ($p<0.001$), but systolic wall thickening itself did not change due to a decrease in the duration of systole.

Table 7.3 shows the base-line values of the myocardial wall thickness variables and their changes after flow reduction and after administration of oxyfedrine or saline. Immediately after reduction of the LAD-flow EDT decreased by about 10% ($p<0.05$). The mean velocity of systolic wall thickening ($\bar{v}_{swt}$) dropped to nearly zero, which, in turn, resulted in a nearly complete loss of systolic wall thickening (swt; table 7.3). However, the myocardial wall started to thicken during early diastole (17% of EDT). In the saline-treated animals this pattern did not change in the 25 min period of ischaemia.

In the oxyfedrine treated animals EDT returned towards its

Table 7.3. Segmental wall thickness variables at base-line (A), after 10 min of LAD flow reduction (B), and the effects of saline (C) or oxyfedrine, 0.15 $mg.kg^{-1}$ (D) on these variables.

Myocardial wall thickness variable	Units	Base-line values (A) n=17	Values at 10 min of ischaemia (B) n=17	Percentage change from (B) at 25 min ischaemia	
				Saline-treated (C) n=6	oxyfedrine-treated (D) n=11
End-diastolic thickness (EDT)	mm	10.30 ± 0.29	9.29 ± 0.18 a	3.2 ± 2.5 a	11 ± 3 b
End-systolic thickness (EST)	mm	15.4 ± 0.6	9.8 ± 0.3 a	2.0 ± 1.4 a	24 ± 7 bc
Systolic thickening (swt)	% of EDT	49 ± 4	5.2 ± 2.3 a	-0.8 ± 1.4 ad	11 ± 4 cd
Mean velocity of swt ($\bar{v}_{swt}$)	s^{-1}	1.55 ± 0.17	0.17 ± 0.09 a	0.02 ± 0.06ad	0.45 ± 0.14 abcd
Diastolic thickening (dwt)	% of EDT	0	16.6 ± 2.5 a	11 ± 11	-8 ± 29

a: different from base-line value (A), $p < 0.05$; b: different from (B), $p < 0.05$; c: different from saline-treated group (C), $p < 0.05$; d: absolute changes.

base-line value and $\bar{v}_{swt}$ more than doubled ($p<0.05$; table 7.3; fig.7.2). Despite the increase in $\bar{v}_{swt}$, swt returned to only 18±5% of EDT, due to the decreased duration of systole (tables 1 and 3). The combined effect on EST was an increase of about 25%. On the other hand, dwt decreased to 9% of EDT (not significant).

7.3.4. Arteriovenous shunting and renal blood flow

The amount of microspheres which entered the lungs can be used as a measure of peripheral arteriovenous anastomotic blood flow (shunting; Johnston and Saxena, 1978; Saxena, 1978). The bronchial artery flow, which is less than 2% of cardiac output (CO; Forsyth et al., 1968), is thereby neglected. The average of total shunting was 30±5% of CO (n=11), ranging from 6.5 to 61.5% of CO. The reduction of the LAD-flow did not affect total shunting, nor did the administration of oxyfedrine during flow reduction. The observed decrease in CO during ischaemia, thus, caused a reduction in tissue perfusion.

The flow to both kidneys was measured, merely as a check on the adequacy of mixing of microspheres with blood. We never observed a difference in perfusion between the right and the left kidney of more than 1%. At the base line the total kidney flow was 2.98±0.23 $cm^3.min^{-1}.g^{-1}$, constituting 9.3±1.2% of CO. During ischaemia the flow decreased to 2.59±0.20 $cm^3.min^{-1}.g^{-1}$ ($p<0.05$), being 9.3±1.1% of CO. The continuation of flow reduction or the administration of oxyfedrine did not result in a change in flow or in the percentage of CO delivered to the kidneys.

7.4. DISCUSSION

The base-line values of the haemodynamic variables and their behaviour during flow reduction compare well with that reported earlier in many investigations (Schamhardt et al., 1979; Verdouw et al., 1979a; Verdouw et al., 1980). Production of acute stenosis of a coronary artery, as shown here, leads to a diminution of cardiac contractility, cardiac output, a fall in arterial blood pressure and a reduction of the blood flow to the ischaemic segment of the myocardium (Schamhardt et al., 1979; Verdouw et al., 1979a; Verdouw et al., 1980).

The administration of oxyfedrine during flow reduction led to marked changes in global haemodynamics. LVdP/dtmax increased by 86% to a level which was 25% above base line, reflecting an improvement in the overall myocardial contractility. Since LVdP/dtmax is sensitive to changes in heart rate and blood pressure, it could be argued that the increase in LVdPdt/max was caused by increases in any of these two parameters. However, raising heart rate by atrial pacing from 100 to 120 beats.min^{-1} in this preparation resulted in only a 6% increase in LVdP/dtmax (Verdouw et al., 1980). Although no data are available concerning the effect of blood pressure on LVdP/dtmax in the open chested pig, the findings in other species make it very unlikely, that a 13% elevation of arterial blood pressure can account for an increase

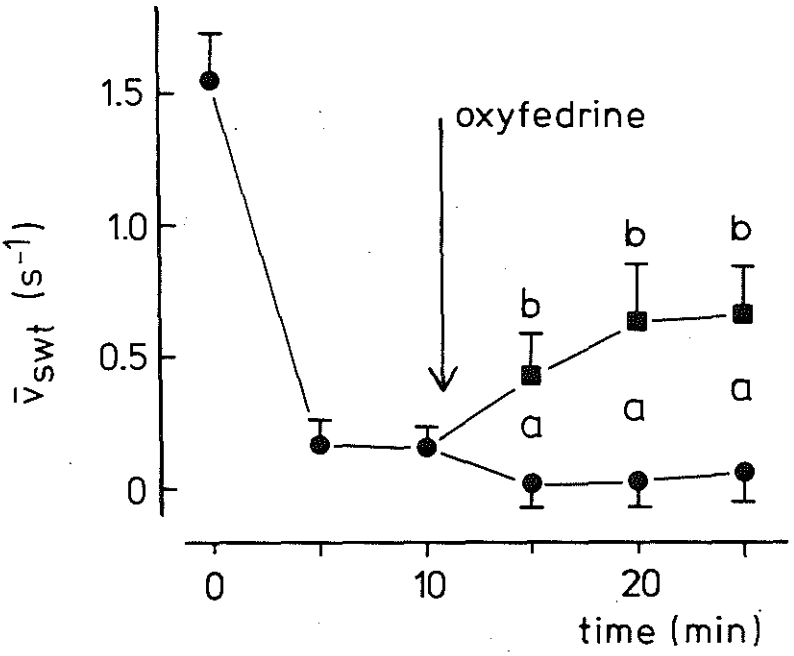

fig.7.2. Mean velocity of systolic myocardial wall thickening ($\bar{v}_{swt}$, computed as the systolic wall thickening, divided by the duration of systole) at base line, during coronary flow reduction and after the administration of oxyfedrine (0.15 $mg.kg^{-1}$). $\bar{v}_{swt}$ was reduced after the onset of LAD-flow reduction and remained depressed after saline-treatment (n=6). Administration of oxyfedrine (n=11) significantly increased $\bar{v}_{swt}$. a: different from saline-treated group ($p<0.05$); b: different from values at 10 min of flow reduction ($p<0.05$). Values are given as mean±SEM.

of more than 80% in LVdP/dtmax. Therefore, the conclusion that the positive inotropic properties of oxyfedrine are largely responsible for the changes in LVdP/dtmax seems warranted.

Reduction of the LAD-flow also resulted in a decrease in the ratio between endocardial and epicardial perfusion (endo/epi-ratio), so that the endocardium encountered a more severe ischaemia than the epicardium (Schamhardt et al., 1979). No differences were observed between the blood flow and the endo/epi-ratio in the central core of the ischaemic region and in the border zone adjacent to the normally perfused myocardium. The absence of collaterals in the domestic swine heart could explain this finding (Gregg and Fisher, 1963; Schaper, 1971; Fedor et al., 1978).

The blood flow to the ischaemic zone increased considerably after administration of oxyfedrine, although the extravascular screw clamp on the LAD was not readjusted. The flow to the post-stenotic segment of the myocardium, where the arterioles shall be maximally dilated, can be increased by either an elevation of the perfusion pressure and/or an enhancement of the perfusion time (i.e. the diastolic time per minute). In our preparation administration of oxyfedrine moderately increased the perfusion pressure (13%) and, despite the induced tachycardia, the perfusion time per minute (9%; table 7.1). The magnitude of these factors probably does not fully account for the observed increase in the blood flow to the ischaemic zone. It seems that a relaxation of the circular fibres in the LAD plays an important role in view of the evidence that oxyfedrine can effectively relax the large coronary arteries (Kukovetz and Poech, 1970).

A progressive reduction in coronary artery flow is accompanied by a progressive loss of systolic segmental shortening (Wyatt et al., 1975; Waters et al., 1977) and systolic myocardial wall thickening (Parmley et al., 1980; Verdouw et al., 1980). However, little is known about the effects of improved perfusion on the function of the ischaemic zone. Moreover, an improvement in the former does not necessarily mean an improvement of the latter (Komer et al., 1979; Schamhardt et al., 1979). This is especially true for those interventions which not only affect oxygen supply but also alter the oxygen demand. In this study, the end-diastolic myocardial wall thickness (EDT) decreased during flow reduction, but returned to base line after oxyfedrine. Whether this is merely a reflection of a drug-induced decrease in end-diastolic left ventricular volume (left ventricular end-diastolic pressure) is unclear. The end-systolic wall thickness (EST) is determined by EDT, the velocity of systolic wall thickening and the duration of systole. During ischaemia, the velocity of wall thickening decreased to virtually zero. Consequently, EST did not deviate very much from EDT. The pattern is characteristic of severe ischaemia (Verdouw et al., 1980).

After the administration of the drug the velocity of wall thickening in the ischaemic area increased to 50% of base line, despite an increase in at least two factors (heart rate and blood pressure), which augment the myocardial oxygen demand. Indeed, Tomoike et al. (1978) reported that an increase in heart rate during

myocardial ischaemia further worsened the regional perfusion of the ischaemic segment. Therefore it must be assumed that the increase in oxygen supply to the ischaemic zone exceeds the increase in oxygen demand.

The administration of oxyfedrine increased the coronary flow to about 60% of its base-line value and improved the segmental myocardial performance, although it remained markedly depressed. This is not surprising, since gradual coronary flow reduction below 60% of control resulted in an almost complete loss of systolic wall thickening (Gallagher et al., 1979; Verdouw et al., 1980). A larger increase in flow is necessary to restore the normal pattern of myocardial wall thickening (Verdouw et al., 1980). This is especially true, since oxyfedrine not only increases myocardial oxygen supply, but also the oxygen demand.

We conclude that the administration of oxyfedrine (0.15 $mg.kg^{-1}$) to pigs with a partially occluded coronary artery enhanced LVdP/dtmax, heart rate, and the blood flow to the non-ischaemic region of the heart. However, unlike other sympathomimetic agents (isoprenaline, dopamine, dobutamine; (Vatner and Baig, 1979)), oxyfedrine also increased the blood supply to, and improved the function of the ischaemic zone, despite the concomitant increase myocardial oxygen demand. It is thus conceivable that the drug could be useful in acute myocardial ischaemia, particularly in patients where the overall haemodynamic condition is unstable.

7.5. Acknowledgment

The authors gratefully acknowlegde the technical assistance of Mrs. M.A. Kroos, Miss A.M. Rutteman and R.H. van Bremen. We thank the "Interuniversitair Cardiologisch Instituut" for the use of their computer for the analysis of wall thickness tracings. Chemiewerk Homburg A.G. supplied the oxyfedrine (Ildamen®).

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Regional Myocardial Perfusion and Wall Thickness and Arteriovenous Shunting After Ergotamine Administration to Pigs with a Fixed Coronary Stenosis

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Journal of Cardiovascular Pharmacology
1:673–686

Reprinted from

Journal of Cardiovascular Pharmacology

Raven Press / 1140 Avenue of the Americas / New York, NY 10036

REGIONAL MYOCARDIAL PERFUSION AND WALL THICKNESS AND ARTERIOVENOUS SHUNTING AFTER ERGOTAMINE ADMINISTRATION TO PIGS WITH A FIXED CORONARY STENOSIS

Summary. The haemodynamic effects of the antimigraine drug ergotamine, considered contraindicated in patients with coronary artery disease, were studied in pigs with a normal myocardial circulation (doses of 8,16, and 32 $\mu g.kg^{-1}$, i.v.) and in pigs with an acute coronary stenosis (8 $\mu g.kg^{-1}$). In both groups of animals, ergotamine decreased heart rate, cardiac output, and arteriovenous anastomotic blood flow while increasing aortic blood pressure and systemic vascular resistance. No effects on total ventricular blood flow and its distribution within the myocardium were found in normal animals. In animals with a clamp on the left anterior descending coronary artery (LAD), the blood flow to the LAD-perfused area was reduced from 1.10 ± 0.16 to 0.67 ± 0.05 $cm^3.min^{-1}.g^{-1}$. The endocardium was affected more than the epicardium and the endo/epi flow ratio decreased from 1.18 ± 0.05 to 0.74 ± 0.07. Ergotamine increased the blood flow to the ischaemic zone towards normal values, and the endo/epi flow ratio to 1.05 ± 0.21. However, myocardial wall thickness parameters, which showed functional deterioration during ischaemia, did not change after ergotamine. The present study provides no clear support for cardiovascular contraindications of ergotamine administration.

8.1 INTRODUCTION

Ergotamine tartrate is widely and effectively used for the treatment of acute attacks of migraine. However, the drug is contraindicated in patients suffering from coronary artery disease, hypertension, or peripheral vascular disease, since it increases systemic vascular resistance and may facilitate induction of coronary artery spasm in susceptible persons (Saxena and de Vlaamschleuter, 1974; Nickerson and Collier, 1975; Clark et al., 1978; Griffith et al., 1978; Johnston and Saxena, 1978). Total myocardial blood flow reportedly decreases after ergotamine administration, but this may be secondary to the bradycardia which the drug invariably causes (Saxena and de Vlaamschluter, 1974; Nickerson and Collier, 1975; Clark et al., 1978; Griffith et al., 1978; Johnston and Saxena, 1978). Redistribution of the blood flow within the myocardium by ergotamine, irrespective of changes in total coronary flow, could lead to local myocardial ischaemia. The present study was undertaken to investigate

the effects of ergotamine on regional myocardial perfusion and function in pigs with normal or restricted coronary inflow. In addition, the effect of ergotamine on the blood flow through arteriovenous anastomoses (AVAs) was studied, since closure of these shunts has been suggested as a possible mechanism of action of the drug against migraine (Johnston and Saxena, 1978; Saxena, 1978; Spierings and Saxena, 1979).

8.2. METHODS

8.2.1. General preparation

Young Yorkshire pigs (20 to 35.5 kg) were prepared as previously described (Verdouw et al., 1979a). Briefly, they were anaesthetized with azaperone/metomidate (2/8 $mg.kg^{-1}.h^{-1}$), intubated, and mechanically ventilated with O_2:N_2O=1:2. Pancuronium bromide was administered by infusion (4 $mg.h^{-1}$) to achieve neuromuscular blockade. Respiratory rate and tidal volume were adjusted or sodium bicarbonate (1.5%) was infused to keep arterial blood gases within the normal range ($7.35<pH<7.45$; $35<pCO_2$ [mmHg]<45; $90<pO_2$ [mmHg]<140). An 8 French (F) pressure transducer-tipped Millar catheter was inserted via the left carotid artery into the left ventricle. A 6F Millar catheter was inserted via the right femoral artery into the abdominal aorta. An 8F standard catheter was positioned in the superior vena cava for infusion of drugs and fluid or withdrawal of blood samples. Another 8F catheter was placed in the left femoral artery for withdrawal of an arterial reference sample during microsphere injection. A 7F balloon-tipped thermodilution catheter was passed via the left femoral vein into the pulmonary artery for the measurement of cardiac output. The chest was opened via a midsternal split to expose the heart, and a catheter was placed in the left atrial appendage for the injection of microspheres. A 1.0-1.5 cm segment near the origin of the left anterior descending coronary artery (LAD) was dissected free from the surrounding tissue and a calibrated electromagnetic flow probe and a screw clamp were placed around the vessel. Heparin (5,000 U i.v.) was administered routinely.

The following haemodynamic variables were calculated from the tracings written on a Gould Brush 480 recorder: heart rate (HR); left ventricular systolic pressure (LVSP) and end-diastolic pressure (LVEDP); the maximum rate of rise and rate of fall of ventricular pressure (LVdP/dtmax, LVdP/dtmin, respectively); mean aortic blood pressure (MBP); cardiac output (CO); systemic vascular resistance, computed as:

$$SVR=(MBP-RAP)/CO$$

with RAP the mean right atrial pressure; stroke volume, computed as cardiac output divided by heart rate and LAD flow with the electromagnetic flow meter. Coronary vascular resistance was calculated as:

$$CVR=(MBP-RAP)/\dot{Q}m$$

where $\dot{Q}m$ is the flow per gram of tissue of the LAD-perfused area, measured with microspheres. The double product HRxLVSP was calculated and used as an index for the oxygen demand of the heart (Brettschneider, 1971). When simultaneous measurements of cardiac output and total AVA flow (see below) were made, the nutritional part of cardiac output was calculated by subtracting the latter from cardiac output values.

8.2.2. Myocardial wall thickness

In 16 animals -three with a normal myocardial circulation and 13 which were later subjected to myocardial ischaemia- a 5 MHz ultrasonic transducer (Krautkramer-Branson, Lewistown, Pa., USA) was sutured onto the epicardium in the LAD-perfused area for continuous measurements of the regional myocardial wall thickness. Wall thickness tracings were analyzed using a previously described technique (ten Cate et al., 1979). Attention was focussed on: end-systolic wall thickness (EST), end-diastolic wall thickness (EDT), the maximum wall thickness (maxT) and the time interval (tmaxT) between the closure of the aortic valves and maximum thickness. When maximum thickness was reached before the closure of the aortic valves, this interval was counted negative. From the above measurements systolic and diastolic wall thickening (swt and dwt, respectively) were calculated by the following formulas:

$$swt = (EST-EDT)/EDT \times 100\%$$

$$dwt = (maxT-EST)/EDT \times 100\%$$

The calculations for the diastolic wall thickening were performed only when maximum thickness was reached after closure of the aortic valves.

8.2.3. Regional myocardial blood flow measurements

Total blood flow to and regional flow within the heart were measured with the microsphere method (Hales, 1974; Heymann et al., 1977). To provide a microsphere density of at least 400 microspheres in the smallest tissue samples (Buckberg et al., 1971), a sufficient number ($1-2\times10^6$) of 13.4 ± 1.2 micron microspheres (3M Co) labelled with ^{125}I, ^{141}Ce, and ^{85}Sr were injected into the left atrium. Care was taken to prevent aggregation of the microspheres by adding a drop of Tween 80 and by mechanical and ultrasonical agitation prior to injection. An arterial reference sample (23 $cm^3.min^{-1}$ over 90 sec) was withdrawn to calibrate flow measurements. The equivalent of blood withdrawn was returned to the animal as plasma replacement (Haemaccel®). After completion of the experiment, the LAD-perfused area was visualised by injecting 1.5 cm^3 of methylene blue dye into the LAD. The heart was excised, washed, and fixed in 4% formalin for 24 h. Epicardial fat, large vessels, valves, and cordae tendinae were removed. The LAD-perfused area was cut out. Border zones, half the

width of the myocardium, perfused by the LAD, adjacent to the LAD area, were defined. From the remaining tissue, the central section, equal in size to the LAD area, was used as control, while any other pieces were counted as "rest". Transmural pieces were divided into four layers of approximately equal thickness, from endocardium to epicardium, and denoted as endocardium 1 and 2, epicardium 1 and 2. The radioactivity in each tissue sample was determined in a gamma-spectrometer at energy window settings corresponding to the photo peaks of the isotopes used. Rough counts were corrected by a digital computer for background counts and overlap from radiation with higher energy (Hales, 1974; Heymann et al., 1977). Tissue flow ($\dot{Q}t$) was calculated from tissue radioactivity (It), arterial withdrawal rate ($\dot{Q}w$) and blood radioactivity (Iw) as:

$$\dot{Q}t=\dot{Q}w(It/Iw)$$

All flow values were normalized to flow per gram wet weight of tissue. The endo/epi ratio was calculated by dividing the weight-normalized endocardium 1 flow by the normalized epicardium 2 flow.

8.2.4. Arteriovenous anastomotic blood flow

Since bronchial artery flow is less than 1.5% of cardiac output (Kaihara et al., 1968; Buckberg et al., 1971) and the lungs are able to trap more than 96% of the microspheres reaching the pulmonary artery (Forsyth et al., 1968; Kaihara et al., 1968; Spierings et al., 1979; our observations in 4 pigs), radioactivity in the lungs was used as an index of total systemic arteriovenous anastomotic blood flow (Saxena, 1978; Saxena et al., 1978). Both lungs were dissected out and counted in their entirety for radioactivity. Additionally, fractional shunting (FS; Archie et al., 1973) in the superior vena cava was calculated by the formula:

$$FS = (Isvc/Ia) \times (\dot{Q}a/\dot{Q}svc)$$

where I denotes radioactivity in arterial (a) or superior vena caval (svc) blood and $\dot{Q}$ is the corresponding withdrawal rate.

8.2.5. Experimental protocols

In all animals, the base-line values for the measured variables were obtained after the animal had been in a stable haemodynamic state for at least 30 min. To establish a dose-response curve ergotamine tartrate (Gynergen, Sandoz) was administered via the superior vena cava in nine anaesthetized pigs in doses from 8 to 32 $\mu g.kg^{-1}$. Since microspheres with three different labels were available, the regional haemodynamic measurements were made after no more than two doses of the drug in any given animal.

In another series of experiments the mean LAD flow (electromagnetic flow meter reading) was reduced to approximately 40% of control by tightening the screw on the clamp for a period of 30 min. When the flow tended to increase, due to vasodilation, the clamp was

Table 8.1. Base-line haemodynamic variables in anaesthetized pigs.

Haemodynamic variable	Unit	Normal coronary flow (n=9)	Fixed coronary stenosis	
			Untreated animals (n=6)	Treated animals (n=7)
HR	min^{-1}	91 ± 5	98 ± 6	83 ± 5
CO	$dm^3.min^{-1}$	3.57 ± 0.19	3.8 ± 0.4	3.05 ± 0.26
SV	cm^3	40.0 ± 2.8	38.4 ± 2.2	36.9 ± 2.6
MBP	mmHg	81.9 ± 2.7	93 ± 3	88 ± 4
SVR	$mmHg.min.dm^{-3}$	23.7 ± 1.7	25.5 ± 1.9	31 ± 3
LVSP×HR	$mmHg.min^{-1}$	8900 ± 500	10600 ± 900	8700 ± 400
LVEDP	mmHg	4.3 ± 0.9	8.0 ± 1.2	5.0 ± 1.2
LVdP/dtmax	$mmHg.s^{-1}$	2400 ± 250	3300 ± 400	2900 ± 300
LVdP/dtmin	$mmHg.s^{-1}$	-1500 ± 110	-2100 ± 300	-1740 ± 200
Peripheral AVA flow	% CO $dm^3.min^{-1}$	24 ± 6 0.82 ± 0.21	32 ± 7 1.19 ± 0.27	22.5 ± 2.7 0.68 ± 0.10
Nutritional CO	$dm^3.min^{-1}$	2.74 ± 0.29	2.6 ± 0.4	2.37 ± 0.20
SVC AVA flow	% SVC flow	-	44 ± 12	48 ± 7
tot. myoc. flow	$cm^3.min^{-1}.g^{-1}$	0.93 ± 0.08	1.38 ± 0.28	0.80 ± 0.08
endo/epi-ratio		1.09 ± 0.06	1.19 ± 0.04	1.20 ± 0.05

Treated animals received 8 $\mu g.kg^{-1}$, i.v. ergotamine after 16 min of coronary flow reduction. Abbreviations: HR: heart rate; CO: cardiac output; SV: stroke volume; MBP: mean aortic blood pressure; SVR: systemic vascular resistance; LVSP: left ventricular systolic pressure; LVEDP: left ventricular end-diastolic pressure; LVdP/dtmax: maximum rate of rise of left ventricular pressure; LVdP/dtmin: maximum rate of fall of LVP; peripheral AVA flow: flow through peripheral arteriovenous anastomoses (AVA), calculated from radioactivity in the lungs; SVC: superior vena cava; nutritional CO: CO minus peripheral AVA flow; tot. myoc. flow: total myocardial blood flow; endo/epi-ratio between the perfusion of endol and epi2, see text.

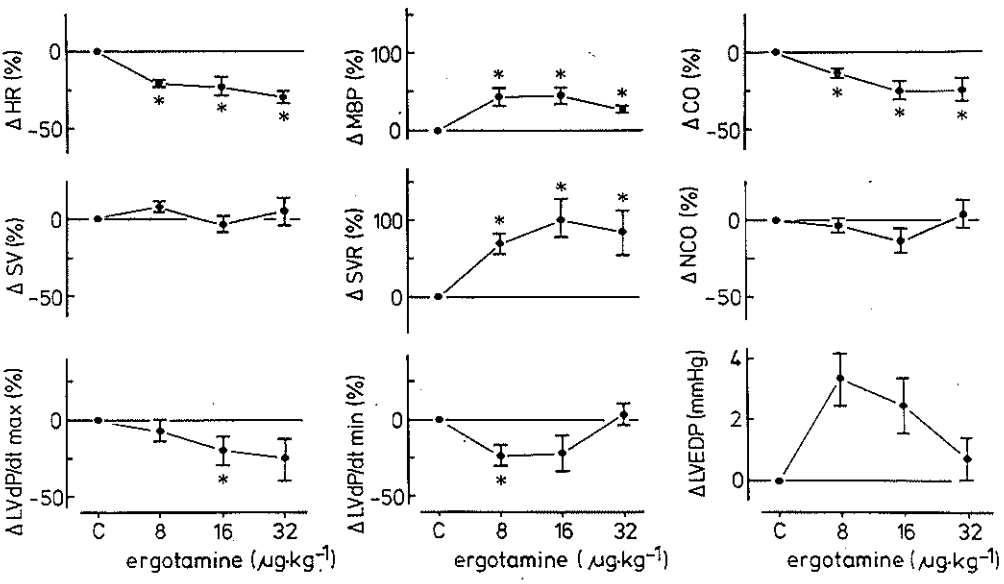

fig.8.1. Percent changes (mean+SEM) from base-line values of haemodynamic parameters (table 8.1) in pigs which received cumulative doses of ergotamine. All doses groups n=5, except for the nutritional cardiac output (NCO), calculated in 4 animals in each group. Asterisks denote $p<0.05$ vs. base-line values (Student's paired t-test). C denotes base-line measurements; Δ, change from base-line value. Abbreviations: see legend of table 8.1.

Table 8.2. Effect of ergotamine on total and regional myocardial haemodynamics in anaesthetized pigs (n=9) with a normal coronary circulation.

Regional blood flow	Base-line value	% change from base-line value by ergotamine ($\mu g.kg^{-1}$, i.v.)		
		8	16	32
LAD blood flow				
E.M. flowmeter	44 ± 5	30 ± 11 a	10 ± 14	-5 ± 12
Microspheres	32.4 ± 2.5	24 ± 11	16 ± 15	0 ± 10
Total myoc. fl.	0.93 ± 0.08	21 ± 9	13 ± 14	-2 ± 8
Endocardium 1	0.93 ± 0.08	26 ± 12	17 ± 15	-2 ± 9
Endocardium 2	1.00 ± 0.08	25 ± 11	12 ± 14	-4 ± 10
Epicardium 1	0.95 ± 0.08	19 ± 9	9 ± 13	-3 ± 8
Epicardium 2	0.88 ± 0.09	24 ± 10	17 ± 16	-2 ± 9
Endo/epi-ratio	1.11 ± 0.06	1 ± 3	-2 ± 6	0.8 ± 2.1

LAD-flow is given in $cm^3.min^{-1}$, other flows in $cm^3.min^{-1}.g^{-1}$. Total myoc. fl., total myocardial blood flow. Endocardial and epicardial blood flows were calculated for the total left ventricle. Endo/epi-ratio was calculated as the ratio between the flows to endocardium 1 and epicardium 2. a, significantly different from base-line values ($p<0.05$, Student's paired t-test).

readjusted. This was only necessary within the first five min of flow reduction. The haemodynamic and wall thickness variables were measured at 14 and 25 min of ischaemia. A group of 11 animals served as controls and were not treated during the 30 min of ischaemia, while another group (n=9) received 8 $\mu g.kg^{-1}$ ergotamine after 16 min of flow reduction. Reduction of the LAD flow led to ventricular fibrillation in 5 out of 11 untreated pigs and in 3 of 9 animals which received 8 $\mu g.kg^{-1}$ ergotamine. The difference in the incidence of ventricular fibrillation was not statistically significant. No attempts were made to defibrillate these animals. One of the three pigs in the ergotamine group fibrillated after the systemic haemodynamic and myocardial wall thickness measurements, but before the injection of microspheres. Except for this, all other fibrillating animals were excluded from the study.

8.2.6. Data analysis

The Student's paired t-test (Snedecor and Cochran, 1967) was used for statistical analysis of ergotamine- and myocardial ischaemia-induced changes in the different variables compared with the corresponding base-line values. The Mann-Whitney U-test (Siegel, 1956) was employed for comparison of ergotamine-induced changes in the variables between treated and untreated animals. $p<0.05$ was considered statistically significant. All values presented are means$\pm$standard error of the mean (SEM).

8.3. RESULTS

8.3.1. Base-line values of haemodynamic parameters

Table 8.1 lists systemic haemodynamic and regional myocardial flow values in the three groups of animals prior to any ergotamine treatment or coronary flow reduction. There was general agreement between the base-line values in the 3 groups, except that the total myocardial flow was non-significantly higher in the untreated series. The data presented agree well with those reported earlier (Rivas et al., 1976; Verdouw et al., 1977; Fedor et al., 1978; Most et al., 1978; Verdouw et al., 1979a).

8.3.2. Effects of ergotamine during normal perfusion

8.3.2.1. Systemic haemodynamics

The effects of ergotamine on the systemic haemodynamic variables are shown in fig.8.1. The response was relatively independent of the dose. Although stroke volume did not change, there was a 25% reduction in cardiac output as a result of a similar decrease in heart rate. No significant change was noticed in the nutritional cardiac output. Mean aortic blood pressure increased by 40% due to a doubling of the systemic vascular resistance. LVdP/dtmax decreased gradually by 25% with increasing doses of ergotamine, while LVdP/dtmin decreased

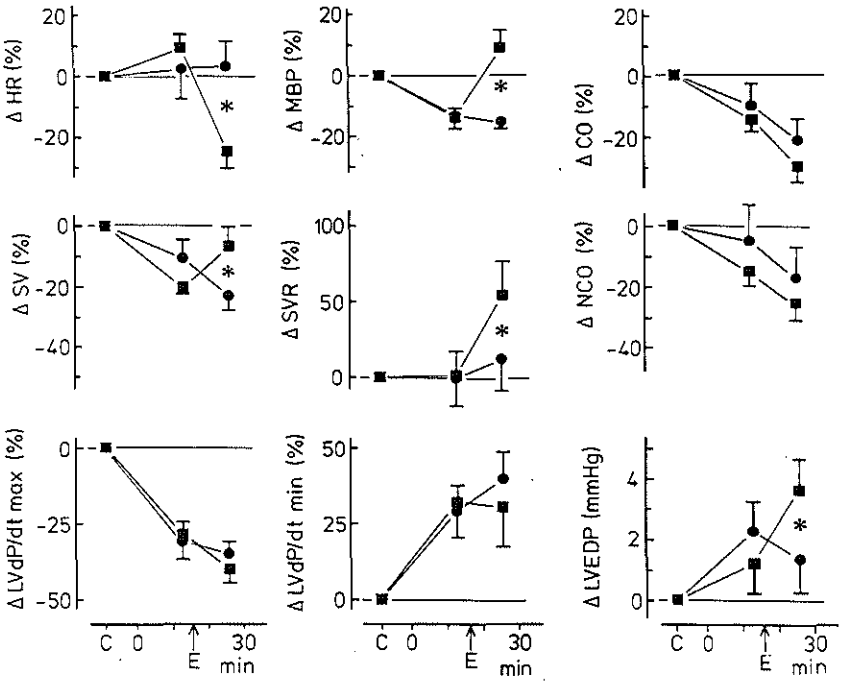

fig.8.2. Percent changes in haemodynamic parameters (mean+SEM) during ischaemia for untreated (●) and ergotamine-treated (■) pigs. Abbreviations and base-line values: see legend of table 8.1. Ergotamine ($8\,\mu g.kg^{-1}$) was administered at E. Asterisks denote $p<0.05$, treated vs. untreated animals (Mann-Whitney U-test).

(i.e., became more negative) at lower doses but returned to its base-line value after a dose of 32 $\mu g.kg^{-1}$.

8.3.2.2. Total and regional myocardial blood flow

Table 8.2 shows the effects of ergotamine on total and regional myocardial blood flow. The blood flow in the LAD coronary artery measured with the electromagnetic flow meter (44±5 $cm^3.min^{-1}$) was higher than the microsphere value (32±2 $cm^3.min^{-1}$). Apart from a moderate increase in the electromagnetic LAD flow after the lowest dose of ergotamine, total myocardial blood flow did not change significantly after drug administration. The index of myocardial oxygen demand, the product of heart rate and LVSP (Brettschneider, 1971), was also not appreciably modified by ergotamine; the changes after 8, 16, and 32 $\mu g.kg^{-1}$ being 10±5, 6±5, and 16±6%, respectively. The effects of ergotamine on the regional distribution of myocardial blood flow were also not striking. The myocardial blood flow to the four layers from endocardium to epicardium remained at approximately 1.0 $cm^3.min^{-1}.g^{-1}$; the endo/epi ratio being 1.11±0.06 at the base line. Ergotamine administration did not cause any change in the distribution of the intramyocardial blood flow, so there were also no changes in the endo/epi ratio (table 8.2).

8.3.2.3. Myocardial wall thickness

The effect of cumulative doses (8, 16, and 32 $\mu g.kg^{-1}$, i.v.) of ergotamine on the myocardial wall thickness were studied in three pigs. Whether or not the heart rate, which decreased after ergotamine, was paced back to a frequency of 120 beats.min^{-1}, all doses of ergotamine failed to cause any significant change in end-diastolic thickness, systolic thickening or diastolic thickening. Only a modest (5-10%) decrease was noticed in end-systolic thickening and maximum thickness with the highest two doses of ergotamine, probably indicating some negative inotropic action of the drug.

8.2.3.4. Total peripheral arteriovenous shunting

As shown in table 8.1, 24% of the microspheres injected into the left atrium, traversed through arteriovenous anastomoses and were recovered in the lungs. Following administration of ergotamine in doses of 8, 16, and 32 $\mu g.kg^{-1}$, i.v., the calculated total AVA flow (0.82±0.21 $dm^3.min^{-1}$ at base line) was reduced by 44±8, 54±5, and 77±5%, respectively. Since there was no significant change in the nutritional cardiac output, the decrease in cardiac output was essentially at the expense of total peripheral AVA flow.

8.3.3. Effects of ergotamine during myocardial ischaemia

8.3.3.1. Systemic haemodynamic changes

The changes from base line in haemodynamic variables (table 8.1) during ischaemia are shown in fig.8.2. After 15 min of ischaemia, heart rate and systemic vascular resistance had not changed. Mean

Table 8.3. Effect of ergotamine on total and regional myocardial blood flow in pigs in which the LAD-flow was reduced.

Myocardial blood flow ($cm^3.min^{-1}.g^{-1}$)	Base-line value (A) n = 13	Value at 14 min of ischaemia (B) n = 13	% changes from (B) at 25 min of ischaemia	
			Untreated n = 6	Treated n = 6
Total myoc. fl.	1.07 ± 0.15	0.95 ± 0.09	-9 ± 8	5 ± 8
Ischaemic zone				
Endocardium 1	1.14 ± 0.17	0.50 ± 0.04 a	-11 ± 13 a	70 ± 40 c
Endocardium 2	1.21 ± 0.17	0.54 ± 0.04 a	-9 ± 11 a	80 ± 40 c
Epicardium 1	1.15 ± 0.17	0.57 ± 0.04 a	-5 ± 11 a	58 ± 21 bc
Epicardium 2	0.99 ± 0.16	0.71 ± 0.05	-5 ± 10 a	19 ± 16
Endo/epi-ratio	1.18 ± 0.05	0.74 ± 0.07 a	-4 ± 11 a	60 ± 40
Non-ischaemic zone				
Endocardium 1	1.26 ± 0.18	1.41 ± 0.17	-12 ± 11	-9 ± 5
Endocardium 2	1.37 ± 0.20	1.52 ± 0.18	-10 ± 11	-12 ± 4 b
Epicardium 1	1.22 ± 0.17	1.34 ± 0.15	-10 ± 9	-12 ± 5 b
Epicardium 2	1.01 ± 0.15	1.18 ± 0.14 a	-10 ± 8	-15 ± 5 b
Endo/epi-ratio	1.27 ± 0.04	1.23 ± 0.05 a	-3 ± 6	6.7 ± 2.3 b

Total myoc. fl., total myocardial blood flow. Endo/epi-ratio was calculated as the ratio between the flow to endocardium 1 and epicardium 2; a, significantly different from base-line value ($p < 0.05$); Student's paired t-test); b, significantly different from values at 14 min of ischaemia in the corresponding animals ($p < 0.05$; Student's paired t-test); c, significantly different from changes in the untreated group ($p < 0.05$; Mann-Whitney U-test).

Table 8.4. Effect of ergotamine (8 µg.kg^{-1}, i.v.) on myocardial wall thickness of the ischaemic region during LAD-flow reduction in pigs.

Myocardial wall thickness parameters	Base-line value (A) n = 13	Value at 14 min of ischaemia (B) n = 13	% changes from (B) at 25 min of ischaemia	
			Untreated n = 6	Treated n = 6
EST (mm)	17.4 ± 0.5	10.6 ± 0.5 a	1.5 ± 2.1	-1 ± 4
EDT (mm)	11.6 ± 0.4	10.3 ± 0.4 a	1.4 ± 2.3	-0.8 ± 2.1
maxT (mm)	17.8 ± 0.5	12.4 ± 0.6 a	0.7 ± 2.9	3 ± 4
tmaxT (msec)	-26 ± 3	151 ± 10 a	-2 ± 5	26 ± 9 b
swt (%)	52 ± 4	4.2 ± 1.8 a	60 ± 40	50 ± 110
dwt (%)	0	15.1 ± 1.9	-4 ± 18	17 ± 21

EST: end-systolic thickness; EDT: end-diastolic thickness; maxT: maximum thickness; tmaxT: time in relation to the closure of aortic valve at which maximal thickness is achieved; swt: systolic thickening; dwt: diastolic thickening; a, b and c as in table 8.3.

Table 8.5. Effect of ergotamine (8 $\mu g \cdot kg^{-1}$) on arteriovenous anastomotic (AVA) flow in pigs.

AVA flow	base-line value (A) n=13	value at 14 min of ischaemia (B) n=13	Percentage change from (B) at 25 min of ischaemia	
			untreated n=6	treated n=5
Total peripheral AVA flow				
% of CO	27 ± 4	27.0 ± 2.7	2 ± 8	-25 ± 9 bc
$dm^3 \cdot min^{-1}$	0.92 ± 0.15	0.81 ± 0.15	-10 ± 10	-41 ± 6 bc
AVA flow in SVC				
% of SVC flow	46 ± 6 d	49 ± 5 d	23 ± 19 e	-35 ± 8 bcf

Total peripheral AVA flow was measured from the microsphere content of the lungs; SVC, superior vena cava; b and c as in table 8.3; d, n=10; e, n=4; f, n=6.

aortic blood pressure, total cardiac output and nutritional cardiac output, and stroke volume decreased by about 10%, while LVEDP increased by 1-2 mmHg. LVdP/dtmax decreased by 25% and LVdP/dtmin became less negative by the same amount. During the subsequent period of ischaemia, heart rate did not change, cardiac output and stroke volume tended to decrease, while LVdP/dtmin became 10% less negative. Administration of ergotamine to ischaemic animals resulted in a decrease in heart rate by 30%. Stroke volume increased by 15%, while the cardiac output decreased by 15%. LVEDP rose by 2 mmHg, while MBP increased by 25%. LVdP/dtmax, LVdP/dtmin, and nutritional cardiac output were decreased, but the changes, as compared to those in the untreated series, were not significant.

8.3.3.2. Total and regional myocardial blood flow

The blood flow to the area perfused by the LAD coronary artery before the flow reduction was 43±7 $cm^3.min^{-1}$ (flow meter, n=12) or 41±6 $cm^3.min^{-1}$ (microsphere method, n=13). During LAD flow reduction, the electromagnetic flow meter indicated a decrease to 39% of control, while the reduction computed from microsphere measurement was to 58% of control. Regional blood flow values obtained at 14 min of ischaemia showed that there was a more marked reduction of blood flow in the endocardium than in the epicardium. As a result, the endo/epi ratio decreased significantly (table 8.3). The hard core of ischaemia in the center of the LAD area showed flow values as low as 8% of control. The flow in the nonischaemic zone and in the border zone (not shown) tended to increase, without changes in the total myocardial blood flow.

Administration of ergotamine (8 $\mu g.kg^{-1}$, i.v.) redistributed the coronary blood flow. The total flow to the ischaemic region increased from 0.57±0.05 to 0.76±0.09 $cm^3.min^{-1}.g^{-1}$ (35%), as compared with the base-line value of 0.82±0.11 $cm^3.min^{-1}.g^{-1}$ in the 6 treated animals. The blood flow value to the ischaemic zone returned to normal in 2 of these animals. The increase in flow favoured the endocardium rather than the epicardium, so that the endo/epi ratio returned towards normal (table 8.3).

8.3.3.3. Myocardial wall thickness

Immediately after onset of flow reduction, the myocardial wall lost its ability to thicken during systole (normally about 50% of end-diastolic thickness; table 8.4). Instead, an early diastolic thickening by 15% of end-diastolic thickness was observed. Maximum wall thickness was reached about 150 msec after the closure of the aortic valves, in contrast to 26 msec before closure during normal perfusion. This pattern remained stable during the entire ischaemic period. Following treatment with ergotamine, segmental myocardial function neither improved (not even in the 2 animals in which the flow had returned to normal) nor deteriorated. Systolic thickening remained nearly absent. Diastolic thickening, which appeared during ischaemia, remained essentially unchanged after ergotamine administration. The only conspicious change with the drug was that

the incidence of maximal wall thickness was shifted further into diastole (tmaxT=193±10 msec).

8.3.3.4. Arteriovenous anastomotic flow

As in normal pigs, a quarter of the cardiac output was shunted through the peripheral AVAs, resulting in an AVA flow of 0.92±0.15 $dm^3.min^{-1}$ (table 8.5). Similarly, one-half of the superior vena caval blood was shunted through AVAs in the head region (SVC shunting). Myocardial ischaemia had no effect on either total or SVC shunting. Ergotamine treatment, however, significantly reduced the total peripheral shunting by 25% and SVC shunting by 35% . The changes in the AVA flows were significantly different in the treated compared with the untreated pigs.

8.4. DISCUSSION

8.4.1. Systemic haemodynamics

The profile of cardiovascular actions of ergotamine and related alkaloids depends on the existing vascular tone (Aellig and Berne, 1969). On the one hand, these drugs decrease the activity of the sympathetic nervous system, and on the other hand, they possess a direct vasoconstrictor action. In the present series of experiments, the vascular tone appears to have been low, as reflected by the level of the intrinsic blood pressure. Consequently, the direct vasoconstrictor effect of the drug prevailed, thus increasing the systemic vascular resistance and blood pressure. Since stroke volume did not change, the observed decrease in cardiac output was caused largely by the heart rate-lowering effect of ergotamine. The bradycardic effect of ergot alkaloids, which is independent of the changes in the blood pressure (Clark et al., 1978), has been shown to be due mainly to a presynaptic dopaminergic inhibition of the cardioaccelerator nerve (Schotsyk, 1978; Saxena and Cairo-Rawlins, 1979). It is interesting that the nutritional cardiac output was less affected by the drug, which, as reported earlier (Johnston and Saxena, 1978; Saxena, 1978), has a remarkably small effect on tissue blood flow.

The inotropic potency of ergotamine can be estimated from the LVdP/dt tracings. LVdP/dtmax not only depends on the inotropic state of the myocardium, but may also be modified by the loading conditions and the heart rate. However, in domestic swine, the increase in heart rate by atrial pacing induced very little effect on this variable (Verdouw et al., 1980). Thus the small decrease in LVdP/dtmax observed in the present study may suggest a slight negative inotropic effect of ergotamine. LVdP/dtmin, which is frequently used as an index of myocardial relaxation, also depends on LVSP (Frederiksen et al., 1978). Therefore, the increases in LVdP/dtmin after ergotamine administration were very likely secondary to the pressor effects of the drug.

8.4.2. Myocardial blood flow

Myocardial blood flow was measured in the present study by two methods: the electromagnetic flow meter and radioactive microspheres. The LAD flow determined at the base line by the two methods in all pigs of the three series were 44±4 (n=21) and 38±4 (n=22), respectively. The Spearman rank correlation between the two measurements, though not excellent, was statistically significant ($r=0.63$, $p<0.001$). The variability in the measurements must be due to at least two factors : (a) an error in the exact localization of the LAD-perfused area and (b) animal to animal variability in the calibration factor of the flow meter probe. However, it was found that the changes in the base-line flow values due to ischaemia and/or ergotamine administration showed much better correlation ($r=0.91$, $p<0.001$). These correlations indicate the reliability of the data produced by the two methods.

Total myocardial blood flow was not noticably affected by ergotamine. Although both heart rate and MBP changed markedly, the product of heart rate and LVSP, an index of myocardial oxygen demand (Brettschneider, 1971), was unaltered or only slightly increased. However, in previous experiments (Johnston and Saxena, 1978) in which ergotamine caused bradycardia without any concomitant change in the blood pressure, the drug did decrease the total myocardial blood flow. It can be concluded that ergotamine had no effect on the myocardial blood flow autoregulatory function.

Even when total organ blood flow is unchanged, the intra-organ distribution of blood flow may change substantially. Since the endocardium is highly vulnerable to ischaemia, particular attention was paid to the distribution of flow from endocardium to epicardium. Ergotamine administration to animals with normally perfused hearts did not result in changes in the intramyocardial blood flow distribution. Reduction of the LAD flow resulted in a decrease in the endo/epi ratio, so that the endocardium encountered a relatively more severe ischaemia than the epicardium. Treatment with ergotamine not only increased total myocardial blood flow, but also improved the distribution pattern. Although the poststenotic pressure was not measured, it might be expected that in a perfusion pressure dependent system [the screw clamp is responsible for about 96% of the "vascular resistance" (Gould et al., 1975)], the changes in the MBP and poststenotic pressure (Schaper, 1971) were parallel; thus the improvement in the myocardial perfusion was partly due to the higher perfusion pressure. In addition, an increase in the duration of the diastolic perfusion time, as a result of bradycardia after ergotamine, may be responsible for the beneficial change in the endo/epi ratio.

8.4.3. Myocardial wall thickness

In agreement with earlier data from this laboratory, reduction of the LAD flow to 40% of control resulted in a loss of systolic thickening and appearance of a diastolic "bulging" (Verdouw et al., 1980). Despite a return towards base-line values of the blood flow to the ischaemic zone after ergotamine, there was no improvement in the

performance of the ischaemic ventricular wall as assessed by echographic measurement of the wall thickness. The effects of reperfusion on myocardial performance are not yet fully understood. Conflicting reports have appeared, demonstrating little or complete recovery of the haemodynamic and metabolic function of the myocardium on reperfusion for varying periods after total or partial occlusion of the LAD, both in the pig (Althaus et al., 1977; Verdouw et al., 1979b) and in the dog (Maroko et al., 1972; Lang et al., 1974; Heyndrickx et al., 1975; Ramanathan et al., 1978). The period for which the flow returned to normal after ergotamine might be inadequate for improving myocardial wall function. However, it is also possible that the increase in the afterload and wall stress following ergotamine, sustains the abnormal metabolism (and thus lack of systolic wall thickening) of the poststenotic ischaemic segment of the myocardium, despite an improvement in the blood flow. Similarly, a recent study (Komer et al., 1979) reports that haemodynamic improvements with nitroglycerin were unaccompanied by any beneficial influences on the wall thickness in acutely ischaemic canine myocardium. It appears that changes in wall thickness are a sensitive index of myocardial ischaemia.

8.4.4. Arteriovenous anastomotic flow

The use of 15 micron radioactive microspheres in studies of arteriovenous shunting has been discussed in a number of previous reports (Forsyth et al., 1968; Kaihara et al., 1968; Buckberg et al., 1971; Archie et al., 1973; Hales, 1974; Heymann et al., 1977; Johnston and Saxena, 1978; Saxena, 1978; Saxena et al., 1978; Spierings and Saxena, 1979; Verdouw et al., 1979a). The data presented in this paper indicate that, at least under the experimental conditions of this study, a large part of the cardiac output is "wasted" through nonnutritional channels. A very high proportion of the blood supply to the head tissues is shunted to the vena cava superior. This has also been reported for other species (Johnston and Saxena, 1978; Saxena, 1978; Spierings and Saxena, 1979). The AVAs, which appear to play a major role in the pathophysiology of migraine (Heyck, 1969; Johnston and Saxena, 1978; Saxena, 1978; Spierings and Saxena, 1979), were greatly constricted after administration of ergotamine.

In conclusion, our data show that the regional myocardial blood flow pattern in animals with acute reduction of the LAD flow was improved by ergotamine. However, segmental wall motion in the ischaemic zone, regarded widely as a sensitive marker of myocardial ischaemia, was unaffected after the administration of the drug. The present study does not provide clear supporting evidence for cardiovascular contraindications of ergotamine. Nevertheless, it is possible that in patients with coronary atherosclerosis who respond to ergotamine with a marked pressor effect without bradycardia, the increased oxygen demand may not be successfully met by a corresponding increase in myocardial blood flow.

8.5. Acknowledgements

T.M. van der Hoek participated during the Elective Research Program in the third-year curriculum of this faculty. The authors gratefully acknowledge the technical assistance of Mrs. M.A. Kroos, Miss A.M. Rutteman and R.H. van Bremen. The analysis of wall thickness tracings was performed using the computer belonging to the "Inter Universitair Cardiologisch Instituut". Ergotamine was supplied by Sandoz B.V., Uden. Dr. M.J. Parnham and J. de Beurs assisted in the preparation of the manuscript.

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European Journal of Pharmacology, 64 (1980) 209—220

THE EFFECTS OF ALINIDINE, AN N-ALLYL DERIVATIVE OF CLONIDINE, ON REGIONAL MYOCARDIAL PERFUSION AND PERFORMANCE IN THE PIG WITH OR WITHOUT ATRIAL PACING *

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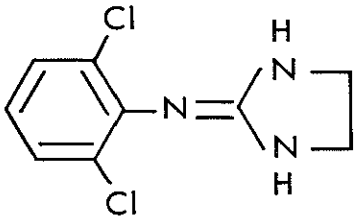
Received 10 December 1979, revised MS received 5 March 1980, accepted 18 March 1980

THE EFFECTS OF ALINIDINE, AN N-ALLYL-DERIVATIVE OF CLONIDINE, ON REGIONAL MYOCARDIAL PERFUSION AND PERFORMANCE IN THE PIG WITH OR WITHOUT ATRIAL PACING

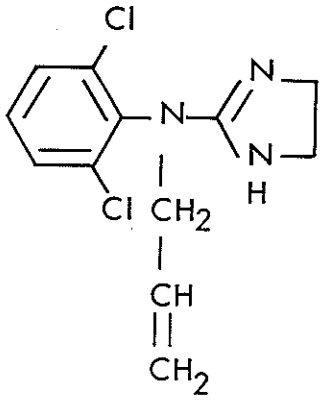
Summary. The effects of alinidine (0.2 - 6.0 $mg.kg^{-1}$), an N-allyl-derivative of clonidine, were investigated on systemic and regional haemodynamics, in particular myocardial perfusion and performance, in the domestic pig, during or in the absence of atrial pacing. The drug had a pronounced bradycardic action and also caused dose-dependent reductions in the maximum rate of rise in left ventricular pressure (maxLVdP/dt), cardiac output (CO), arterial blood pressure and in the mean velocity of systolic wall thickening ($\bar{v}_{swt}$) in the absence of atrial pacing. Since the duration of systole was prolonged by alinidine, the total wall thickening during systole (swt) remained unchanged until the highest dose was given. When the heart rate was kept constant by atrial pacing, there were no changes in the maxLVdP/dt, CO or $\bar{v}_{swt}$ with lower doses (<0.4 $mg.kg^{-1}$) of alinidine. With higher doses, however, there was a significant reduction in these variables, demonstrating a clear negative inotropic action of the drug. The decrease in CO was entirely at the expense of its nutrient fraction (NCO), since systemic arteriovenous anastomotic flow remained unchanged. However, the reduction in NCO did not hamper tissue oxygenation either because of autoregulation within blood vessels (cerebral and renal), or because the tissues were able to extract more O_2 from the blood. Similarly, despite the reduction of myocardial perfusion, no imbalance in the myocardial oxygen supply-demand relationship was noticed due to a simultaneous reduction of the myocardial work in both unpaced and paced hearts. Moreover, the changes in the intramyocardial blood flow were quite uniform. It is concluded that alinidine has a negative chronotropic and, in higher doses, a negative inotropic action. The cardiovascular profile of the drug suggests that it could be useful in patients with ischaemic heart disease.

9.1. INTRODUCTION

Recent studies have shown that the most outstanding feature of the pharmacological profile of alinidine (St 567), an N-allyl- derivative of clonidine, 2-N-allyl-N-(2,6- dichlorophenyl)- amino- 2-imidazoline (fig.9.1), is a slowing of the heart rate, leading to a diminution of the cardiac output (Kobinger et al., 1979 a,b). In contrast to clonidine, alinidine does not appreciably lower the arterial blood



clonidine



alinidine

fig.9.1. Chemical structure of clonidine and its N-allyl derivative alinidine.

pressure, nor does it seem to act via the central nervous system. The evidence, so far, indicates that the drug depresses the automaticity of the sinoatrial node directly, i.e. without influencing the autonomic nervous system (Kobinger et al., 1979 a,b; Lillie et al., 1979; Tritthart and Windisch, 1979). The specific bradycardic action of alinidine suggests that this agent, by decreasing myocardial oxygen demand, could have a beneficial effect in patients with ischaemic heart disease.

The present study was designed to investigate the effects of alinidine on systemic and regional haemodynamics, in particular myocardial perfusion and performance, in the domestic pig during or in the absence of atrial pacing. The radioactive microsphere technique was applied for the study of organ perfusion (Heymann et al., 1977; Schamhardt et al., 1979), while regional myocardial function was estimated from continuous myocardial wall thickness tracings (ten Cate et al., 1979; Verdouw et al., 1980).

9.2. MATERIALS AND METHODS

9.2.1. General

Pigs (25 to 30 kg) were sedated with 120 mg azaperone (stresnil®) i.m. Subsequently, they received 150 mg metomidate (hypnodil®) via a dorsal ear vein. After intubation, the animals were ventilated with a Bennett respirator (BA-4) using a mixture of 33% O_2 and 67% N_2O. Respiratory rate and tidal volume were adjusted, when required, to keep arterial blood gases (determined with an ABL-1 Acid-Base Laboratory, Radiometer, Copenhagen) within the normal range: $7.35<pH<7.45$; $35<PCO_2$ [mmHg]<45 and $90<PO_2$ [mmHg]<150. Recordings from peripheral ECG leads were monitored throughout the study. An 8 French (F) double lumen catheter was placed in the superior vena cava for continuous administration of the anaesthetics (8 $mg.kg^{-1}.h^{-1}$ azaperone and 2 $mg.kg^{-1}.h^{-1}$ metomidate) and a muscle relaxant (4 $mg.h^{-1}$ pancuronium bromide), while a single lumen catheter was placed in the inferior vena cava for administration of alinidine. A 7 F triple lumen, balloon-tipped thermodilution catheter (Edwards Laboratories, Santa Ana, Calif., USA) was placed in the pulmonary artery via the right femoral vein, for the determination of cardiac output and pulmonary artery pressure and for the withdrawal of mixed venous blood. Left ventricular and central aortic pressures were obtained from catheters tipped with micromanometers (7 F Millar). Arterial blood samples were withdrawn through an 8 F catheter placed in the upper part of the abdominal aorta. A heating pad maintained the temperature of the animals between 36 and 37.5 C.

The heart was exposed via a midsternal split, the left mammary artery and vein were ligated and sections of the fourth and fifth rib were removed. Excessive bleeding was prevented by electric cautery. The left anterior descending coronary artery (LAD) was dissected at the origin and a precalibrated electromagnetic flow probe (Skalar, Delft, The Netherlands) was placed around the vessel. Zero flow

reference measurements were obtained by occluding the LAD for a short period with a pair of atraumatic forceps. The great cardiac vein was canulated and a polyethylene catheter was inserted. The catheters were flushed with heparin to prevent clotting. Finally, a pacing wire was fixed on the right atrium.

9.2.2. Myocardial wall thickness

Myocardial wall thickness was monitored with a 5MHz ultrasonic transducer (Krautkramer-Branson, Lewistown, Pa., USA) sutured onto a part of the epicardial surface perfused by the LAD. The signals were displayed continuously on a modified EchocardioVisor ultrasonoscope (Organon Teknika, Oss, The Netherlands). At suitable intervals, the tracings were also recorded on photographic paper for subsequent analysis.

9.2.3. Regional blood flow

Total blood flow to, or regional blood flow within a number of organs (heart, brain, kidneys and lungs) was measured with the radioactive microsphere method (Heymann et al., 1977; Schamhardt et al., 1979). About $1\text{-}2\times10^6$ microspheres (15 ± 5 micron diam., 3M Company, Minneapolis, Minn., USA) labelled with either ^{125}I, ^{141}Ce, ^{85}Sr or ^{95}Nb, were injected through a canula inserted into the left atrial appendage over a period of 10-15 s. Arterial blood was withdrawn (10 $ml \cdot min^{-1}$) for a period of 90 s, starting a few seconds before the microsphere injection, in order to calibrate flow measurements.

9.2.4. Collection of data before and after alinidine

After completion of the surgical procedure, a minimum of 30 min was allowed for stabilization of the preparation, before base-line data were obtained at the animal's own sinus rhythm. These data included pressures in the left ventricle, central aorta, right atrium and pulmonary artery, cardiac output and recordings of the myocardial wall thickness. Arterial and coronary venous samples (1.5 ml) were withdrawn for the determination of O_2saturation with an oxymeter (American Optical Company, Bedford, Mass., USA). After collection of these data the heart rate was raised to 120 $beats \cdot min^{-1}$ for 5 min by atrial pacing. Just before the end of the pacing period all previous measurements were repeated and, in addition, microspheres were injected in order to measure regional blood flow.

After the base-line measurements, two doses of 0.2 $mg \cdot kg^{-1}$ alinidine (as hydrobromide) were injected i.v. with a 15 min interval. These injections were followed by doses of 0.8, 1.6 and 3.2 $mg \cdot kg^{-1}$, i.v. every 30 min. The animals, thus, received 0.2, 0.4, 1.2, 2.8 and 6.0 $mg \cdot kg^{-1}$ of alinidine in cumulative doses. All cardiovascular variables, except the regional flow measured with the microspheres, were determined both at the animal's own sinus rhythm and at the fixed rate of 120 $beats \cdot min^{-1}$. The radioactive microspheres were given at the base line and only during atrial pacing, after the cumulative doses of 0.4, 1.2 and 2.8 $mg \cdot kg^{-1}$ alinidine, to study the effect of

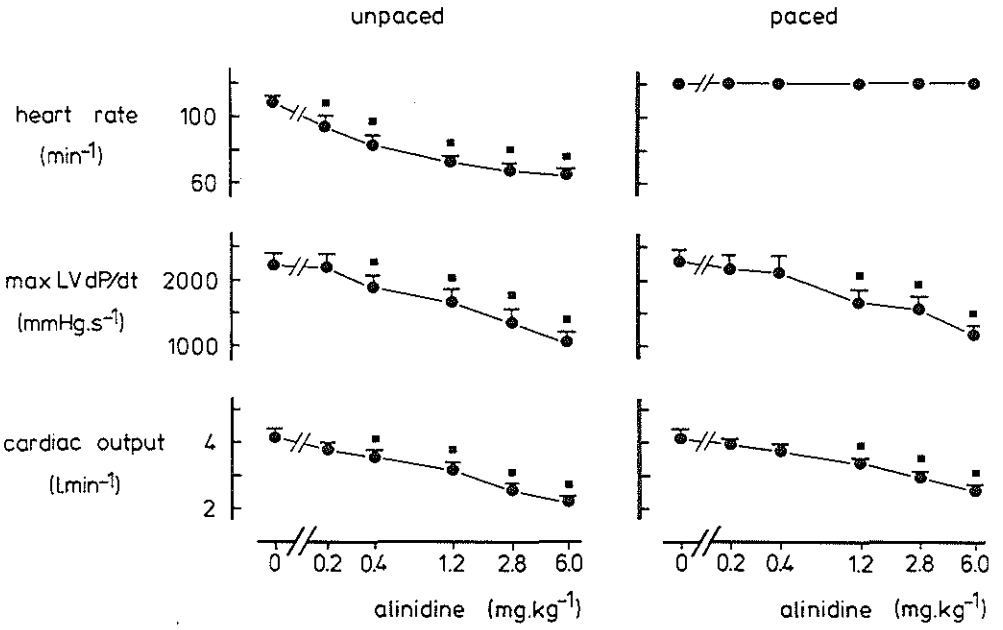


fig.9.2. The effect of alinidine on heart rate, the maximum of the rate of rise in left ventricular pressure (maxLVdP/dt) and cardiac output in unpaced and paced hearts. It is shown that alinidine, besides causing bradycardia, decreases maxLVdP/dt and cardiac output both in the absence of and during atrial pacing. Absolute values (mean±SEM) are depicted. ■, significantly ($p<0.05$) different from the base line.

the drug on regional circulaton without the influence of drug-induced bradycardia.

9.2.5. Data recording and analysis

9.2.5.1. General

A Brush Gold recorder (type MK 480) was used for recording (at a paper speed of 200 $mm.s^{-1}$ of the appropriate tracings of the ECG, the pressures in the left ventricle (and its first derivative), central aorta, right atrium and pulmonary artery and flow in the LAD. Thermodilution curves were recorded at a paper speed of 10 $mm.s^{-1}$. The following variables were obtained from the tracings: heart rate, P-Q intervals, QRS width, Q-T interval, left ventricular systolic and end-diastolic pressure (LVEDP), the maximum rate of rise in left ventricular pressure (maxLVdP/dt), mean aortic pressure (MBP), right atrial pressure (RAP), mean pulmonary artery pressure and cardiac output (CO). For an accurate measurement of LVEDP, the gain of the left ventricular pressure signal was magnified 2.5 times during recording. Vascular resistances were calculated by dividing the driving pressure (MBP-RAP) by the corresponding flows.

9.2.5.2. Myocardial wall thickness

The echocardiogram, the pressures in the left ventricle and in the root of the aorta and the rate of change of ventricular pressure were recorded simultaneously at a paper speed of 50 $mm.s^{-1}$ on linagraph direct print photographic paper (Eastman Kodak) using a Honeywell fiberoptic system (LS6). The tracings were analyzed with a digital computer as described previously (van Zwieten et al., 1979). Following measurements of the end-diastolic (EDT) and the end-systolic (EST) wall thicknesses and the duration of systole, the systolic wall thickening (swt) and the mean velocity of systolic wall thickening $\overline{v}_{swt}$ were determined. End-diastole was defined as the onset of the upstroke of LVdP/dt, while end-systole was taken as the occurrence of the incisura on the central aortic pressure. Systolic wall thickening was calculated as:

$$swt=((EST-EDT)/EDT)x100\%$$

while $\overline{v}_{swt}$ was defined as the ratio of (EST-EDT) and the duration of systole.

9.2.5.3. Radioactive microsphere data

After completion of the experiment, the heart, lungs, kidneys, and brain were excised and handled as described in detail elsewhere (Schamhardt et al., 1979). Transmural pieces from the heart were divided into four layers of equal thickness from endocardium to epicardium and named sequentially as endo 1, endo 2, epi 1 and epi 2. The endo/epi ratio was calculated from the weight-normalized flows to the two peripheral layers, i.e. endo 1: epi 2. The blood flow calculated on the basis of the microsphere content of the lungs

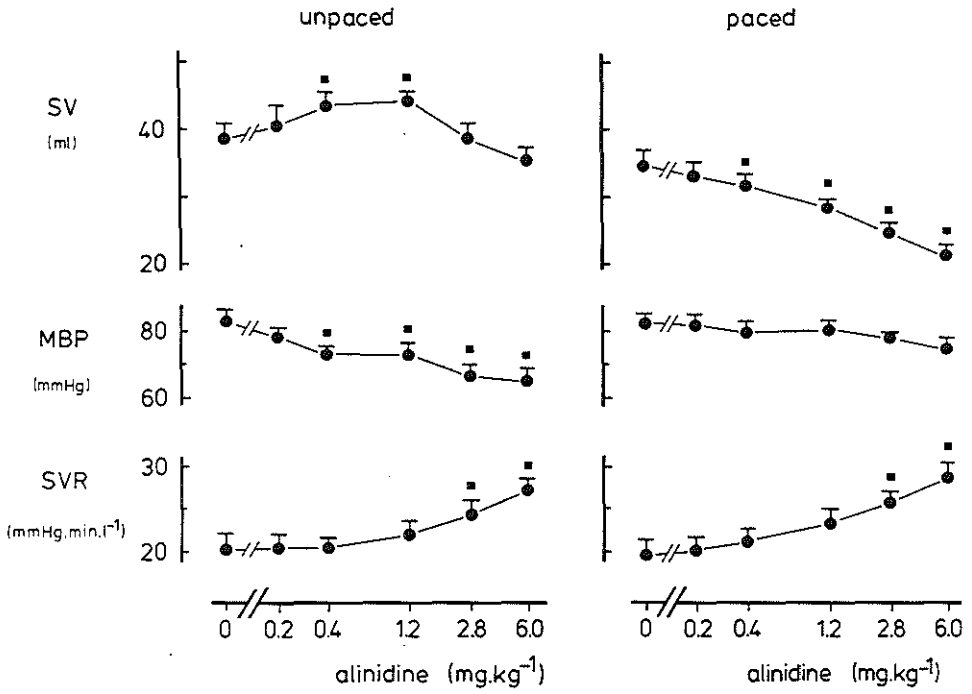


fig.9.3. The effects of alinidine on the stroke volume (SV), mean arterial blood pressure (MBP) and total systemic vascular resistance (SVR) in the absence of and during atrial pacing. Note that the stroke volume increased (with lower doses) when the heart was not paced, but decreased during pacing, in response to alinidine. MBP was decreased only in the absence of atrial pacing while SVR showed an increase irrespective of pacing. Absolute values (mean±SEM) are depicted. ■, significantly ($p<0.05$) different from the base line.

represents almost entirely blood shunted through peripheral arteriovenous anastomoses (AVAs; Johnston and Saxena, 1978; Saxena, 1978; Saxena et al., 1978; Schamhardt et al., 1979) since the bronchial artery flow is only about 1% of the cardiac output (Forsyth et al., 1968). Thus, the nutrient (capillary) fraction of cardiac output (NCO) can be estimated by substracting peripheral AVA-flow from cardiac output.

9.2.5.4. O_2extraction and O_2consumption

Precise determination of O_2extraction and O_2consumption, using the O_2content of the blood, could not be carried out since haemoglobin concentration was not measured routinely. In three experiments, however, we established that the haemoglobin concentration did not changed appreciably during the course of the experiments. Thus, the indices of myocardial O_2extraction (MO_2ext) and myocardial O_2consumption (MO_2cons) were calculated with the following formulae:

$$MO_2ext = ((artSO_2-corvenSO_2)/artSO_2)x100\% \quad (1)$$

$$MO_2cons = (artSO_2-corvenSO_2)xLAD\text{-}flow \quad (2)$$

where $artSO_2$ and $corvenSO_2$ denote the oxygen saturation of arterial and coronary venous blood, respectively. The index of total body O_2consumption was calculated using formula (2) with the substitution of mixed venous O_2saturation ($mixvenSO_2$) and cardiac output (CO) for $corvenSO_2$ and LAD-flow, respectively. Since part of oxygen in the mixed venous blood arrives in the veins without extraction of oxygen by the tissues (shunting), the following equation can be derived:

$$COx(artSO_2-mixvenSO_2)=NCOx(artSO_2-nvbSO_2) \quad (3)$$

where NCO denotes the nutrient fraction of the cardiac output. Hence, $nvbSO_2$ (oxygen saturation of the nutrient fraction of cardiac output after passage through the tissue) was calculated as:

$$nvbSO_2= CO/NCOxmixvenSO_2+(1-CO/NCO)xartSO_2 \quad (4)$$

Finally, the index of total body O_2extraction (TO_2ext) was calculated by the formula:

$$TO_2ext = ((artSO_2-nvbSO_2)/artSO_2)x100\% \quad (5)$$

9.2.6. Statistical analysis

Data have been presented as mean±SEM. Student's paired t-test (Snedecor and Cochran, 1967) was used to determine whether alinidine-induced changes differed significantly from the base-line values. Only $P<0.05$ (two-tailed) was considered to be statistically significant.

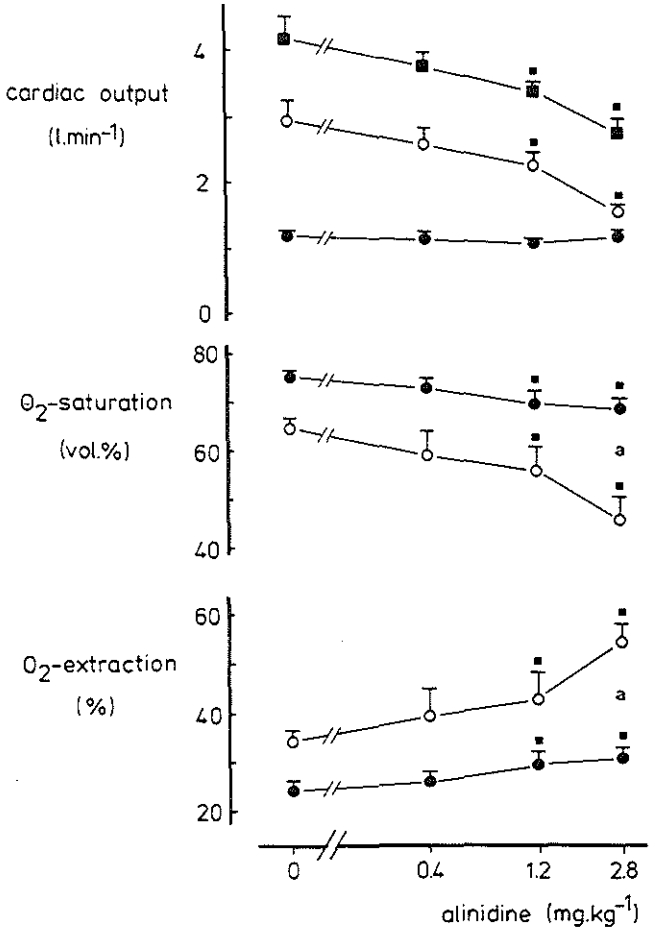


fig.9.4. The effects of alinidine on cardiac output (■) and its nutrient (○) and non-nutrient (●) fractions, O_2saturation and total body O_2extraction from the nutritional (○) and the mixed venous blood (●) in 8 pigs. The O_2saturation and O_2extraction in the nutrient fraction of the venous blood were derived using formulae (4) and (5) (section 9.2.5.4). Alinidine decreased NCO and the nutritional venous O_2saturation but increased O_2extraction from the nutrient arterial blood. After 6 mg.kg^{-1}, the nutritional venous O_2saturation decreased more than the mixed venous O_2saturation, and the nutritional venous O_2extraction increased more than the mixed venous O_2extraction (a, $p<0.05$). ■ , significantly ($p<0.05$) different from the base line.

9.3. RESULTS

9.3.1. General

Complete data were obtained from eight animals. One other animal died of sudden ventricular fibrillation, 10 min after the administration of 0.2 $mg.kg^{-1}$ alinidine. Although spontaneous ventricular arrhythmias are not frequently observed in anaesthetized domestic pigs, they do occur. Therefore, the cause of death could not be ascribed to alinidine administration. The animal was not defibrillated, and was excluded from the study. In the other animals, conduction disturbances were occasionally seen immediately after the administration of the two highest doses, but they disappeared after a few minutes.

9.3.2. Heart rate and electrocardiographic changes

The major change after alinidine administration was a prolongation of the PP' interval, since heart rate decreased significantly and dose dependently (fig.9.2). As described in detail by Kobinger et al. (1979b), there were proportional changes in the Q-T interval. In order to eliminate the secondary influence on these ECG parameters due to the bradycardia the P-Q, QRS and the Q-T intervals were also determined after the heart rate had been paced to 120 $beats.min^{-1}$. Except for a tendency of the Q-T interval to increase by about 10% after the highest dose, no consistent changes in the ECG parameters were observed.

9.3.3. Systemic and pulmonary haemodynamics

9.3.3.1. Myocardial contractility and cardiac output

The effects of the various doses of alinidine, at the animal's own heart rate and after atrial pacing to a frequency of 120 $beats.min^{-1}$, on maxLVdP/dt and cardiac output are shown in fig.9.2. Both maxLVdP/dt and cardiac output had already decreased significantly at a dose of 0.4 $mg.kg^{-1}$ alinidine and decreased to about half the base-line value at the highest dose in unpaced hearts. Although doses of upto 0.4 $mg.kg^{-1}$ did not affect these variables in paced hearts, higher doses produced decreases which were similar in magnitude in the paced and unpaced hearts.

9.3.3.2. Stroke volume, blood pressure and vascular resistance

Fig.9.3 depicts the changes produced by alinidine in the stroke volume, mean arterial blood pressure and total systemic vascular resistance. It is obvious that the stroke volume followed the same pattern as the cardiac output when the heart was paced at 120 $beats.min^{-1}$. However, a strikingly different pattern was observed at the animal's own heart rate. At lower doses of alinidine the stroke volume increased (by upto 16%), while it returned towards the base-line value with higher doses of the drug. The mean arterial pressure decreased gradually in response to the drug only when the heart had not been paced. During pacing, the blood pressure did not

Table 9.1. Effect of alinidine on regional myocardial perfusion in 8 pigs.

Perfusion variable	Base-line value	Percentage change by alinidine ($mg \cdot kg^{-1}$)				
		0.2	0.4	1.2	2.8	6.0
LAD-flow (unpaced) [1]	61 ± 6	-9 ± 4	-20 ± 6 *	-33 ± 8 *	-49 ± 7 *	-51 ± 7 *
LAD-flow (paced) [1]	62 ± 7	-3 ± 4	-11 ± 6	-21 ± 9 *	-32 ± 9 *	-44 ± 6 *
Myocardial O_2 extraction [2,3]	70 ± 2	-5 ± 2 *	0 ± 4	1 ± 4	5 ± 4	8 ± 4
Myocardial O_2 demand [2,4]	25 ± 3	-18 ± 6 *	-37 ± 6 *	-52 ± 6 *	-63 ± 7 *	-74 ± 5 *
Myocardial blood flow [5]						
Left ventricle	1.31 ± 0.10	-	-5 ± 10	-24 ± 6 *	-33 ± 5 *	-
Endocardium 1	1.33 ± 0.10	-	-7 ± 11	-24 ± 6 *	-29 ± 6 *	-
Endocardium 2	1.44 ± 0.11	-	-6 ± 11	-23 ± 6 *	-31 ± 6 *	-
Epicardium 1	1.32 ± 0.10	-	-4 ± 11	-23 ± 5 *	-32 ± 5 *	-
Epicardium 2	1.20 ± 0.10	-	-2 ± 10	-24 ± 6 *	-32 ± 6 *	-
Endo/epi-ratio [6]	1.12 ± 0.04	-	-5 ± 4	0 ± 3	5 ± 5	-

1, LAD-flow: $ml \cdot min^{-1}$; 2, measured at animal's own heart rate, i.e. without atrial pacing; 3, calculated as: $(artSO_2 - cor.ven.SO_2)/ artSO_2$; 4, calculated as: heart rate ($b \cdot min^{-1}$) x maxLVdP/dt ($mHg \cdot s^{-1}$) x LV systolic pressure (mHg); 5, measured with microspheres during atrial pacing ($ml \cdot min^{-1} \cdot g^{-1}$); 6, ratio of endocardium 1 and epicardium 2 flows; *, significantly ($p<0.05$) different from the corresponding base-line values.

change significantly (fig.9.3). Since the changes in the mean arterial blood pressure were relatively smaller than those in the cardiac output, the calculated systemic vascular resistance was increased by alinidine in both situations - paced and unpaced.

9.3.3.3. LV end-diastolic, right atrial and pulmonary pressures

The base-line value for left ventricular end-diastolic pressure (LVEDP) was 7.1±0.8 mmHg. This value gradually increased to 9.6±1.2 mmHg after 6.0 $mg.kg^{-1}$ ($P<0.05$), when measured at the animal's own rhythm. However, at a rate of 120 $beats.min^{-1}$, no changes in LVEDP were observed, as the values at the base line and after the highest dose were both 6.0±0.6 mmHg. This indicates that the slight increase in LVEDP after alinidine administration was due to bradycardia caused by the drug. As with LVEDP, neither right atrial, nor pulmonary arterial pressure showed appreciable changes after alinidine.

9.3.3.4. Total O_2availability and O_2consumption

Since artSO_2 and the haemoglobin concentration did not change appreciably during the course of our experiments, the total amount of O_2, available to the tissues, was mainly dependent on the fractionation of the total cardiac output (CO) into the nutrient (NCO) and the non-nutrient (AVA) parts. The fraction of the microspheres entrapped by the lungs (29±3, 32±4, 33±3 and 43±2% before and after 0.4, 1.2 and 2.8 $mg.kg^{-1}$ alinidine, respectively) mainly represent the amount of blood shunted through AVAs (Forsyth et al., 1968; Johnston and Saxena, 1978; Saxena et al., 1978). As shown in fig.9.4, the NCO, calculated as the difference between CO and AVA-flow, was decreased by alinidine without a concomitant change in AVA-flow. This reduction in O_2availability was, however, compensated for by an increased extraction of O_2 from arterial blood: the SO_2 of mixed venous blood gradually decreased and O_2consumption remained unchanged.

9.3.4. Regional haemodynamics

9.3.4.1. Myocardial perfusion

Myocardial blood flow in the present experiments was measured by two methods: radioactive microspheres (only during pacing) and electromagnetic flow probe on LAD (continuously). The base-line blood flow ($ml.min^{-1}.g^{-1}$) to the right ventricle (1.11±0.09, n=8) and to the atria (1.22±0.15, n=8) was in both cases, as expected, less than that to the left ventricle (1.47±0.10, n=8). The base-line LAD-flows ($ml.min^{-1}$) in the unpaced (61±6) and in the paced (62±7) hearts were not significantly different. However, after alinidine, LAD-flow decreased parallel with heart rate, but when the heart was paced, LAD-flow increased significantly, the changes being 7±3, 15±5, 19±5, 41±11 and 23±7, respectively, after 0.2, 0.4, 1.2, 2.8 and 6.0 $mg.kg^{-1}$.

Table 9.1 presents the data pertaining to myocardial perfusion before and after alinidine administration. Both LAD-flow (in the absence of or during atrial pacing) and left ventricular blood flow

Table 9.2. Effect of alinidine on cerebral and renal haemodynamics in 7 pigs[1].

Haemodynamic variable	Base-line value	% Change by alinidine ($mg.kg^{-1}$)		
		0.4	1.2	2.8
Cerebral blood flow				
% CO	0.53 ± 7	13 ± 13	7 ± 10	53 ± 16*
$ml.min^{-1}.g^{-1}$	0.25 ± 0.02	-4 ± 12	-9 ± 10	-5 ± 14
Cerebral vascular[2] resistance	350 ± 40	-3 ± 11	9 ± 8	-3 ± 12
Renal blood flow				
% CO	11.0 ± 1.4	12 ± 8	5 ± 7	21 ± 9*
$ml.min^{-1}$	3.9 ± 0.5	1 ± 7	-15 ± 6	-19 ± 9
Renal vascular resistance[2]	25 ± 6	0 ± 6	20 ± 11	28 ± 15

1, measurements were made during atrial pacing; 2, calculated by dividing the driving pressure (MBP-RAP) (mmHg) by blood flow ($ml.min^{-1}.g^{-1}$); *, significantly ($p < 0.05$) different from base-line values.

(measured during atrial pacing using microspheres) decreased in a dose dependent manner as did the heart rate and the maxLVdP/dt. The reduction in the blood flow to the different layers was of a similar magnitude and, thus, the endo-epi ratio did not change. Moreover, the decrease in the myocardial blood flow did not cause an imbalance between myocardial O_2demand and O_2supply (table 9.1). At low doses of alinidine there was a slight decrease in O_2extraction ($P<0.05$) but at higher doses (>2.8 $mg.kg^{-1}$) myocardial O_2extraction tended to increase although these changes did not achieve statistical significance.

9.3.4.2. Cerebral and renal haemodynamics

Cerebral blood flow was not influenced by alinidine despite the pronounced decrease in cardiac output (table 9.2). It appears that there was autoregulation within the cerebral vessels and that at the 2.8 $mg.kg^{-1}$ dose they received a significantly higher fraction of cardiac output. The blood flow measured in each of the two kidneys in the 8 experiments showed an excellent correlation with each other (Spearman rank correlation coefficient = 0.97; $p<0.001$, $n=32$). As with cerebral blood flow, autoregulaton occurred within the renal blood vessels to some extent and, as a consequence, the total renal blood flow did not change significantly after alinidine (table 9.2).

9.3.5. Myocardial wall thickness

The effect of alinidine on myocardial wall thickness variables is shown in fig.9.5. End-systolic wall thickness remained unaffected by the drug and, hence, the changes in systolic wall thickening (swt) followed closely those in the end-systolic wall thickness. At the animal's own sinus rhythm, the decrease in swt only became significant after the highest dose (6 $mg.kg^{-1}$) of the drug. However, when the heart was paced at 120 $beats.min^{-1}$, a significant decrease in swt was already measureable after 1.2 $mg.kg^{-1}$, despite similar decreases in the mean velocity of thickening ($\bar{v}_{swt}$) in paced and unpaced hearts. Both $\bar{v}_{swt}$ and the duration of systole determine swt, and an inspection of fig.9.5 reveals that the duration of systole was not affected when the heart rate was kept constant, but did increase significantly when the rate was allowed to decrease. Hence the increase in the duration of systole allowed the myocardium to contract for a longer period so that only minor changes were observed in swt in unpaced hearts.

9.4. DISCUSSION

The N-allyl-derivative of clonidine (alinidine), like some veratrum- and harmala-alkaloids (Krayer, 1949; Kosterlitz et al , 1955; Innes et al., 1956; Zetler, 1968; Carpentier, 1977), has been shown to cause bradycardia by exerting a direct salutary action on the sinoatrial-node (Kobinger et al., 1979 a,b; Lillie et al., 1979; Tritthart and Windisch, 1979) In comparison to the Ca^{2+}-antagonist verapamil, and the antiarrhythmics quinidine and lidocaine, this compound has reportedly a more specific negative chronotropic than

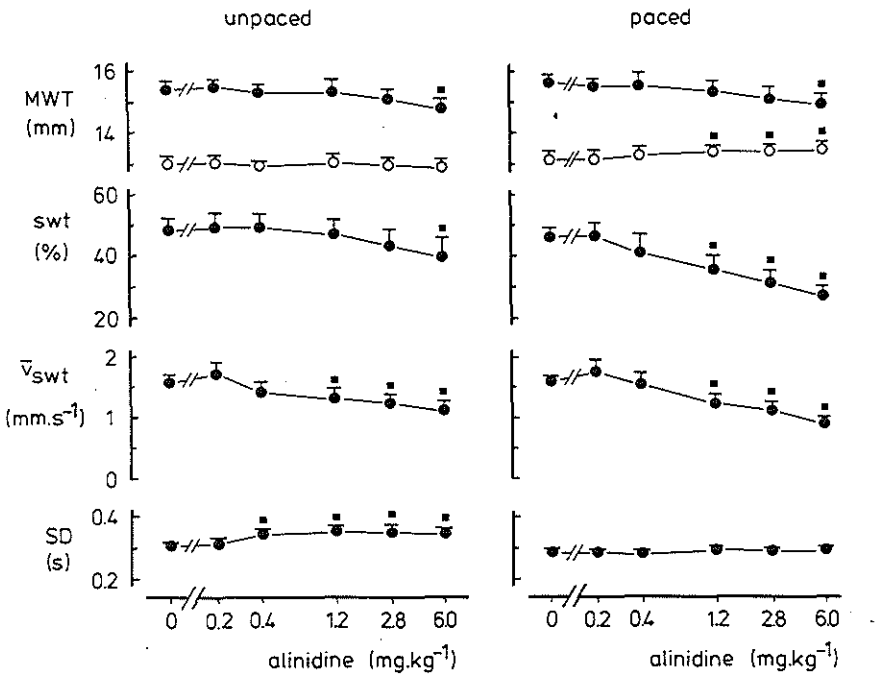


fig.9.5. The effect of alinidine, in the absence of and during atrial pacing on myocardial wall thickness variables. MWT, myocardial wall thickness, end-systolic (●) and end-diastolic (○) thickness; swt, systolic wall thickening; $\bar{v}_{swt}$, mean velocity of systolic wall thickening; SD, duration of the isovolumic contraction and the left ventricular ejection. ■, significantly ($p<0.05$) different from the base line.

negative inotropic action (Kobinger et al., 1979b). In the present study, which was designed to investigate the cardiovascular profile of alinidine in detail, we confirm that the drug produced a pronounced bradycardia, together with a decrease in maxLVdP/dt. Since the effects on maxLVdP/dt were independent of the preload (LVEDP), the heart rate and the afterload (mean arterial blood pressure), it can be concluded that the drug, in addition to causing bradycardia, also depressed myocardial contractility. Our data obtained from pigs (fig.9.2) also suggest that alinidine may be a little more effective on heart rate than on myocardial contractility.

The reduction of the mean velocity of systolic myocardial thickening ($\bar{v}_{swt}$) in both paced and unpaced hearts, is in agreement with our conclusion that alinidine depresses myocardial contractility. In spite of a depression of myocardial contractility, defined in terms of the rate of contraction, no changes (except with the highest dose) in the systolic wall thickening (swt) were observed in the unpaced hearts, because the duration of the systole and its electrical counterpart (the Q-T interval) were prolonged by alinidine (see our results, and Kobinger et al., 1979a). This probably resulted in an increase in stroke volume when contractility was decreased with lower doses of alinidine (upto 1.2 $mg.kg^{-1}$). However, when the heart rate was held constant, the duration of systole and the Q-T interval changed only marginally, so that all myocardial parameters (maxLVdP/dt, cardiac output, stroke volume, systolic wall thickening and its first derivative) decreased simultaneously.

The decrease in cardiac output was due to both negative chronotropic and negative inotropic effects of the drug. The resultant fall in mean arterial blood pressure, which was almost absent during atrial pacing due to the altered systolic-diastolic ratio, was restricted to some extent by an elevation of total systemic vascular resistance. The elevation of systemic vascular resistance seems to be mediated via a baroreceptor reflex, since alinidine, unlike clonidine, is devoid of any appreciable central or peripheral alpha-adrenergic action (Kobinger et al., 1979a).

The use of radioactive microspheres revealed that, under the present experimental conditions and as reported previously (Schamhardt et al., 1979), about 25-30% of the cardiac output traversed through the non-nutritional channels (AVAs). It is remarkable that this percentage was significantly increased to 40% after 2.8 $mg.kg^{-1}$ alinidine. Consequently, the AVA-flow remained unchanged after the drug and the reduction of the cardiac output was entirely attributable to its nutrient fraction. Indeed, there are circumstances under which differential effects have been observed on the distribution of cardiac output into nutritional (NCO) and non-nutritional (AVA) fractions (Hales et al., 1978; Johnston and Saxena, 1978; Schamhardt et al., 1979). It is noteworthy that the rather selective reduction of NCO did not adversely affect either the oxygenation of vital organs (brain and heart) or renal blood flow or, perhaps, renal perfusion. The cerebral, and to some extent, the renal blood vessels, exhibited autoregulation in order to derive a higher fraction of cardiac output,

while most other tissues of the body extracted a higher fraction of O_2 from the blood. Both myocardial O_2supply (blood flow) and O_2demand decreased in the same proportion after alinidine, as indicated by a relatively constant myocardial O_2extraction. There was no intramyocardial redistribution of coronary blood flow as the transmural changes in myocardial blood flow were quite uniform.

Lastly, it must be noted that changes in the ratio of nutritional and shunt flow can affect the interpretation of the mixed venous blood O_2saturation (SO_2) values which are often employed clinically in the assessment of total body O_2balance. A simple calculation reveals that a mixed venous SO_2 of 65 vol% in the presence of 25% shunt flow (and 98 vol% artSO_2) means that the SO_2 of the nutritional venous blood is only 54 vol%. Thus, mixed venous SO_2 may provide incorrect information about total O_2extration when the amount of AVA-flow is altered.

In summary, alinidine at doses less than 0.4 $mg.kg^{-1}$, had a marked bradycardic action. This was associated with a relatively small negative inotropic action of the drug. Despite a reduction in myocardial perfusion, there was no deterioration in the myocardial oxygen balance. At higher doses of alinidine the negative inotropic action was more pronounced which, together with bradycardia, substantially decreased cardiac output. The reduction of cardiac output did not hamper tissue oxygenation either because of autoregulation within the specific vascular beds or because the tissues were able to extract more oxygen from the blood. The decrease in myocardial O_2demand with only a minor decrease in myocardial performance could prove to be beneficial for patients with ischaemic heart disease.

9.5 Acknowledgements

The expert technical assistance of Mrs. M.A. Kroos- de Haan and Mr. Robert van Bremen are gratefully acknowledged. The "Interuniversitair Cardiologisch Instituut" is thanked for the use the PDP11/10 computer for the analysis of the echocardiographic tracings. Alinidine was generously supplied by C.M. Boeringer Sohn, Ingelheim, Western Germany. Dr. M.J. Parnham kindly made linguistic corrections.

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IMPROVEMENT OF PERFUSION AND FUNCTION OF ISCHAEMIC PORCINE MYOCARDIUM AFTER REDUCTION OF HEART RATE BY ALINIDINE

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Submitted for publication in: Journal of Cardiovascular Pharmacology

IMPROVEMENT OF PERFUSION AND FUNCTION OF ISCHAEMIC PORCINE MYOCARDIUM AFTER REDUCTION OF HEART RATE BY ALINIDINE

Summary. The effect of alinidine, a compound that reduces heart rate by a direct salutory action on the sino-auricular node, was studied on perfusion and function of the ischaemic myocardium in 10 open-chested pigs. Myocardial ischaemia was produced by narrowing the left anterior descending coronary artery (LAD) to an extent that systolic myocardial wall thickening (ultrasound) was reduced to 30% of the base-line value. Following LAD-stenosis the transmural blood flow and the endo/epi ratio in the affected segment of myocardium was reduced by about 50% from 1.42±0.15 to 0.68±0.11 $ml.min^{-1}.g^{-1}$ and from 1.05±0.08 to 0.59±0.10, respectively. The flows to the non-ischaemic control area decreased uniformly by about 15% and similar moderate reductions were noticed in the global haemodynamic parameters.

Alinidine, in a dose (0.4 - 0.9 $mg.kg^{-1}$) that decreased the heart rate by about 25%, caused a marked redistribution of ischaemic flow in favour of the endocardium: the endo/epi ratio increased to 1.08±0.12. Concomitantly, systolic myocardial wall thickening of the hypokinetic myocardial segment returned to half its base-line value. The flow to the control area was decreased transmurally by about 30% so that, despite the presence of the stenosis, the whole heart was nearly homogenously perfused after alinidine. Right atrial pacing to the pre-drug heart rate only partially reduced the effects of alinidine on endo/epi ratio (0.86±0.09) of the ischaemic myocardium; wall thickness variables did not change.

It is concluded that alinidine ameliorates the imbalance between oxygen supply and demand in the post-stenotic segment of the myocardium mainly by reducing its metabolic needs. Though the prolongation of diastolic perfusion time through heart rate reduction plays an important role in the favourable redistribution of myocardial blood flow other factors, such as negative inotropic effects of the drug, may also be involved.

10.1 INTRODUCTION

Heart rate is a prime determinant of the myocardial oxygen demand (Weber and Janicki, 1979). Therefore, an increase in heart rate further aggravates the imbalance between myocardial oxygen demand and supply especially when the coronary blood flow is already impaired (Tomoike et al., 1978; Vatner and Baig, 1979; Kumada et al., 1980). On the other hand, a decrease in heart rate is beneficial for which

purpose beta-adrenoceptor blocking agents are frequently employed. Indeed, it has been shown that these drugs can improve the regional myocardial function by reducing myocardial oxygen demand and concomitantly causing a redistribution of perfusion in favour of the jeopardized myocardium (Becker et al., 1975; Theroux et al., 1976; Vatner et al., 1977; Berdeaux et al., 1978; Tomoike et al., 1978; Buck et al., 1979; Kumada et al., 1980). However, the beta-adrenoceptor blockers invariably decrease overall myocardial function and may, at times, cause an unacceptable reduction in cardiac output thereby precipitating acute myocardial failure. In the latter case, the restoration of contractile function cannot, or only at the cost of very high doses, be realized by the use of beta-adrenergic stimulants. Furthermore, in the group of patients suffering from bronchial asthma, beta-adrenergic blockade is contraindicated.

Another means to reduce heart rate is alinidine, a recently developed N-allyl-derivative of clonidine, which directly suppresses the rate of discharge of the sino-auricular node (Kobinger et al., 1979a; 1979b; Tritthart and Windisch, 1979). This compound leaves the autonomic regulation of the heart intact and has the potential advantage that its negative chronotropic action predominates over its negative inotropic property (Kobinger et al., 1979a; 1979b; Verdouw et al., 1980b). In order to obtain additional support for the view that heart rate reduction is beneficial in acute stages of myocardial ischaemia, we investigated the effects of alinidine on regional myocardial performance and perfusion in the porcine heart. Myocardial perfusion was measured using radioactive microspheres, while regional function was assessed from myocardial wall thickness tracings.

10.2 MATERIALS AND METHODS

10.2.1. General

Young Yorkshire pigs (20 to 32 kg) were prepared as described in detail elsewhere (Schamhardt et al., 1979; Verdouw et al., 1980a). The pressures in the left ventricle and in the central aorta were measured with pressure transducer-tipped Millar catheters. Cardiac output was monitored with an electromagnetic flow meter (Skalar, Delft, The Netherlands) and calibrated by thermodilution. After opening the chest, the proximal part of the left anterior descending coronary artery (LAD) was dissected free from the surrounding tissue and a J-shaped screw clamp was adjusted around the vessel. Proximal to the clamp an electromagnetic flow probe was placed to monitor the profile of the blood flow in LAD. A 5 MHz ultrasound crystal (Krautkramer- Branson, Lewistown, Pa., USA) was sutured on the epicardium in the LAD-perfused area. A pacing wire was fixed on the right atrium. The left atrial appendage was catheterized for the injection of microspheres.

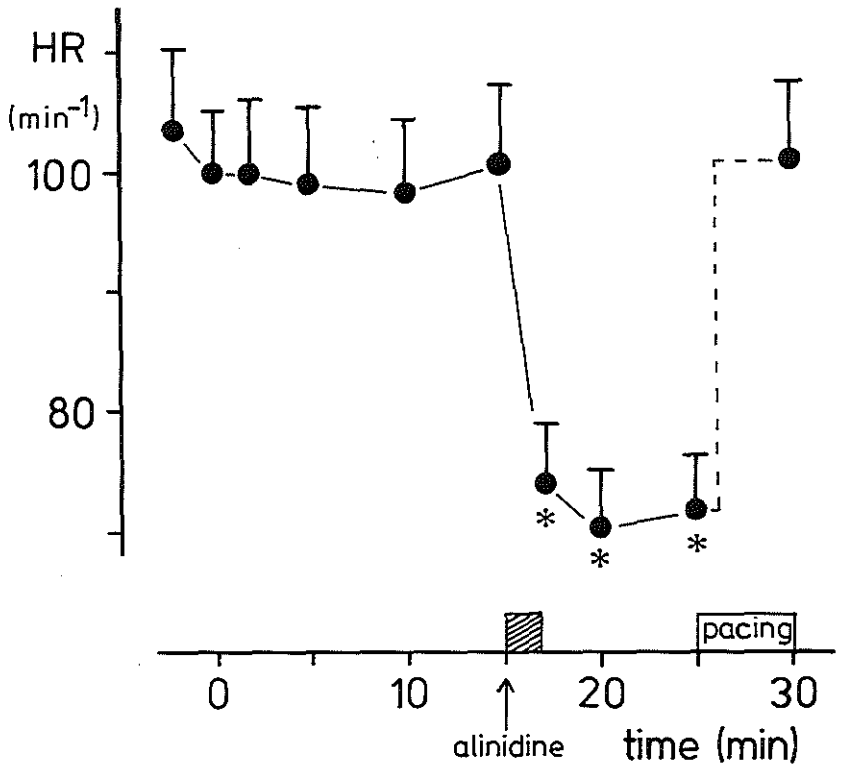

fig.10.1. Illustration of experimental protocol from data on heart rate (HR). After base-line measurements (t=-2), coronary blood flow was reduced to lower segmental wall thickening below 15%. Alinidine was injected to obtain a reduction in heart rate of about 25%. Heart rate was then returned to its pre-drug level by atrial pacing to study the effects of alinidine without drug-induced bradycardia. Data are presented as mean $\pm$ SEM. *, $p<0.05$ vs. pre-alinidine value (Student's paired t-test).

10.2.2. Regional myocardial blood flow

Myocardial blood flow distribution was measured using the microsphere technique (Heymann et al., 1977; Schamhardt et al., 1979). Just before the injection of about $2x10^6$ microspheres (15±5 micron diameter, 3M Co., labelled with either ^{125}I, ^{141}Ce, ^{85}Sr, or ^{95}Nb) the withdrawal of an arterial reference sample was started (flow rate 19.5 $cm^3.min^{-1}$) and continued for a period of about one min. After termination of the experiment, the LAD-area was visualized by the injection of 1.5 cm^3 of methylene blue dye. The heart was excised and fixed in 4% formalin for at least 24 h. Epicardial fat and large vessels were removed. The atria and the right ventricle were separated from the left ventricle, including the intraventricular septum. The blood flows to the central core of the LAD-perfused region on which the wall thickness probe had been sutured and to a two to four centimeter segment in the posterior wall were calculated. Left ventricular samples were divided into four layers of equal thickness from endocardium to epicardium and named sequentially as endocardium1, endocardium2, epicardium1 and epicardium2. The endo/epi blood flow ratio was calculated from the weight normalized flows to the two peripheral layers, i.e. endocardium1: epicardium2.

10.2.3. Myocardial wall thickness

The wall thickness tracings, written on direct print photographic paper (Eastman Kodak) using a fibreoptic system (Honeywell LS6), were analyzed as previously described (Verdouw et al., 1980a). From the end-diastolic (EDT) and end-systolic (EST) wall thicknesses, the systolic wall thickening (swt) was calculated as:

$$swt=((EST-EDT)/EDT)x100\%,$$

while the mean velocity of swt ($\bar{v}_{swt}$) was defined as:

$$\bar{v}_{swt}=(EST-EDT)/(EDTxDS),$$

where DS is the duration of the isovolumic contraction phase plus the ejection time. End-diastole was defined as the onset of the upstroke of the rate of rise of left ventricular pressure, while end-systole was taken as the occurence of the incisura on the central aortic pressure tracing.

10.2.4. Experimental protocol and drug administration

The experimental protocol is illustrated by the data on heart rate, as shown in fig.1. After stabilization of at least 30 min, base-line measurements of systemic haemodynamic, regional myocardial perfusion and wall thickness variables were made (t=-2 min) and the screw on the J-clamp around the LAD was tightened (t=0) to reduce LAD-flow to a level that the systolic myocardial thickening decreased below 15%. Within five to ten min the preparation was stable and the measurement of haemodynamic and wall thickness variables were repeated. Alinidine was injected at 15 min in a dose to obtain a reduction in heart rate

Table 10.1. The effects on systemic haemodynamic variables of coronary flow reduction and alinidine administration in ten pigs.

Haemodynamic variable	Unit	Base-line value A	Ischaemia B	Ischaemia + alinidine C	Ischaemia + alinidine + atrial pacing D (d)
Heart rate	min^{-1}	104 ± 6	98 ± 6	72 ± 5 ab	105 ± 6
Cardiac output	$dm^3 \cdot min^{-1}$	3.7 ± 0.4	3.13 ± 0.29 a	2.64 ± 0.25 ab	2.98 ± 0.28 abc
Stroke volume	cm^3	35.6 ± 1.8	31.9 ± 1.9 a	36.7 ± 2.0 b	28.2 ± 1.8 abc
Mean arterial blood pressure	mmHg	84 ± 5	75 ± 3 a	68.7 ± 2.6 ab	80 ± 4 c
Systemic vascular resistance	$mmHg \cdot min \cdot dm^{-3}$	25 ± 3	25.6 ± 2.5	28.1 ± 2.7 ab	29 ± 3 ab
LV end-diastolic pressure	mmHg	9.1 ± 0.8	10.2 ± 1.2	13.2 ± 1.2 ab	8.6 ± 1.3 c
LV dP/dt max	$mmHg \cdot s^{-1}$	3000 ± 300	2230 ± 200 a	1760 ± 160 ab	1740 ± 160 ab

Abbreviations: LV, left ventricular; dP/dt max, maximum rate of rise; a, $p<0.05$ vs. A; b, $p<0.05$ vs. B; c, $p<0.05$ vs. C; d, n=9. Data are presented as mean ± SEM. Statistics are carried out using the Student's paired t-test (two tailed).

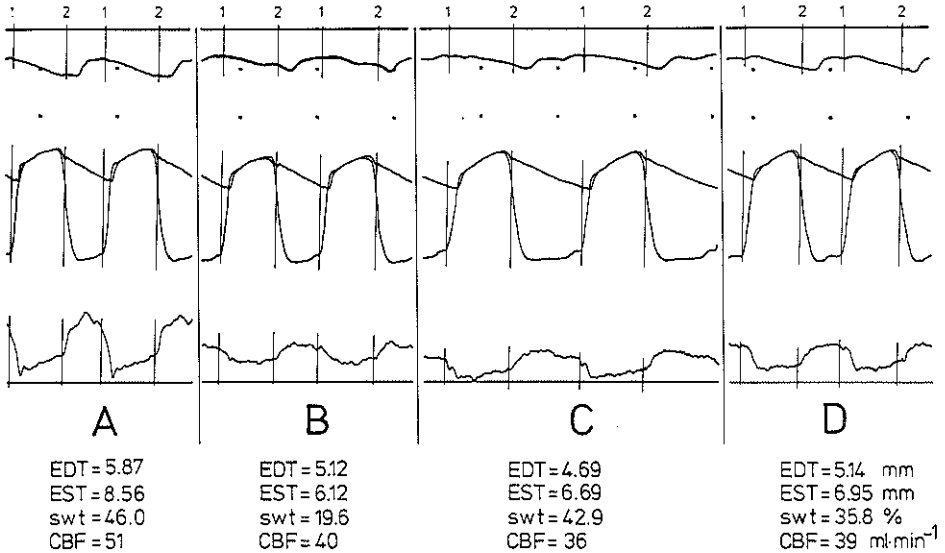

fig.10.2. Myocardial wall thickness, left ventricular- and aortic pressures and phasic coronary blood flow (CBF) at the base line (A) and during coronary stenosis before (B) and after alinidine (C) and during atrial pacing to the pre-alinidine heart rate (D) (redrawn from the origional tracings). Systolic myocardial wall thickening and phasic coronary blood flow were markedly reduced during partial coronary constriction. After alinidine the end-diastolic wall thickness (EDT) and the pronounced protodiastolic thickening decreased so that most wall thickening (swt) occured in systole. The systolic fraction of CBF decreased. Atrial pacing did hardly affect the pattern of wall thickening, but increased both the systolic and the diastolic fraction of CBF. Due to the decrease in the duration of diastole, the total flow was only marginally augmented. The time/depth markers correspond with 0.5 s/10 mm. Abbreviations: EST, end-systolic myocardial wall thickness; 1, end-diastole; 2, end-systole.

Table 10.2. Effect on regional myocardial blood flow variables of coronary flow reduction and alinidine administration in ten pigs.

Regional blood flow variable	Base-line value A	Ischaemia B	Ischaemia + alinidine C	Ischaemia + alinidine + atrial pacing D (d)
Central core ischaemic area				
Endo 1	1.33 ± 0.13	0.45 ± 0.07 a	0.73 ± 0.10 ab	0.78 ± 0.11 ab
Endo 2	1.45 ± 0.14	0.52 ± 0.09 a	0.80 ± 0.11 ab	0.90 ± 0.13 ab
Epi 1	1.53 ± 0.16	0.78 ± 0.15 a	0.82 ± 0.10 a	1.02 ± 0.16 a
Epi 2	1.36 ± 0.19	0.87 ± 0.14 a	0.65 ± 0.08 ab	0.84 ± 0.12 a
Transm.	1.42 ± 0.15	0.68 ± 0.11 a	0.75 ± 0.09 a	0.89 ± 0.13 a
Endo/epi	1.05 ± 0.08	0.59 ± 0.10 a	1.08 ± 0.12 b	0.86 ± 0.09 bc
Control area				
Endo 1	1.50 ± 0.17	1.21 ± 0.12 a	0.86 ± 0.07 ab	1.15 ± 0.09 ac
Endo 2	1.58 ± 0.19	1.25 ± 0.11 a	0.90 ± 0.07 ab	1.18 ± 0.07 ac
Epi 1	1.49 ± 0.17	1.20 ± 0.10 a	0.85 ± 0.06 ab	1.17 ± 0.06 ac
Epi 2	1.33 ± 0.16	1.06 ± 0.11 a	0.75 ± 0.06 ab	1.02 ± 0.05 c
Transm.	1.47 ± 0.17	1.17 ± 0.11 a	0.83 ± 0.06 ab	1.13 ± 0.06 ac
Endo/epi	1.16 ± 0.08	1.15 ± 0.09	1.15 ± 0.08	1.13 ± 0.09

Blood flow values are presented in $cm^3 \cdot min^{-1} \cdot g^{-1}$. Abbreviations: Endo, endocardium; Epi, epicardium; Transm., transmural; Endo/epi, the ratio between Endo 1 and Epi 2 blood flows; a, $p<0.05$ vs. A; b, $p<0.05$ vs. B; c, $p<0.05$ vs. C; d, n=9. Data are presented as mean ± SEM. Statistics are carried out using the Student's paired t-test (two tailed).

of about 25%. The required dose ranged from 0.4 to 0.9 $mg.kg^{-1}$ (0.57±0.07, mean±SEM). When the heart rate had stabilized, all determinations were carried out again. Subsequently, the heart rate was increased by right atrial pacing to the pre-drug level and the final measurements were done.

Previous studies from this laboratory using the same techniques have shown that, when treated with 0.9% NaCl, systemic haemodynamic variables and regional myocardial blood flow and performance remain stable for at least 30 min after the induction of ischaemia (Schamhardt et al., 1979). Nevertheless, we performed two additional experiments where 0.9% NaCl was administered in stead of alinidine. Again, no appreciable changes were noticed in any of the measured variables.

10.2.5. Statistical analysis

Statistical analysis was carried out using the Student's paired t-test (two-tailed; Snedecor and Cochran, 1967). Differences with $p>0.05$ were considered to be not statistically significant (NS). Data have been presented as mean ± SEM.

10.3. RESULTS

10.3.1. Systemic haemodynamics

The data on haemodynamic variables are listed in table 1. The reduction in LAD blood flow caused an immediate decrease in mean arterial blood pressure (11%), cardiac output (15%), stroke volume (10%) and in the peak rate of rise of left ventricular pressure (LVdP/dtmax; 24%). Heart rate, systemic vascular resistance and left ventricular end-diastolic pressure (LVEDP) did not change significantly.

Treatment with alinidine (0.4-0.9 $mg.kg^{-1}$) caused a decrease in heart rate by 27%. The magnitude of the fall in cardiac output (15%) was less than that of heart rate since the drug increased stroke volume by 15% despite the decrease in LVdP/dtmax. The effects on mean arterial blood pressure were minimal (-7%, $p<0.05$) because of a 10% increase in systemic vascular resistance. Left ventricular end-diastolic pressure increased from 10.2 to 13.2 mmHg ($p<0.05$).

Pacing heart rate back to its pre-alinidine value caused an 11% increase in cardiac output despite a 22% decrease in stroke volume. Since the systemic vascular resistance had not changed, it caused a concomitant increase in mean arterial blood pressure by about 22%. No effects on LVdP/dtmax could be noticed, while LVEDP returned to its base-line value.

10.3.2. Regional myocardial blood flow

Figure 2 shows the profile of LAD blood flow observed in one of the experiments during its four phases: at the base line (A), after LAD stenosis (B), after heart rate reduction by alinidine (C) and following atrial pacing (D). It is shown that coronary stenosis

Table 10.3. Effect on myocardial wall thickness variables of coronary flow reduction and alinidine administration in seven pigs.

Wall thickness variable	Unit	Base-line value A	Ischaemia B	Ischaemia + alinidine C	Ischaemia + alinidine + atrial pacing D
End-diastolic wall thickness	mm	8.5 ± 0.7	7.6 ± 0.6 a	7.5 ± 0.7 a	7.7 ± 0.8 a
End-systolic wall thickness	mm	12.9 ± 1.3	8.7 ± 0.8 a	9.7 ± 0.9 a	9.6 ± 1.1 a
Systolic wall thickening (swt)	%	51 ± 4	15 ± 4 a	29 ± 4 ab	25 ± 4 ab
Mean velocity of swt	s^{-1}	1.70 ± 0.23	0.49 ± 0.15 a	0.78 ± 0.10 ab	0.76 ± 0.15 ab
Duration of systole	ms	318 ± 23	326 ± 25	383 ± 27 ab	345 ± 23 bc

Duration of systole has been defined as the time of isovolumic contraction plus ejection. Abbreviations: a, $p<0.05$ vs. A; b, $p<0.05$ vs. B; c, $p<0.05$ vs. C. Data are presented as mean ± SEM. Statistics are carried out using the Student's paired t-test (two tailed).

caused a decrease in diastolic flow, but also slightly increased the flow in systole. Alinidine enhanced the duration of diastole, so that the flow in that period increased, while the systolic flow decreased. Atrial pacing was followed by increases in both the systolic and the diastolic flow. However, due to the decrease in the duration of diastole, the total flow was only minimally augmented. Detailed data concerning the regional myocardial blood flow distribution are presented in table 2. Coronary flow reduction resulted in a decrease by 53% in transmural flow to the central core of the ischaemic area. The endocardium was affected more than the epicardium and, as a result, the endo/epi blood flow ratio decreased by 42%. The flow to the control area decreased transmurally to 83% of its base-line value, evenly distributed over all myocardial layers.

Alinidine caused an increase in the blood flow to the endocardial layers of the ischaemic area from 35 to 55% of their base-line values, but did not affect the flow to the epicardium. Consequently, the endo/epi blood flow ratio returned to its base-line value. The flow to the non-ischaemic area decreased by 28% and the endo/epi-ratio remained unchanged. The return of heart rate by atrial pacing to its pre-drug level was not followed by any appreciable increase in the ischaemic flow, while the endo/epi-ratio decreased ($p<0.05$), and the flow to the control area increased by 31% ($p<0.05$; table 2), without an effect on the endo/epi-ratio.

10.3.3. Segmental myocardial wall thickness

The effects of LAD stenosis, alinidine-administration and atrial pacing on the myocardial wall thickness parameters are shown in fig. 2 and table 3. The reduction of coronary blood flow resulted in a decrease in the systolic myocardial wall thickening (swt) from 51% to 15% (i.e. a reduction to about 30% of its base-line value). This was primarily due to the decrease in end-systolic wall thickness since the end-diastolic thickness decreased only by 12%. The velocity of systolic wall thickening decreased to the same extent as swt but the duration of systole was not altered.

Alinidine did not affect the end-diastolic wall thickness. The end-systolic wall thickness increased only slightly ($p>0.05$) despite a significant increase in both the mean velocity of systolic wall thickening and the duration of systole. However, it resulted in an increase in swt from 15 to 29% ($p<0.05$). Despite the decrease in the duration of systole, no effects on wall thickness variables could be observed after atrial pacing to the pre-alinidine heart rate.

10.4. DISCUSSION

The experimental model as has been employed in the present experiments attempts to simulate the clinical setting in angina pectoris. As is common in the clinical situation, stenosis of the left anterior descending coronary artery (LAD) in the porcine heart produced a well defined area of hypokinetic myocardium. Since the

global haemodynamics, the perfusion and performance of the ischaemic region and the blood flow to the control unaffected myocardium have been measured simultaneously, the present experimental set-up seems especially suitable to investigate the effects of pharmacological interventions, such as heart rate reduction by alinidine, which may have a possible therapeutic application in angina pectoris.

The administration of alinidine had qualitatively similar effects on systemic haemodynamic variables in pigs with intact (Verdouw et al., 1980b) or with stenosed LAD (present experiments). In both situations the drug caused bradycardia, hypotension, a reduction of cardiac output and LVdP/dtmax and an elevation of LVEDP. Similarly, the blood flow to the normal myocardium was reduced, without any change in endo/epi flow ratio. This was probably a consequence of autoregulatory response following a reduction in myocardial oxygen demand to an extent that compared well with that in non-ischaemic myocardium (Verdouw et al., 1980b). In striking contrast, however, alinidine increased the endocardial perfusion thereby elevating the endo/epi-ratio and improved the mean velocity and the magnitude of systolic myocardial wall thickening of the hypokinetic portion of the myocardium. It is pertinent to point out that heart rate reduction through beta-adrenoceptor blockade has also been shown to improve the oxygen demand/supply relationship in the ischaemic myocardium in both anaesthetized (Becker et al., 1975; Berdeaux et al., 1978; Buck et al., 1979) and conscious dogs at rest (Theroux et al., 1976; Vatner et al., 1977; Tomoike et al., 1978) or during exercise (Kumada et al., 1980). As with alinidine, beta-adrenoceptor blocking agents elevate the endo/epi blood flow ratio (Becker et al., 1975; Vatner et al., 1977; Berdeaux et al., 1978; Buck et al., 1979).

The amelioration of the imbalance between oxygen supply and demand in the ischaemic segment of the myocardium may be due to several factors. Like propranolol (Becker et al., 1975; Theroux et al., 1976; Berdeaux et al., 1978; Tomoike et al., 1978; Buck et al., 1979; Kumada et al., 1980), alinidine reduced the myocardial oxygen demand by a reduction of heart rate, a factor which is a major determinant of myocardial oxygen consumption (Weber and Janiki, 1979). In addition, the drug-induced reduction in systolic blood pressure, LVdP/dtmax and cardiac output contribute towards the lowering of the oxygen requirement. Although slight elevations of LVEDP and the mean velocity of systolic wall thickening in the ischaemic segment of the myocardium may have resulted in some increase in its metabolic needs (Burns and Covell, 1972; Weber and Janiki, 1979), such an increase shall be less than the reduction due to the other factors.

As revealed by the microspheres, the alinidine-induced redistribution of the blood flow in favour of the endocardium of the LAD-area is due to the prolongation of diastolic time, since it is well known that an increase in diastolic time enhances endocardial flow (Downey and Kirk, 1974; Hess and Bache, 1976; Archie, 1975). The concomitant decrease in the epicardial flow is perhaps due to the more marked autoregulatory reserve of the epicardium (Gross and Winbury, 1973; Pasyk et al., 1976; Guyton et al., 1977; Gallagher

et al., 1980). It is apparent in the present experiments (see fig. 2) that alinidine increased the diastolic fraction of the blood flow in the stenosed LAD. Atrial pacing decreased the diastolic flow and the endo/epi ratio in the ischaemic myocardial segment. However, as found with some beta-adrenoceptor blockers (Gross and Winbury, 1973; Parratt and Marshall, 1977; Berdeaux et al., 1978; Tomoike et al., 1978), atrial pacing to pre-drug heart rate did not completely reverse the beneficial effects of alinidine on the endo/epi flow ratio. This implies that other factors are also involved. One such factor could be the negative inotropic effect of the drug in the normally perfused myocardium as shown by the decrease in the LVdP/dtmax and mean velocity of systolic wall thickening and the lack of enhancement in these parameters following atrial pacing (Kobinger et al., 1979a; 1979b; Verdouw et al., 1980b). The negative inotropy would diminish oxygen requirements of the normal regions thereby making the oxygen needs and the perfusion of the whole myocardium more uniform.

Lastly, it may be mentioned that there was no complete recovery of the function of the hypokinetic segment of the myocardium after alinidine. Though it is possible that a higher dose of the drug may have improved the performance of the ischaemic myocardium further, it must be realized that reperfusion following a partial coronary stenosis does not fully restore the myocardial function, probably because of cellular death (Ramanathan et al., 1978; Verdouw et al., 1979).

In conclusion, following heart rate reduction by alinidine, paradoxical to the decreases in the normal myocardium, the blood flow to and the function of the ischaemic myocardial segment were considerably increased. The effects on global haemodynamics and ischaemic flow and function are similar with those obtained after the administration of a beta-adrenoceptor blocking agent like propranolol.

10.5. Acknowledgements

The expert technical assistance of Miss A.M. Rutteman and R.H. van Bremen is gratefully acknowleged. The "Interuniversitair Cardiologisch Instituut" is thanked for the use of their PDP11/10 computer, used in the analysis of wall thickness tracings. Boehringer Ingelheim (W.G.) generously supplied alinidine (St 567).

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11. SUMMARY AND CONCLUSIONS

11.1. Introduction

During the normal function of the heart, as described in chapter 1, the blood delivered by the venous circulation is pumped against the arterial blood pressure into the systemic circulation. The energy for this process is derived from the oxidation of substrates, which are delivered to the heart by the coronary blood flow. During ventricular systole, myocardial perfusion is impeded by the intramyocardial pressure, so that the endocardial blood flow is almost completely inhibited. Any debt that may have been incurred is, however, fully repaid during ventricular diastole. A close link exists between regional perfusion and function, so that within seconds after alteration of coronary blood flow, the affected segment of the heart adapts its ability to participate in the contractile process.

A number of changes in overall and segmental myocardial function occurs after (partial) occlusion of a coronary artery (chapter 2). The complex of biochemical, mechanical and regional perfusion deficits is denoted as myocardial ischaemia. A consequence of ischaemia is the occurrence of ventricular arrhythmias which can lead, under certain circumstances, to fatal ventricular fibrillation. Locally, the ischaemic myocardial segment exhibits a reduction in contractile function, as reflected by the immediate loss of systolic wall thickening. The regional blood flow decreases, first in the endocardial layers and later in the epicardium, but the effects on global haemodynamic function depend on the site, the size and the degree of ischaemia in the area of flow reduction. They reach from an absence of any measurable effect upto a complete failure of the ischaemic heart to maintain cardiac output and blood pressure. The functional significance of interarterial collateral channels in the perfusion of the ventricle becomes more important during gradual coronary occlusions.

A discrepancy might be expected between global haemodynamics and local contractile function in the presence of regional myocardial ischaemia. As the normally perfused region exerts tension on the ischaemic area it can, therefore, further impair the reduced local function. On the other hand, due to the same mechanism, a relative improvement of the function of the ischaemic zone can be overshadowed by the (hyper-) activity of the normally perfused region. A measure for the adequacy of regional function is the local myocardial oxygen demand and supply relationship. Elevations in heart rate, wall tension, intra ventricular volume and contractility augment the myocardial oxygen demand, while improvements in coronary (collateral) blood flow may enhance the oxygen supply. To judge the efficacy of interventions which may improve the oxygen demand and supply relationship it is, therefore, essential to be able to determine the

global as well as the local contractile function, together with the regional myocardial perfusion.

A large amount of literature is available concerning interventions on the ischaemic heart (chapter 3). Mechanical assist by intra aortic counter pulsation reduces myocardial oxygen demand by lowering the resistance to the ejection of blood into the aorta. Variable effects on total and regional myocardial blood flow have been reported (3.2.1). Increasing arterial blood pressure will cause an elevation of oxygen demand and may jeopardize ischaemic myocardium, but it also augments coronary blood flow. Comparable counteracting effects may be expected when myocardial contractility is increased by inotropic stimulation: both augmentation and reduction in ischaemic flow and function have been described (3.2.3).

Another approach is to relieve the components which determine the myocardial oxygen demand. Decreasing heart rate as well as myocardial contractility by beta-adrenergic blockade is a classic example (3.3.1). Systemic and/or venous vasodilation also will reduce the myocardial oxygen demand and lead to a relief of the load to the ischaemic segment. On the other hand, such a decrease in the arterial blood pressure lowers coronary blood flow and, therefore, oxygen supply. A last example is the impediment of contraction by the use of calcium antagonistic drugs which reduce the myocardial oxygen needs, but may also cause an unacceptable loss of contractile function. The optimal combination in a given clinical or experimental circumstance cannot be deduced a priori, since it depends on a number of factors, as mentioned above.

To clarify some of these aspects, we studied by a relatively arbitrary choice the effects of (i) positive inotropic stimulation by oxyfedrine (chapters 6 and 7), (ii) decreasing heart rate, cardiac output and concomitantly increasing the afterload by ergotamine (chapter 8) and (iii) a lower heart rate selectively induced by alinidine (chapters 9 and 10) on the oxygen demand and supply relationship in the partially ischaemic porcine left ventricle.

11.2. Materials and methods

The model in which these studies were carried out and the methodology applied was nearly identical in all studies. Anaesthetized pigs (±25 kg) were ventilated with O_2:N_2O=1:2 and catheterized with Millar catheters having a pressure transducer at the tip in the left ventricle and the aorta and with a thermodilution catheter in the pulmonary artery. The latter served for the measurement of cardiac output. The chest was opened to expose the heart and an electromagnetic flowprobe was placed around the left anterior descending cornary artery (LAD). Myocardial ischaemia could be induced by tightening a screw clamp around the LAD.

A practical problem proved to be the quantification of the regional function of the normally perfused and the ischaemic segment of the heart. A highly sensitive method was found in the determination of the myocardial wall thickness changes during the cardiac contraction by means of ultrasound reflection (see 1.3.2.4). It is well

established that local underperfusion immediately causes decreases in segmental wall thickening. Newly developed miniature ultrasound crystals, directly sutured onto the epicardium, made the measurement technique reliable and reproducible. When these crystals are sutured on the normally perfused as well as on the ischaemic regions, local function can be directly assessed, while the global haemodynamic variables provide insight in the overall functioning of the partially ischaemic heart.

Regional myocardial blood flow can be determined accurately with the radioactive microsphere technique. To this end, plastic spheres of about 15 micron diameter, labelled with gamma radiation emitting radionuclides, are injected into the left atrium. The spheres mix with the arterial blood and are distributed in proportion with the local blood flow. Since their size is larger than the blood cells, these spheres are entrapped in the arterioles or capillaries of the body. The amount of radioactivity in a certain piece of tissue then reflects, provided that the microspheres are homogeneously mixed in the blood, the local blood flow. This microsphere technique can be used to study the distribution of cardiac output, to determine total and regional organ blood flow, to measure cardiac output and to derive variables that are associated with arteriovenous anastomotic blood flow. The prerequisites of the microsphere technique and the computer programs which we have developed to calculate blood flows from radioactivity data have been described in chapters 4 and 5.

11.3. Results

(i) oxyfedrine

Beta-adrenergic stimulation with oxyfedrine was employed to investigate the effects of a coronary vasodilating agent together with inotropic stimulation on global haemodynamics and metabolism of myocardium, made ischaemic by coronary flow reduction. The myocardial lactate metabolism was assessed as a measure of the degree of ischaemia (chapter 6). Both before and during myocardial ischaemia, induced by reduction of the LAD-flow to 25% of its base-line value, 0.2 $mg.kg^{-1}$ oxyfedrine considerably increased the maximum rate of rise of left ventricular pressure (LVdP/dtmax), an index of myocardial contractility, but also augmented heart rate, while it did not affect the mean arterial blood pressure and cardiac output. The increase in the myocardial oxygen demand did not result in a more anaerobic myocardial metabolism, as the lactate balance did not change. It is concluded that oxyfedrine can be useful in the treatment of pump failure resulting from acute myocardial ischaemia.

Global haemodynamic variables do not completely reflect the effects of interventions on partially ischaemic hearts. Therefore, the effects of oxyfedrine were also investigated on the regional myocardial blood flow distribution together with the wall thickness variables in the fraction of the heart that was made ischaemic by a fixed stenosis around the LAD (chapter 7). As in the previous study, LVdP/dtmax and heart rate were increased after the administration of

0.15 mg.kg^{-1} oxyfedrine. However, the drug increased both the flow to the normal, non-ischaemic myocardium as well as the flow to and the function of the ischaemic segment, despite a concomitant increase in the myocardial oxygen demand.

(ii) ergotamine tartrate

Ergotamine, a drug effectively used for the treatment of acute attacks of migraine, is generally contraindicated in patients suffering from coronary artery disease, since it increases the afterload and may induce coronary spasm. However, the drug concomitantly causes bradycardia, which decreases myocardial oxygen demand. Furthermore, it has been shown to close arteriovenous anastomoses (AVAs) in cat's head. These effects could be beneficial to improve the myocardial oxygen demand/supply relationship. Therefore, ergotamine was studied in the porcine heart preparation without myocardial ischaemia to obtain a dose response relationship and, in the ischaemic heart, to determine the effects on segmental flow and function (chapter 8). In doses of 8, 16 and 32 g.kg^{-1} ergotamine decreased heart rate, cardiac output and total systemic AVA-flow, while increasing systemic vascular resistance and blood pressure. No effects on myocardial blood flow and its distribution were found. Eight g.kg^{-1} ergotamine given during myocardial ischaemia increased the blood flow and the endo/epi ratio of the ischaemic segment towards normal values. Disappointingly, the wall thickness variables, which had deteriorated during flow reduction, remained depressed.

(iii) alinidine hydrobromide

Heart rate is a main determinant of myocardial oxygen demand. An N-allyl derivative of clonidine, alinidine, almost selectively lowers heart rate by a direct action on the sinoauricular node. Therefore, the drug could be useful to restore the myocardial oxygen demand and supply relationship during myocardial ischaemia. First, we determined a dose-response relation in pigs with a normal myocardial circulation (chapter 9) and found, that a dose of 0.4 mg.kg^{-1} was able to reduce heart rate by about 20% without significant effects on myocardial contractility and other haemodynamic variables. Myocardial perfusion decreased transmurally, parallel with the reduction in myocardial oxygen demand. The segmental wall function remained unaltered. At higher doses, the negative inotropic action of alinidine was more pronounced which caused a marked decrease in cardiac output.

In a second study (chapter 10) the effects on myocardial perfusion and performance of heart rate reduction by alinidine were studied when administered during myocardial ischaemia. After stabilization of the flow and function of the ischaemic left ventricle, alinidine was infused to obtain a reduction in heart rate of at least 25% (dose: 0.4-0.9 (0.57±0.07) mg.kg^{-1}). The effects on global haemodynamic variables were similar to those obtained from nonischaemic hearts. However, the ischaemic flow redistributed in favour of the endocardium, so that the whole left ventricle was nearly homogeneously

perfused, despite the presence of the coronary stenosis. However, the wall thickness variables only partially recovered.

11.4. Discussion

Each of these pharmacological studies accents a special aspect of cardiac and coronary regulatory mechanisms. Therefore, their potential clinical application and relevance will be discussed here. The most important result from the studies with oxyfedrine is, that inotropic and mild chronotropic stimulation of the partially ischaemic porcine heart improves both the flow to and the function of the ischaemic segment, even though it does not require the metabolism to shift to more anaerobic pathways. The frequently reported worsening of ischaemia in the area of flow reduction was not found with oxyfedrine. However, it must be noted that oxyfedrine did not cause a very large increase in myocardial contractility, while the concomitant increase in heart rate was no more than 20%. Furthermore, the administration of oxyfedrine caused a decrease in left ventricular end-diastolic pressure and, in turn, in myocardial oxygen demand. Thus, mild inotropic stimulation can be achieved during myocardial ischaemia without apparent cost.

Although ergotamine and ergonovine are substances derived from the same family, they exert different actions on the heart and the cardiovascular system. Ergonovine is frequently used to induce variant angina through induction of coronary spasm. Ergotamine, on the other hand, is used to treat migraine. The drug is thought to be contraindicated in cardiovascular diseases because of its properties to increase blood pressure and, secondly, to reduce cardiac output and heart rate and possibly also because of the assumed induction of coronary spasm. Since ergotamine decreased the myocardial oxygen demand by reducing heart rate, but increased oxygen demand by elevating blood pressure, which, in turn, may increase oxygen supply via the augmentation of coronary blood flow, it was considered of interest to study ergotamine in the partially ischaemic heart. Furthermore, another property of the drug is to reduce arteriovenous anastomotic (AVA) blood flow which might reduce excessive and "unproductive" cardiac output. The latter point was confirmed by our study, since the observed decrease in cardiac output was completely at the expense of AVA-flow. The normally encountered values for AVA-flow from 10 to 60% of cardiac output (see 1.2) provides room for speculations on the functional significance of this flow in the regulation of cardiac output. Should this concept be true, it could be a potent source of reserve in peripheral tissue perfusion in circumstances where oxygen requirements are increased. That energy is wasted in the pumping of blood through AVA's, however, sounds unlikely, since it also may play a role in the regulation of body temperature. The contrary effects of ergotamine on the ischaemic segment of the heart suggest an equilibrium between the increases in oxygen demand and supply, as the wall thickness hardly changed, while perfusion markedly increased, even favouring the endocardium over the epicardium. Furthermore, this study failed to provide clear evidence

for contraindications of ergotamine usage in cardiovascular disorders.

The selective decrease in heart rate by alinidine caused a marked decrease in the myocardial oxygen demand, as reflected by a concomitant drop in coronary blood flow. The drug also caused a decrease in myocardial contractility when given in doses in excess of 0.4 $mg.kg^{-1}$. However, there was a beneficial redistribution of myocardial perfusion in favour of the endocardium, without changes in the pattern of myocardial wall thickening when given during myocardial ischaemia. These observations compare favourably with those of beta-adrenoceptor blocking agents, where the primarily desired effect is a reduction in myocardial oxygen consumption. Furthermore, alinidine can be used to lower heart rate in patients who do not tolerate any beta-adrenoceptor blockade. It must be mentioned, however, that if the vicious circle of sympathetic cardiac stimulation induced by myocardial ischaemia itself, is not blocked, alinidine may not be as useful as it now appears. Nevertheless, it can be concluded that alinidine ameliorates the imbalance between myocardial oxygen demand and supply in the post-stenotic segment by reducing its oxygen requirements.

11.5. Perspectives

The studies reported in this thesis provide answers to a number of questions concerning the behaviour of the heart under local and severe ischaemia. The ratio between endocardial and epicardial perfusion (endo/epi ratio) is near to unity under conditions of normal myocardial perfusion. The exact value of this ratio, however, depends on the size of the microspheres (see 4.3.2). This shows that the architecture of the coronary system limits the interpretation of the data on regional blood flow. On the other hand, the distribution of microspheres of different sizes over the cross section of an artery has been described to be inhomogeneous, so that these variations are not surprising. Also the behaviour of microspheres in the presence of a flow limiting coronary stenosis is unpredictable. Furthermore, the endo/epi ratio decreases considerably when a stenosis is applied in such a way that it only minimally reduces flow. Thus there remain pitfalls in the interpretation of the endo/epi ratio. An in-vitro study of an arterial segment with an extravascular device to reduce the lumen of the vessel can be used to study the behaviour of microspheres in a stenosed coronary artery. A (computer-) analysis of the coronary circulation including variations in intra-myocardial tissue pressure and distorsions of the ventricular wall during contraction and ejection may provide further insight in the regional myocardial blood flow distribution.

The dissection of a coronary artery for the placement of a flow probe and/or a flow limiting device to induce ischaemia is another source of difficulties, since it is hard to avoid damaging the local ventricular innervation. Since the innervation of the not affected, non-ischaemic tissue remains intact, this imbalance in control systems may affect the results.

As far as the pattern of myocardial wall thickening during

ischaemia is concerned, systolic thickening diminishes and the wall continues to contract during diastole (chapters 7 and 8). In fact, the maximal wall thickness in this period may approach the value of EDT during normal perfusion. Whether the diastolic thickening is a (partially) active event or not, it may worsen the perfusion of the ischaemic segment because of the generation of intramyocardial tissue pressure which impedes diastolic flow. At any rate, the origin and significance of diastolic myocardial wall thickening during ischaemia has to be studied further.

Another unsolved problem is the presence of the high arteriovenous anastomotic blood flow in our preparation. By using microspheres of different sizes, it can prove possible to locate the site of shunting. The dependency on anaesthetic agents, sympathetic tone and the age of the animals are means which may provide more insight in the phenomenon of arteriovenous shunting. Its role in the human circulation can only be guessed at.

As discussed in chapter 10, heart rate remains a prime determinant of the myocardial oxygen demand. Whenever positive inotropic therapy is required to stabilize the patient's haemodynamic condition, the concomitant increase in heart rate frequently interferes. Therefore, the combination of positive inotropic stimulation with heart rate reduction by alinidine is very promising, and, particularly, if it can be shown to suppress the reflex tachycardia after systemic vasodilation, so frequently required in the clinic. Ergotamine may be useful when high cardiac output is associated with low blood pressures, the "high output syndrome", because a large fraction of this cardiac output may be shunt flow. This, in fact, may be a consequence of peripheral dysregulation.

All results obtained during the course of these investigations demonstrate the usefulness of the animal model to study the effects of pharmacological interventions on the perfusion and performance of the ischaemic myocardium. Since a major limitation remains that the animals are anaesthetized, attempts should be made to perform such experiments in conscious, chronically instrumented animals.

12. SAMENVATTING EN CONCLUSIES

12.1. Inleiding

Tijdens de normale hartactie, zoals beschreven in hoofdstuk 1, wordt het bloed dat door de aderen wordt aangevoerd, onder de arteriele druk uitgepompt in de systemische circulatie. De energie voor dit proces wordt betrokken uit de oxidatie van substraten, die naar het hart zijn gevoerd door de coronaire bloedstroom. Tijdens de ventriculaire systole wordt de myocardiale doorbloeding belemmerd door de intra myocardiale druk, zodat de doorbloeding van het endocardium vrijwel volledig wordt afgesneden. Een eventueel tekort wordt echter aangevuld tijdens de ventriculaire diastole. Er bestaat een sterke relatie tussen de regionale doorbloeding en de regionale functie, zodat binnen enkele seconden na een verandering in de coronair doorbloeding het betrokken gedeelte van het hart zijn vermogen om deel te nemen aan het contractie proces aanpast.

Een groot aantal veranderingen vindt plaats in totale en regionale myocardiale functie na een (gedeeltelijke) afsluiting van een coronair arterie (hoofdstuk 2). Het complex van biochemische, mechanische en regionale doorbloedings verslechteringen wordt myocardiale ischemie genoemd. Een gevolg van ischemie is het ontstaan van aritmieen van ventriculaire oorsprong, die onder bepaalde omstandigheden kunnen leiden tot fatale ventrikel fibrillatie. Het ischemische gedeelte van het hart vertoont ogenblikkelijk een plaatselijke verminderingen van de actieve systolische wandverdikking. De regionale doorbloeding neemt het eerst af in de endocardiale lagen en pas later in het epicardium, maar de invloed op de globale hemodynamische functie hangt af van de plaats, de afmeting en de mate van ischemie in het slecht doorbloede gebied. Alles tussen geen meetbaar effect en een volledig falen van het ischemische hart om het hart minuut volume en de arteriele bloeddruk te handhaven is mogelijk. De functionele betekenis van collateraal kanalen tussen de coronair arterien onderling voor de doorbloeding van de ventrikel wordt van meer belang bij een graduele coronair arterie afsluiting.

In aanwezigheid van regionale myocardiale ischemie ontstaat een tegenstrijdigheid in het gedrag van de locale functie en de globale hemodynamische variabelen. Daar het normaal doorbloede gebied kracht uitoefent op het ischemische gebied, kan het daardoor de verminderde locale functie verder verslechteren. Aan de andere kant kan hetzelfde mechanisme verklaren dat een relatieve verbetering in de functie van het ischemische gebied overschaduwd wordt door de (verhoogde) activiteit van het normaal doorbloede gebied. Een maat voor het al dan niet goed functioneren van een gedeelte van de ventrikel is het locale evenwicht tussen zuurstof behoefte en aanbod. Een verhoging van hart frequentie, wandspanning, intra ventriculair volume en contractiliteit verhoogt de zuurstof behoefte, terwijl een toename in

de coronair (collateraal) doorbloeding het zuurstof aanbod kan vergroten. Om de werkzaamheid te beoordelen van ingrepen, die de zuurstof balans kunnen verbeteren, is het absoluut noodzakelijk om zowel de locale functie als de regionale doorbloeding te kunnen bepalen.

Een grote hoeveelheid literatuur is beschikbaar over interventies op het ischemische hart (hoofdstuk 3). Mechanische ondersteuning door middel van een ballon catheter in de aorta vermindert de zuurstof behoefte van het hart door een verlaging van de weerstand waarmee het bloed in de aorta wordt gepompt. Wisselende invloeden op de totale en de regionale myocardiale doorbloeding zijn beschreven (3.2.1). Een stijging van de arteriele bloeddruk leidt tot een verhoging van de zuurstof behoefte en kan het ischemische gebied in gevaar brengen, maar aan de andere kant resulteert deze verhoging wel in een toename van de coronair doorbloeding. Op dezelfde wijze kunnen tegenstrijdige invloeden verwacht worden van een verhoging van de contractiliteit met positief inotrope stimulatie: zowel toe- als afnamen in de doorbloeding van het ischemische gebied zijn waargenomen (3.2.3).

Een andere benadering volgt uit het verminderen van de invloed van de factoren die de zuurstof behoefte van het hart bepalen. Een verlaging van hart frequentie en myocardiale contractiliteit door middel van beta-adrenoceptor blokkade is een klassiek voorbeeld (3.3.1). Vasodilatatie van het arteriele en/of het veneuze vaatbed veroorzaakt ook een vermindering van de myocardiale zuurstof behoefte en een verlichting van de belasting op het ischemische gebied. Aan de andere kant leidt een dergelijke ingreep ook tot een vermindering in de coronair doorbloeding en daardoor in het locale zuurstof aanbod. Een laatste voorbeeld is het blokkeren van de contractie door gebruik te maken van medicamenten met calcium antagonistische werking. Deze verlagen de zuurstof behoefte, maar kunnen ook een onaanvaardbare daling in de contractiliteit veroorzaken. De optimale combinatie in een gegeven klinische of experimentele omstandigheid kan niet a priori voorspeld worden, aangezien deze, zoals boven vermeld, van een groot aantal factoren afhankelijk is.

Teneinde een aantal van deze aspecten op te helderen bestudeerden wij de invloed van (i) positieve inotrope stimulatie door middel van oxyfedrine (hoofdstukken 6 en 7), (ii) verlagen van de hartfrequentie en het hart minuut volume met daarbij een verhoging van de arteriele bloeddruk met ergotamine (hoofdstuk 8) en (iii) een selectieve verlaging van de hartfrequentie met alinidine (hoofdstukken 9 en 10) op de relatie tussen zuurstof behoefte en aanbod in het (ischemische) varkenshart.

12.2. Proefdier model en meetmethoden

Het model waarin deze studies werden uitgevoerd en de toegepaste technieken waren nagenoeg gelijk in alle studies. Genarcotiseerde varkens (±25 kg) werden beademd met $N_2O:O_2=2:1$ en gecatheteriseerd met Millar catheter-tip manometers in de linker ventrikel en de aorta en met een thermodilutie catheter in pulmonaal arterie. Deze laatste werd gebruikt voor het bepalen van het hart minuut volume. De thorax

werd geopend, het hart vrijgeprepareerd en een electromagnetische bloedstroom meetkop aangebracht om de afdalende tak van de linker coronair arterie (LAD). Myocardiale ischemie kon geinduceerd worden door het aandraaien van een schroefklem om de LAD. Een practisch probleem is het quantificeren van de regionale functie in het normaal geperfundeerde en het ischemische gedeelte van het hart. Een bijzonder gevoelige methode werd gevonden in de bepaling van de veranderingen in de myocardiale wanddikte uit de reflectie van ultrageluid (zie 1.3.2.4). Het is algemeen bekend dat een plaatselijk doorbloedings tekort direct wordt gevolgd door een verminderde functie. Nieuw ontwikkelde miniatuur ultrageluid kristallen die direct op het hart genaaid worden, maakten de meetmethode betrouwbaar en reproduceerbaar. Wanneer kristallen op zowel het normaal geperfundeerde als het ischemische gebied worden aangebracht kan de locale functie direct worden bepaald, terwijl de globale hemodynamische variabelen inzicht geven in het functioneren van het gedeeltelijk ischemische hart als geheel.

De meting van de regionale myocardiale doorbloeding kan uitgevoerd worden door gebruik te maken van de radioactief gelabelde microsphere methode. Daartoe worden plastic bolletjes met een diameter van 15 micron, voorzien van een gamma straling uitzendend radionuclide, ingespoten in het linker atrium. De bolletjes vermengen zich met het arteriele bloed en worden evenredig met de locale bloedstroom verdeeld. Aangezien zij groter zijn dan de bloedcellen lopen de bolletjes vast in de arteriolen of de capillairen in het lichaam. De hoeveelheid radioactiviteit in een stuk weefsel is dan een maat voor de locale bloedstroom, vooropgesteld dat de bolletjes homogeen in het bloed verdeeld waren. De microsphere techniek kan gebruikt worden voor het bestuderen van de verdeling van het hart minuut volume, de totale en regionale doorbloeding van een orgaan, het hart minuut volume zelf en voor het vinden van variabelen die gerelateerd zijn aan de bloedstroom door arterioveneuze anastomoses. De voorwaarden waaronder de microsphere techniek gebruikt kan worden en de computer programma's die wij ontwikkeld hebben voor het berekenen van de bloedstroom uit radioactiviteits waarnemingen, zijn beschreven in hoofdstukken 4 en 5.

12.3. Resultaten

(i) oxyfedrine

Beta-adrenerge stimulatie met oxyfedrine werd toegepast om de invloed te bestuderen van een coronair vasodilatoir middel tesamen met inotrope stimulatie op de globale hemodynamica en het lactaat metabolisme van het hart, dat ischemisch was gemaakt door een vermindering van de coronair doorbloeding (hoofdstuk 6). Zowel voor als tijdens ischemie, veroorzaakt door een reductie van de LAD-doorbloeding tot 25% van zijn controle waarde, resulteerde de toediening van 0.2 $mg.kg^{-1}$ oxyfedrine in een flinke toename in de maximum stijgsnelheid van de linker ventrikel druk (LVdP/dtmax), een index voor de myocardiale contractiliteit, maar gaf ook een verhoging

van de hartfrequentie, terwijl geen invloed op de gemiddelde bloeddruk en het hart minuut volume werd waargenomen. De toename in de myocardiale zuurstof behoefte werd niet gevolgd door een sterker anaeroob metabolisme van het ischemische hart, aangezien de lactaat balans niet veranderde. Geconcludeerd kan worden dat oxyfedrine bruikbaar kan zijn bij de behandeling van pompfalen ten gevolge van acute myocardiale ischemie.

Globale hemodynamische variabelen geven geen volledig beeld van de invloed van de toediening van farmaca in gedeeltelijk ischemische harten. Daarom werd ook de invloed bestudeerd van oxyfedrine op de regionale myocardiale bloedstroom verdeling tesamen met de wanddikte variabelen in dat deel van het hart dat ischemisch was gemaakt door een vaste stenose om de LAD (hoofdstuk 7). Net als in de vorige studie werden toenamen in LVdP/dtmax en hartfrequentie waargenomen na toediening van 0.15 $mg.kg^{-1}$ oxyfedrine. Het middel veroorzaakte een toename in de bloedstroom naar het normale, niet-ischemische myocardium, maar ook in de bloedstroom naar en de functie van het ischemische gebied, ondanks de aan toediening gepaard gaande toename van de myocardiale zuurstof behoefte.

(ii) ergotamine tartraat

Ergotamine, een veel gebruikt middel om acute aanvallen van migraine te behandelen, heeft een contra indicatie voor patienten, die lijden aan coronaire hartziekten, aangezien het de arteriele bloeddruk verhoogt en spasmen van coronair arterien kan veroorzaken. Het farmacon veroorzaakt echter ook een verlaging van de hartfrequentie, zodat daarmee de zuurstof behoefte van het hart daalt. Bovendien is aangetoond dat het de arterio-veneuze anastomoses (AVAs) in het hoofd van de kat kan sluiten. Deze eigenschappen kunnen de myocardiale zuurstof balans verbeteren. Daarom is ergotamine bestudeerd in het niet-ischemische varkenshart preparaat ten einde de relatie tussen dosis en invloed op de diverse variabelen te bepalen en in het ischemische hart om de invloed na te gaan op de segmentale doorbloeding en functie (hoofdstuk 8). In doses van 8, 16 en 32 $g.kg^{-1}$ verlaagde ergotamine de hartfrequentie, het hart minuut volume en de totale systemische AVA-doorbloeding, terwijl het een stijging in de systemische vasculaire weerstand bewerkstelligde. Er werd geen invloed op de totale en de regionale myocardiale doorbloeding waargenomen. Acht $g.kg^{-1}$ ergotamine, toegediend tijdens myocardiale ischemie, verhoogde de doorbloeding en de endo/epi ratio van het ischemische gebied tot normale waarden. Helaas bleven de wanddikte variabelen, die waren verslechterd tijdens ischemie, afgenomen.

(iii) alinidine hydrobromide

De hartfrequentie is een van de belangrijkste factoren in de myocardiale zuurstof behoefte. Een N-allyl afgeleide van clonidine, alinidine, verlaagt de hartfrequentie nagenoeg selectief door een directe invloed op de sinus knoop. Op grond daarvan zou het middel bruikbaar kunnen zijn om de relatie tussen zuurstof vraag en aanbod

van het hart te verbeteren tijdens myocardiale ischemie. Allereerst bepaalden wij een dosis-responsie relatie in varkens met een normale myocardiale doorbloeding (hoofdstuk 9) en vonden, dat een dosis van 0.4 $mg.kg^{-1}$ in staat was om de hartfrequentie met 20% te verlagen zonder daarbij noemenswaardige invloed uit te oefenen op de overige hemodynamische variabelen. De myocardiale doorbloeding daalde transmuraal, evenredig met de afname in de myocardiale zuurstof behoefte. De segmentale wandfunctie bleef onveranderd. In hogere doses werd de negatieve inotrope invloed van alinidine meer merkbaar en veroorzaakte een flinke daling in het hart minuut volume.

In een tweede studie (hoofdstuk 10) werd de invloed van alinidine op de myocardiale perfusie en functie bestudeerd wanneer het middel werd toegediend tijdens myocardiale ischemie. Nadat de doorbloeding en de functie van het ischemische gebied was gestabiliseerd werd alinidine geinfundeerd teneinde een hartfrequentie daling van minstens 25% te realiseren (dosis: 0.4-0.9 (0.57±0.07) $mg.kg^{-1}$). De invloed op de globale hemodynamische variabelen was vergelijkbaar met de resultaten verkregen in niet-ischemische harten. De ischemische bloedstroom herverdeelde zich echter ten gunste van het endocardium, zodat het gehele linker ventrikel weer nagenoeg homogeen was doorbloed, ondanks de aanwezigheid van de coronair stenose. De wanddikte variabelen herstelden maar ten dele.

12.4. Discussie

Elk van deze farmacologische studies benadert een klein gedeelte van de regel mechanismen van het hart en de coronair doorbloeding. Hun mogelijke klinische toepasbaarheid en het belang daarvan zullen nu nader besproken worden. Het belangrijkste resultaat van de oxyfedrine studies is, dat inotrope en matig chronotrope stimulatie van het gedeeltelijk ischemische hart de doorbloeding en de functie van het ischemische gebied verbetert, terwijl zelfs geen verschuiving naar een meer anaeroob metabolisme wordt veroorzaakt. De veelvuldig beschreven functie verslechtering van het ischemische gebied trad niet op bij oxyfedrine. Er moet echter rekening gehouden worden met het feit dat oxyfedrine geen al te grote toename in de contractiliteit veroorzaakte en dat de tegelijkertijd optredende stijging in de hartfrequentie beperkt bleef tot 20%. Bovendien veroorzaakt de toediening van oxyfedrine een afname van de eind-diastolische druk in de linker ventrikel en daarmee een afname in de myocardiale zuurstof behoefte. Samenvattend kan gesteld worden dat matig inotrope stimulatie tijdens myocardiale ischemie zonder nadelige gevolgen toegepast lijkt te kunnen worden.

Alhoewel ergotamine en ergonovine afkomstig zijn uit dezelfde familie van stoffen, oefenen zij een verschillende invloed uit op het hart en het cardiovasculaire systeem. Ergonovine wordt vaak toegepast om spasmen van coronair arterien, en daarmee "variant angina", op te wekken. Ergotamine daarentegen wordt gebruikt voor het behandelen van migraine. Aan dit middel wordt een contraindicatie toegeschreven voor patienten die lijden aan coronaire hartziekten vanwege zijn bloeddruk verhogende eigenschap en wellicht ook nog vanwege de veronderstelde

inductie van spasmen van coronair arterien. Aangezien ergotamine de zuurstof behoefte van het hart verlaagt door een daling in de hartfrequentie, maar de zuurstof behoefte verhoogt door de stijging in de arteriele bloeddruk, die daarmee op zijn beurt het zuurstof aanbod vergroot door een stijging in de coronaire bloedstroom, leek het interessant om ergotamine te bestuderen in het gedeeltelijk ischemische hart. Bovendien vermindert deze stof de bloedstroom door arterioveneuze anastomoses (AVA's), hetgeen kan leiden tot een verlaging van "verspild" hart minuut volume. Het laatste punt werd bevestigd door onze studie, daar de daling in het hart minuut volume volledig op rekening van de AVA-bloedstroom kwam. De gebruikelijke waarden voor de AVA-bloedstroom van 10 tot 60% van het hart minuut volume (zie 1.2) laat ruimte om te speculeren over de functionele betekenis van deze bloedstroom in de regeling van het hart minuut volume. Indien een dergelijk mechanisme werkzaam zou zijn, kan dit een belangrijke bron van reserve zijn voor de doorbloeding van de perifere weefsels. Het is echter niet waarschijnlijk dat energie verspild wordt aan het rond pompen van bloed door AVA's, aangezien deze ook betrokken zijn bij de regulatie van de lichaams temperatuur. De tegengestelde invloed van ergotamine op het ischemische gedeelte van het hart suggereert dat zich een evenwicht had ingesteld tussen de toename in zuurstof behoefte en aanbod, aangezien de wanddikte variabelen nauwelijks veranderden, ondanks een sterke stijging van de doorbloeding, die in het endocardium zelfs groter was dan in het epicardium. Tenslotte droeg deze studie geen argumenten aan die wijzen op een contraindicatie voor ergotamine in het geval van een slechte cardiovasculaire functie.

De selectieve verlaging van de hartfrequentie met alinidine veroorzaakte een aanzienlijke daling in de myocardiale zuurstof behoefte, zoals af te leiden valt uit de vermindering van de coronair doorbloeding. In doses boven 0.4 $mg.kg^{-1}$ leidde toediening van het middel ook tot een daling in de contractiliteit. Wanneer alinidine werd gegeven tijdens myocardiale ischemie trad er echter een herverdeling op van de bloedstroom in het ischemische gebied ten gunste van het endocardium, overigens zonder dat dit een verbetering gaf in het patroon van de myocardiale wandverdikking. Deze waarnemingen geven een gunstiger beeld vergeleken met die van beta-adrenoceptor blokkerende middelen, waarvan het voornaamste doel is om het myocardiale zuurstof verbruik te remmen. Bovendien kan alinidine gegeven worden aan patienten die geen beta-blokkerende middelen verdragen. Opgemerkt moet worden, dat wanneer de vicieuze cirkel van sympatische stimulatie van het hart ten gevolge van de myocardiale ischemie zelf niet wordt geblokkeerd door alinidine, zijn bruikbaarheid geringer kan zijn dan nu wordt aangenomen. Desalniettemin kan geconcludeerd worden dat alinidine de verstoorde balans tussen zuurstof vraag en aanbod van het ichemische hart verbetert door een verlaging van de zuurstof behoefte van het ischemische gebied.

12.5. Vooruitzichten

De studies zoals beschreven in dit proefschrift geven antwoord op vragen omtrent het gedrag van het hart tijdens regionale, ernstige ischemie. De verhouding tussen de endocardiale- en de epicardiale doorbloeding (endo/epi-ratio) is ongeveer een tijdens normale myocardiale doorbloeding. De precieze waarde hangt echter af van de afmeting van de gebruikte microspheres (zie 4.3.2). Dit bewijst dat de anatomie van het coronair systeem de interpretatie van de getallen betreffende de regionale doorbloeding beinvloedt. Anderzijds is beschreven dat de verdeling van microspheres met verschillende diameter over de doorsnede van een arterie niet homogeen is, zodat deze verschillen geen verbazing behoeven te wekken. Het gedrag van microspheres vlak bij een bloedstroom beperkende coronair stenose is eveneens onvoorspelbaar. Bovendien daalt de endo/epi-ratio al aanzienlijk wanneer een stenose wordt aangebracht, die slechts een geringe daling in de totale bloedstroom veroorzaakt. Al met al blijven er onopgehelderde problemen bij de interpretatie van de eno/epi-ratio. Een in-vitro studie met een arterie en een instrument om de vaatdoorsnede te verkleinen kan gebruikt worden om het gedrag van microspheres in een gedeeltelijk afgesloten coronair arterie te bestuderen. Een (computer-) analyse van de coronair circulatie met inbegrip van de intramyocardiale druk en vervorming van de ventrikel tijdens contractie en uitdrijving van bloed kan een nader inzicht geven in de verdeling van de regionale myocardiale doorbloeding.

Het vrijprepareren van een coronair arterie ten behoeve van de plaatsing van een bloedstroom meetkop en/of een klem om ischemie te veroorzaken, is een andere bron van moeilijkheden, aangezien het daarbij moeilijk te vermijden is, dat de locale innervatie van de ventrikel beschadigd wordt. Aangezien de innervatie van het niet ischemische gebied intact blijft, kan de daardoor veroorzaakte verstoring in de regel systemen de verkregen resultaten beinvloeden.

Bij het bestuderen van het patroon van de myocardiale wandverdikking tijdens ischemie valt op, dat de systolische wandverdikking afneemt en dat de wand blijft verdikken gedurende de diastole (hoofdstukken 7 en 8). In werkelijkheid bereikt de maximale wanddikte de eind-diastolische waarde zoals die tijdens normale perfusie wordt gevonden. Al zou de diastolische wandverdikking geen (gedeeltelijk) actief proces zijn, toch blijft het waar dat deze de diastolische doorbloeding van het ischemische gebied kan belemmeren vanwege de opgewekte intramyocardiale druk. In elk geval moet de bron en het belang van diastolische wandverdikking nader bestudeerd worden.

Een ander onopgelost probleem is de aanwezigheid in ons model van de hoge arterioveneuze bloedstroom. Door gebruik te maken van microspheres met verschillende diameter is het mogelijk om de plaats van de anastomoses te bepalen. De afhankelijkheid van narcose, sympathische activiteit en de leeftijd van de proefdieren zijn een aantal paden die kunnen leiden tot een beter inzicht in het verschijnsel van de bloedstroom door arterioveneuze anastomoses. Het belang hiervan voor de menselijke circulatie blijft zuiver speculatief.

Zoals besproken in hoofdstuk 10 is de hartfrequentie een belangrijke factor die de myocardiale zuurstof behoefte bepaalt. Indien positief inotrope behandeling is vereist om de hemodynamische conditie van een patient te verbeteren, is de tegelijkertijd optredende stijging van de hartfrequentie een schadelijk neven effect. Daarom lijkt de combinatie van positieve inotrope stimulatie en hartfrequentie daling met alinidine veelbelovend, en in het bijzonder indien aangetoond kan worden dat het ook de reflexmatige toename in de hartfrequentie na arteriele vasodilatatie, zoals die veelvuldig in de kliniek noodzakelijk is, kan onderdrukken. Ergotamine kan bruikbaar zijn wanneer een hoog hart minuut volume gepaard gaat aan een lage bloeddruk, het "high output syndrome", aangezien dan een groot deel van het hart minuut volume door arterioveneuze anastomoses zou kunnen vloeien. Een dergelijke situatie kan een gevolg zijn van een verstoring in de perifere regel mechanismen.

Alle resultaten verkregen in de loop van deze onderzoekingen illustreren de bruikbaarheid van het model zoals dit toegepast is bij het bestuderen van farmacologische interventies op de doorbloeding en de functie van het ischemische hart. Aangezien een belangrijke beperking wordt gevormd door het gebruik van proefdieren onder narcose, zal geprobeerd moeten worden om dergelijke experimenten uit te voeren in niet genarcotiseerde proefdieren, die tevoren zijn voorzien van de benodigde instrumenten.

Curriculum Vitae

De schrijver van dit proefschrift werd geboren op 10 november 1949 in Zeist en doorliep aldaar de HBS-B afdeling van het eerste Christelijk Lyceum. De studie in de experimentele natuurkunde aan de Rijks Universiteit in Utrecht werd in 1967 aangevangen. Na het doctoraal examen (december 1973) werd een aanstelling verkregen als wetenschappelijk medewerker bij het Fysiologisch Laboratorium van dezelfde Universiteit. Per september 1976 volgde de overgang naar de Erasmus Universiteit in Rotterdam, Vakgroep Thoraxcentrum, afdeling Experimentele Cardiologie. Daar werden de in dit proefschrift gerapporteerde onderzoekingen uitgevoerd. Aanstellingen ten laste van de Nederlande Hartstichting en van de Vakgroep Farmacologie maakten de bewerking en de afronding van dit proefschrift mogelijk. Per 15 oktober 1980 is de schrijver verbonden aan de Rijks Universiteit in Utrecht, Faculteit Diergeneeskunde, Vakgroep Functionele Morfologie.