PHARMACOLOGICAL MANIPULATION OF ALPHA- AND BETA-ADRENOCEPTORS IN THE ISOLATED PERFUSED RAT KIDNEY. EFFECTS ON RENAL VASCULAR RESISTANCE AND RENIN SECRETION RATE.

Farmacologische beinvloeding van alpha- en beta-receptoren in de geisoleerde geperfundeerde rattenier. Effecten op niervaatweerstand en reninesecretie.

PROEFSCHRIFT

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Aan Jenny, Albert en Victor
Aan mijn moeder, ter
nagedachtenis aan mijn vader

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CHAPTER 1

Introduction:

Almost all drugs and hormones elicit their biologic action by binding to specific cellular recognition sites, these structures are receptors or receptor molecules. A differentiation can be made between receptor-molecule and receptor-site, the latter being that part of the receptor-molecule which interacts with hormones or pharmaca, usually in a direct reversible manner. This implies a certain degree of chemical complementarity of the active agent and its specific receptor-site, and this is the basis for the relationship between the chemical structure of a pharmacon and its biologic action (Ariens 1984; Lefkowitz et al, 1984). Binding to these receptors is followed by activation of intracellular enzyme systems or by ion fluxes and is eventually expressed as characteristic physiological or pharmacological effects.

Adrenoceptors (adrenergic receptors) are those cellular structures, catecholamines and synthetic analogues are capable to bind with.
In 1948 Alquist reported on the different responses he obtained with catecholamines in a variety of tissues. He assumed that the effects were mediated through alpha- and beta-adrenoceptors. Lands (1967) proposed a subdivision of beta-adrenoceptors in β_1- and β_2-adrenoceptors. Later on α-adrenoceptors were shown to exert a different response to various α-agonistic substances and were therefore termed α_1- and α_2-receptors respectively (Langer, 1974; Berthelsen and Pettinger, 1977; Timmermans et al 1980[a], 1981[b]).

Renin was first described in 1898 by Tigerstedt and Bergmann. It is a proteolytic enzyme involved in the renin-angiotensin system which plays an important role in blood pressure regulation. Several factors are recognized which may influence renin secretion. These factors are renal perfusion pressure, sodium concentration at the macula densa, renal sympathetic nerve activity, alterations in extracellular sodium concentration and volume, and various humoral factors (hormones, prostaglandines etc.). Because of the complex interrelation between these factors difficulties are encountered when one attempts to clarify

the interrelation between vasoactivity, sympathetic nerve activity and renin secretion. This is reflected in the literature where considerable controversy remains on which adrenoceptor subtypes are involved in renin secretion. In addition, interactions between the adrenoceptor subtypes and other receptors have been demonstrated. Because of the complexity of these interrelations we carried out experiments in the isolated perfused rat kidney since in such a model several of the aforementioned factors may be controlled.

The main issue of our investigation is which subtype of adrenoceptor could be responsible for the final adjustment in renin secretion. The questions related to this issue are as follows:

1. which adrenoceptors can be defined in the isolated rat kidney by pharmacological methods.

2. which roles can be ascribed to the respective adrenoceptor subtypes in terms of vasoregulation.

3. which adrenoceptors are involved in renin secretion.

4. to what extent are the effects of these adrenoceptors with respect to vasoregulation and renin secretion interdependent.

In the next three chapters the renin-angiotensin system, adrenoceptors in general and in the kidney, and methods will be described.

CHAPTER 2

The renin-angiotensin system.

2.1 Introduction:

Renin is a proteolytic enzyme with a molecular weight of about 44.000 daltons in humans. It acts on the leucine-leucine bond of its substrate, angiotensinogen. This substance is an α_2-globulin, and is synthesized in the liver.
After cleaving of the leucine-leucine bond a decapeptide (angiotensin I) is liberated, which is relatively inactive. This angiotensin I is converted to angiotensin II by a converting enzyme, which is present in blood and tissues, especially in the lungs, but also in the kidney. This angiotensin II (octapeptide) is a potent vasoconstrictor and stimulates aldosterone secretion.
Renin is synthesized and stored in granules of the highly differentiated myoepithelial cells or juxtaglomerular cells in the kidney (Davis and Freeman, 1976). These cells are part of the juxtaglomerular apparatus, which encompasses a tubular and a vascular component (Davis and Freeman, 1976; Barajas, 1979, 1981):

1. the afferent and efferent arterioles of the glomerular hilus.
2. extraglomerular mesangium or Goormatigh cells.
3. macula densa.

Most of the renin containing cells are located in the medial layer of the afferent arterioles (Hartroft and Hartroft, 1961; Barajas and Latta, 1963; Davis and Freeman, 1976; Barajas, 1979), also the efferent arteriole (Barajas and Latta, 1963; Barajas 1971, 1981; Taugner et al, 1982) and extraglomerular mesangium contains granular cells (Barajas, 1981). The macula densa is a portion of the distal tubule which is adjacent to the vascular component at the hilus of the glomerulus (Barajas, 1979).

A gradient of renin is present in the cortex: from the juxtamedullary region towards the outer cortex-zone an increasing concentration of

renin is observed (Davis and Freeman, 1976).
Renin is released from the granular cells of the juxtaglomerular apparatus into the renal bloodstream and renal lymph (Davis and Freeman, 1976), and is predominantly but not completely degraded in the liver (Fagard et al, 1973).
Besides active renin, inactive renin (or prorenin) has been determined in plasma. Activation of inactive renin may be accomplished in several ways, e.g. by acidification at pH 3.0-3.3, followed by dialysis at neutral pH (Derkx et al, 1976), by addition of trypsine, pepsine, kallikreine, plasmine, plasmingen activators (Derkx et al, 1979[a], 1979[b], 1984; Shulkes et al, 1978) or by hypothermia at -5°C (Sealy et al, 1976).

2.2 Renin secretion.

Several mechanisms are involved in renin secretion:

1. renal perfusion pressure (baroreceptor mechanism).
2. sodium concentration at the macula densa.
3. renal adrenoceptors, renal sympathetic nerves and catecholamines.
4. other humoral factors.

2.2.1 Renal perfusion pressure:

The importance of the mean renal perfusion pressure in renin release was first recognized by Tobian et al (1959). They stressed on the role of a baroreceptor in the afferent arteriole. This was confirmed by others (Skinner et al, 1963, 1964; Vander, 1967; Blaine et al, 1971). Renin release varies inversely with local perfusion pressure independently of changes in renal blood flow (Skinner et al, 1964). In the non-filtering kidney (without a functional macula densa), after denervation and adrenolectomy, a fall in the perfusion pressure (due to haemorrhage) stimulated renin secretion (Blaine et al, 1979). This observation was extended by Blaine and Zimmerman (1979), who demonstrated the opposite namely a fall in renin secretion after elevation of renal perfusion pressure.

Several authors have proposed an intrarenal stretch-receptor as a predominant factor in renin-release (Blaine and Davis, 1971; Witty et al, 1972).
Nevertheless, the exact nature of the signal which stimulates renin secretion has not yet been clarified.
Vander (1967) supposed at least three possibilites, e.g. arterial pressure, active afferent arteriolar vascular tone and renal interstitial pressure.
Some authors suggested vasoconstriction to be the responsible stimulus for enhanced renin secretion (Blaine and Davis, 1971; Witty et al, 1972; Fray, 1980), others proposed that rather vasodilatation induces renin secretion (Gutman et al, 1973; Gotshall, 1974).

Davis and Freeman (1976) presumed that the renal vascular baroreceptor responds to alterations in arteriolar wall tension. Several factors may affect the receptor, thereby controlling renin-release, e.g. changes in the diameter of the renal afferent arterioles, alterations in the transmural pressure gradient, renal sympathetic nerve activity which controls renal arteriolar tone, intrinsic myogenic factors and alterations in the elastic components of the vessel wall.
In experiments in the isolated perfused rat kidney Fray (1976) found that vasodilatation and higher perfusion pressure elevate the stretch of the afferent arteriole, thereby decreasing renin secretion. The opposite, vasoconstriction or low perfusion pressure, reduces the stretch, and subsequently, renin secretion is enhanced. Renin secretion would be more sensitive to alterations in the ratio between outer and inner diameter of the afferent arteriole.
Vandongen and Peart (1974[a]) and Fray and Park (1979) found evidence for a predominant role of calcium in renin secretion after stimulation of the baroreceptor.

2.2.2 Sodium concentration at the macula densa:

The macula densa is thought to play a predominant role in renin release, and acts as a sensor that is sensitive to distal tubular sodium (Vander and Miller, 1964; Vander, 1965, 1967; Thurau et al 1967; Davis and Freeman, 1976). A consensus on this mechanism has

not been reached. Vander and Miller (1964) suggested that a reduced sodium load at the macula densa stimulates renin release.
Barajas (1963, 1971) described that the macula densa frequently is more in contact with the efferent arteriole than with the afferent arteriole. He supposed that the sodium concentration at the macula densa in the tubules influences this contact, a reduced sodium load would increase renin release and vice versa.
This was confirmed by Di Bona (1971) and Freeman et al (1974).

Thurau et al (1967, 1982) demonstrated that increasing sodium load stimulated renin secretion. Retrograde perfusion of the macula densa with hypertonic or isotonic sodium chloride solution elevated renin activity in a single JG-apparatus. This, in turn, stimulates angiotensin-II-formation and constricts the afferent arteriole, thereby reducing glomerular filtration and thus, an intrarenal feedback mechanism is assumed to exist.
Gottschalk and Leyssac (1968) proposed that leakage of tubular fluid could explain the observed reduction in glomerular filtration. Cooke et al (1970) provided evidence that increasing sodium load stimulates renin release. In this study diuretics were used, which may have had a direct stimulating effect on renin release.

2.2.3 Renal sympathetic activity.

The kidney receives both sympathetic and parasympathetic nerves, sympathetic fibers being the more prominent ones.
Barajas (1964), Hartroft (1966) and Wagermark et al (1968) demonstrated the occurrence of sympathetic innervation of the juxtaglomerular apparatus. Hartroft (1966) suggested also a similar innervation of the macula densa, though this was not confirmed by Wagermark (1968).
Autoradiography showed that axons associated with the juxtaglomerular apparatus were able to incorporate exogenous tritriated noradrenaline (Barajas, 1981). In addition, it appears that dopaminergic nerves predominate in the preglomerular arteriole, whereas those fibers in approximation to the arcuate arteries contain mostly noradrenaline.

Barajas (1984) described in all portions of the cortical tubular nephron some degree of neural influence. In the distal convoluted tubule a high number of autoradiographically depicted granules in connection with the afferent arteriole was demonstrated, whereas the thick ascending limb of Henle had the highest number granules in contact with the efferent arteriole.

Johnson et al (1971) performed renal nerve stimulation in a non-filtering kidney with exclusion of vasoconstriction by infusion of papaverine and found increased renin-release in these experiments (without stimulation of the macula densa or baroreceptor). This was confirmed by Holdaas et al (1981).
Renal nerve stimulation (without altering renal haemodynamics or urinary sodium excretion) at frequencies between 0.25 and 0.7 Hz stimulates renin secretion at normal perfusion pressure (Osborn et al, 1981; Holdaas et al, 1981; Ammons et al, 1982; Di Bona, 1985; Blair et al, 1985).

Renin secretory responses after suprarenal vasoconstriction or furosemide (Thames and Di Bona, 1979; Osborn et al, 1984; Kopp and Di Bona, 1984) are significantly greater in innervated than denervated kidneys. After infusion of prenalterol (β_1-agonist) in innervated or denervated kidneys (without alterations in renal haemodynamics and sodium excretion) no differences in the renin secretion patterns were observed (Kopp et al, 1981). In innervated and denervated kidneys at different perfusion pressures (90, 130 and 170 mmHg) no differences in renin secretion were observed after low frequency renal nerve stimulation (Kopp and Di Bona, 1984). In experiments with perfusion pressures below the physiological range alpha- and beta-receptors are involved in the process mediating renin secretion after low renal nerve stimulation (Kopp and Di Bona, 1984; Blair et al, 1985; Di Bona, 1985). It was concluded from these observations that both non-neural and neural mechanisms control renin-release, the degree of interaction depending on the level of renal arterial pressure and on the intensity of renal nerve stimulation.

Witty et al (1972) described in dogs with chronic caval constriction a fall in plasma renin-activity after renal denervation. This was confirmed by Gottschall et al (1973). In their experiments sodium depletion reversed the decrease in plasma renin activity, which is in contrast with the data obtained by Mogil et al (1969).
Chronic renal denervation suppressed intrarenal renin-concentration (Tobian, 1964), Cunningham et al (1981) described diminished renin secretion after tilting in renal transplant patients, Davis and Freeman (1976) assessed more pronounced effects on renin-release after acute denervation as compared to chronic denervation.

In 1976 Johnson et al observed decreased renin secretion in sodium depleted, denervated canine kidneys after intrarenal propranolol infusion. In experiments with kidney slices, isolated glomeruli or renal cells suspensions adrenergic agents stimulated renin release (Michelakis et al, 1969; Nolly et al, 1974; Aoi et al, 1974; Johns et al, 1975; Morris et al, 1976; Capponi and Valloton, 1976; Khayat et al, 1981), a similar response was observed in the isolated perfused kidney preparation (Vandongen, 1975).

To conclude: it appears that renal nerves do have an important role in renin secretion but other renin releasing mechanisms may compensate rapidly for the absence of an intact sympathetic innervation (Blaine et al, 1971; Davis and Freeman, 1976). Adrenoceptors of various subtypes mediate the responses to neural and non-neural stimuli. The alpha- and/or beta-adrenoceptors which may be involved in mediation of renin secretion, will be discussed in Chapters 3.5.2.3 and 3.4.2.2.

2.2.4 Humoral factors.

Several humoral factors are involved in renin secretion (plasma sodiumconcentration, potassium, calcium, angiotensin II, vasopressin, parathormone, prostaglandines etc.).Since these factors are not relevant to the central theme of our investigation, we have refrained from discussing them in the present thesis.

CHAPTER 3

Regulation of adrenoceptor stimulation, beta-adrenoceptors, alpha-adrenoceptors

3.1 Regulation of adrenoceptor stimulation.

3.1.1 Mechanisms in noradrenergic transmission.

The sympathetic nerves end in varicosities which contain vesicles formed in the neuronal cell body. These vesicles descend to the peripheral noradrenergic nerve ending: tyrosine is taken up here from the extracellular fluid and converted to dopamine; this in turn is taken up by the storage vesicles and converted to noradrenaline by dopamine-β-hydroxylase (noradrenaline is stored together with dopamine-β-hydroxylase, ATP and chromograffin A) (Starke, 1977; Shepherd and Van Houtte, 1981).
Electric stimulation causes depolarisation of the membrane and influx of calcium; noradrenaline then diffuses towards the synaptic cleft. Besides noradrenaline dopamine-β-hydroxylase is being secreted. This enzyme is not involved in uptake processes. Except for electrical stimulation high potassium concentrations can also evoke depolarisation (Starke, 1977; Westfall, 1977). Furthermore, after increasing pulse frequency intraneuronal calcium concentration increases, thereby occupying "the release receptors" for calcium, which causes inhibition of further noradrenaline release (Starke, 1977).

3.1.2 Regulation of adrenoceptor stimulation by control of noradrenaline release.

Release of transmitter from noradrenergic nerve endings per unit time depends not only on the rate of impuls flow but also on the release of transmitter per impulse. Both inhibitory and excitatory mechanisms act at the somadendritic part of neurons, and in this way regulate frequency of impulses towards noradrenergic nerve endings.
In addition, the quantity of released neurotransmitter is far from constant. Several substances are involved in the process of neuro-

transmitter release. Some of these interact at the somadendritic part of neurons whilst others interact at the adrenergic nerve endings, so a dual receptor regulatory process is proposed (Starke, 1977; Westfall, 1977). In this chapter regulation at peripheral adrenergic nerve endings will be discussed.

Determination of noradrenaline release may be measured in several ways:

1. The amount of liberated neurotransmitter may be assessed by measuring the mechanical or electrical response of the postsynaptic effector cell after pharmacological or electrical stimulation. Some precautions should be taken into account with this method since the response may reflect different mechanisms, for instance, reduced or enhanced transmitter release or alterations in inactivation mechanisms or changes in receptor sensitivity. Combinations of these may also be involved.

2. Measurement of noradrenaline overflow.

3. Determination of stimulated noradrenaline overflow after labelling with radioactive noradrenaline.

Noradrenaline is removed from the synaptic cleft by several distinct mechanisms. The most important of these are (Iversen, 1973; Starke, 1977; Westfall, 1977; Lees, 1981; Shepherd and Van Houtte, 1981):

1. Receptors and other binding sites.

2. Re-uptake in nerve terminals (uptake 1).
   Noradrenaline is either metabolized by monoaminooxidase (MAO) or stored in the vesicles for further release.
   This (uptake 1) process has a high affinity for noradrenaline as compared to that for adrenaline, the transmitter being stored for the main part in the vesicles. Although this system has a low capacity (Cryer et al, 1980), in densily innervated tissues the recapture may amount to 70-80% (Starke, 1977). The latter is also

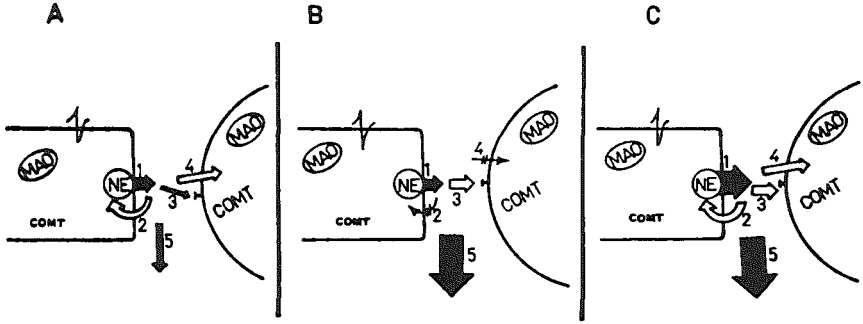
dependent on the width of the junctional cleft (Shepherd and Van Houtte, 1981).

3. Extra-neuronal uptake (uptake 2).
   The neurotransmitter may be inactivated by catecholamine-O-methyl-transferase (COMT) and MAO, which are probably located near the extra-neuronal uptake sites. Uptake 2 is a relatively low affinity, high capacity process. Affinity is higher for adrenaline than for noradrenaline (Cryer et al, 1980; Ziegler et al, 1986), but species differences have been observed. In most tissues uptake 2 plays a relatively unimportant role in proportion to uptake 1.

4. Diffusion away from the receptor site.
   The neurotransmitter presumably enters the venous bloodstream (noradrenaline-overflow). Noradrenaline which enters the circulation is metabolized mainly in the liver and kidney by catecholamine-O-transferase (COMT) and (less importantly) deamination (Iversen, 1973; Langer, 1974; Lees, 1981; Shepherd and Van Houtte, 1981).

It is important to stress the differences between noradrenaline release and noradrenaline overflow:
Release of noradrenaline encompasses any passage of the neurotransmitter across the neuronal membrane, thereafter entering the synaptic cleft.
Noradrenaline overflow (or outflow) refers to increases of noradrenaline or its metabolites in venous blood (or perfusion or incubation fluid), thus representing a diffusion outside the synaptic cleft.
Increments in overflow may be due to a real increase in transmitter release, but also to inhibition of re-uptake processes without a true increase in transmitter release (Starke, 1977; Westfall, 1977).
Even in the unstimulated situation some spontaneous release of noradrenaline (termed basal outflow) occurs. This is in contrast to the increase of the neurotransmitter above the resting levels during and after nerve stimulation (Langer, 1974; Starke, 1977; Westfall, 1977).

Fig. 3.1.1



Schematic representation of transmitter release and transmitter overflow during nerve stimulation.
(A) Normal; (B) increase in overflow due to inhibition of sites of loss, no change in transmitter release; (C) increase in overflow due to an actual increase in transmitter release, sites of loss unaffected. 1, Total amount of transmitter released by nerve stimulation; 2, norepinephrine recaptured by neuronal uptake, subsequently deaminated or stored in the vesicles; 3, fraction of the transmitter released available for activation of the receptors of the effector organ; 4, norepinephrine taken up at extraneuronal sites, subsequently metabolized mostly by COMT; 5, overflow: norepinephrine collected during and after the period of nerve stimulation. NE, norepinephrine; MAO, monoamine oxidase; COMT, catechol-o-methyltransferase.

Several drugs are known to interfere with release of neurotransmitters, other drugs act on uptake processes and some share both properties.

Neuronal uptake (uptake 1) is inhibited by cocaine, desipramine, chloorpromazine and phenoxybenzamine (Iversen, 1973). After stimulation of sympathetically innervated organs or in human fore-arm noradrenaline overflow is potentiated by cocaine or desipramine (Iversen, 1973; Chang et al, 1986), this effect is most marked at low stimulation frequencies and in the presence of low concentrations of cocaine and desipramine. At higher concentrations overflow may be blunted because of a local anaesthetic effect. Non-selective α-adrenoceptor antagonists inhibit uptake 1 processes (Iversen, 1973; Ziegler et al, 1986).

Tyramine indirectly acts like a sympathetic amine by replacing noradrenaline from the store vesicles in the nerve terminals; noradrenaline consequently diffuses into the synaptic cleft.

6-OH-dopamine is taken up in the nerve terminals by an uptake 1 process, thus resulting in a destruction of adrenergic neurons both in the central and peripheral nervous system (Iversen, 1973).

Extra-neuronal uptake (uptake 2) is inhibited by steroids (β-oestradiol, corticosterone), normetanephrine and metanephrine as well as by MAO and COMT inhibitors. Recently, Ziegler et al (1986) described that β-adrenoceptor antagonists inhibit uptake 2 processes.

When uptake 1 is blocked this may result in an increase in the concentration gradient in the synaptic cleft at the surface of the nerve endings. This then promotes stimulation of presynaptic (and postsynaptic) receptors, (Starke, 1977; Westfall, 1977; Cryer et al, 1981).

3.1.3 Role of presynaptic receptors in the regulation of adrenoceptor stimulation by control of noradrenaline release.

Superimposed upon the regulation of sympathetic transmission and effector tissue responses by noradrenaline itself, a complex of non-adrenergic regulatory systems interact with neurotransmitter release.

At least four distinct mechanisms are involved in local regulation of neurotransmitter release (Westfall, 1977).

1. Local feedback mechanism via presynaptically located receptors (autoregulation of neurotransmitter release by noradrenaline).

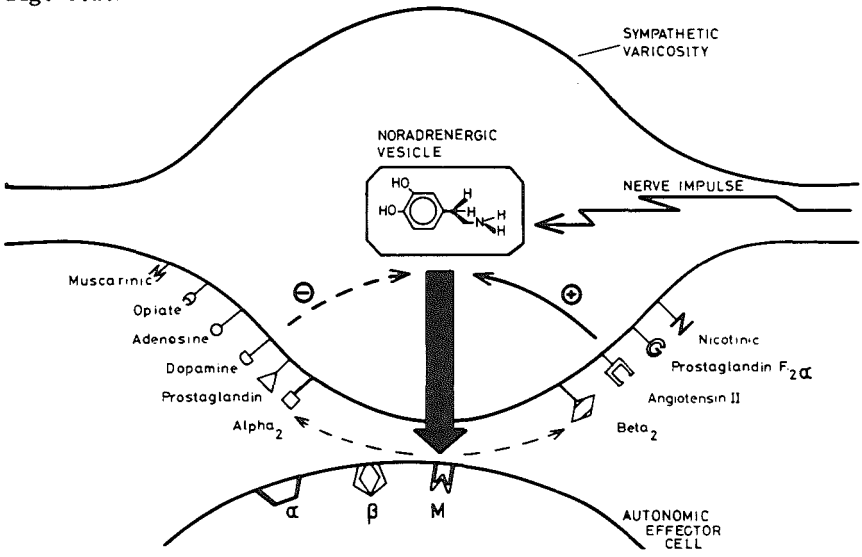
2. Regulation of noradrenaline release by locally produced substances after activation of the postsynaptic receptor in the synapse (transsynaptic regulation) (e.g. by prostaglandines).

3. Regulation of noradrenaline release through simultaneous depolarisation of nicotinic or muscarinic type acetylcholine receptors in the neighbourhood of nerve endings.

4. Control of release by locally synthetized mediators (dopamine) or blood borne substances (angiotensin II).

As depicted in fig. 3.1.2 several hormones and other substances are involved in local regulation of neurotransmitter release. Except for adrenaline and noradrenaline, these will not be discussed further.

Fig. 3.1.2



3.2 Beta-adrenoceptors.

3.2.1 Introduction:

From a historical point of view beta-adrenoceptors will be discussed previously to the alpha-adrenoceptors. In 1967 Lands et al provided evidence as to the existence of two types of β-adrenergic receptors (β_1 and β_2). These were defined according to their relative affinities to adrenaline and noradrenaline. At β_1-adrenoceptors adrenaline and noradrenaline are approximately equipotent whilst adrenaline is distinctly more potent with regard to β_2-adrenoceptors than noradrenaline.
Later on, other drugs, both agonists and antagonists, have been developed with greater affinity to each type of β-adrenoceptor.
Propranolol (non-selective antagonist) is equipotent towards β_1- and β_2-adrenoceptors. Metoprolol, atenolol and practolol (selective β_1-antagonists) are 10 times more potent at β_1-adrenoceptors in heart tissue than at β_2-adrenoceptors in the lung (Minneman, 1979[a]). Thus, "selective" β_1-antagonistic activity implies only modest selectivity (10-20 times). In contrast, prazosin displays a 100- to 1000-fold selectivity with respect to α_1-adrenoceptors (Hoffman and Lefkowitz, 1980[a]; Stiles et al, 1984).

Boissier et al (1971) showed in experiments, performed in dogs pretreated with atropine, that pulmonary β-adrenoceptors behaved in an intermediate fashion as compared with vascular and cardiac β-adrenoceptors with respect to their sensitivity to blockade by practolol.
O'Donnell and Wanstall (1976) observed that the alleged differences between β-adrenoceptors in trachea and blood vessels disappeared after extraneuronal uptake blockade by phenoxybenzamine. Thus, also extraneuronal uptake processes should be taken into account before one assumes differences in receptors. In addition, factors such as affinity of drugs, access to the receptor, the relative hydrophilic or lipophilic nature, susceptibility to degradating enzymes, and chemical stability are important. Moreover, pharmacological specifity of receptors in various organs is not identical (Minneman et al, 1979[a];

Ariens and Simonis, 1983).
Moreover, evidence has been obtained that β_1- and β_2-adrenoceptors coexist in one organ and can mediate possibly similar physiological responses. When we assume the latter, then pharmacological specificity of a particular response will depend on the ratio of β_1- and β_2-adrenoceptors as well as on the relative affinity of pharmaca to both receptor types.

Minneman et al (1979[a]) measured alterations in adenylate-cyclase elicited by several agonists and antagonists and concluded that β-adrenoceptors in rat heart and lung display different pharmacological specifities (in vitro) which is consistent with the existence of two distinct β-adrenoceptors. In subsequent papers Minneman et al (1979[b,c]) established in rats only two β-adrenoceptors, although the relative distribution varies widely in various organs (Minneman, 1979[b]). Further support as to the existence of two β-adrenoceptor types was obtained because of differences in β_1- and β_2-adrenoceptor densities after pharmacological manipulation with 6-OH-dopamine and desmethylimipramine (Minneman, 1979[d]; Minneman and Molinoff, 1980). The latter is also an argument in favour of the existence of these receptors on distinct tissue components e.g. presynaptic or postsynaptic localization (Minneman, 1979[d]; Stiles et al, 1984).

3.2.2 Localization and function of β_1-adrenoceptors.

Heart:
In rat, guinea pig- and rabbit heart the presence of predominantly β_1-adrenoceptors has been established (Minneman, 1979[b]; Lees, 1981; Lefkowitz et al, 1984; Shanks, 1984; Wilffert et al, 1984).
After stimulation of these β_1-adrenoceptors increments in heart rate, contractile force and A-V conduction were observed, furthermore glycogenolysis was promoted.

Lung:
In guinea pig lung, O'Donnel (1978) described a decreasing number of β_1-adrenoceptors going from the central part of the lung in the direction of the periphery concomitantly with decreased sympathetic

innervation.

Adipocytes:
These cells contain β_1-adrenoceptors (Lees, 1981; Shanks, 1984; Wilffert et al, 1984), after stimulation lipolysis is enhanced.

Brain:
β_1-adrenoceptors have been established in several brain regions but large variations (up to 20 fold) in absolute receptor number were observed (Minneman, 1979[b]).
Stimulation of CNS β-receptors elevated heart rate and blood pressure (Korner, 1984), though variable responses were obtained.

Kidney:
Renin release is mediated by β_1-adrenoceptors (Chapter 3.4).

Adrenergic nerve endings:

Beta$_1$-adrenoceptors are recognizable in tissues with sympathetic innervation. In studies in which tyramine was used as an indirectly acting sympathetic amine, only responses were obtained in tissues with β_1-adrenoceptors (Ariens and Simonis, 1983). Minneman et al (1979[d]) described in studies in rat cerebral cortex that 6-OH-dopamine induced a 64% increase in β_1-adrenoceptor density without changes in β_2-adrenoceptor density. Bryan et al (1981) studied in rat, guinea pig and cat atria the influences of neuronal and extra-neuronal uptake inhibitors on isoproterenol evoked responses. Their data supported the hypothesis that β_1-adrenoceptors are innervated, and mediate the axonal release of noradrenaline. Russell and Moran (1980) and Hawthorn and Broadly (1982) likewise provided evidence for the innervation of β_1-adrenoceptors.
In pithed rats Wilffert et al (1982[a]) compared effects of intravenous applied adrenaline on noradrenaline and adrenaline release after dimethylphenylpiperazium (DMPP, this substance causes noradrenaline release from the sympathetic nervous system by stimulation of pre-synaptic nicotinic receptors; furthermore, sympathetic ganglia and the adrenal medulla are stimulated). These effects were studied previously

to and after bilateral adrenalectomy. Catecholamine release evoked by DMPP activated α_1- and β_1-adrenoceptors; after high dosages α_2- and β_2-adrenoceptors were stimulated as well. After removal of the adrenals the latter effect was abolished.
Agreement exists in that β_1-adrenoceptors are mainly stimulated by the neurotransmitter noradrenaline, and are located at the post-synaptic site (Ariens and Simonis, 1983; Man in 't Veld and Schalekamp, 1983; Shanks, 1984; Stiles et al, 1984; Schalekamp and Man in 't Veld, 1985; Bolli et al, 1985).

3.2.3 Beta$_1$-adrenoceptor agonists and antagonists (incomplete list):

β_1-agonists:

- prenalterol (predominant β_1-agonist): Meurer et al, 1980; Altiere et al, 1983; Kopp et al, 1981[a]; Man in 't Veld et al, 1981; Staessen et al, 1983; Vincent et al, 1983; Williams et al, 1983.
- dobutamine
- noradrenaline

β_1-antagonists:

- atenolol (without intrinsic sympathetimimetic activity, ISA): Oates et al, 1978; Distler et al, 1978; Lees, 1981; Wilffert et al, 1982[a]; Vincent et al, 1983; Schalekamp and Man in 't Veld, 1985.

- metoprolol (without ISA):
  Weber et al, 1974; Oates et al, 1978; Meurer et al, 1976; Minneman, 1979[c]; Lees, 1981; Man in 't Veld, 1983; Kopp et al, 1984; Schalekamp and Man in 't Veld, 1985.

- practolol (with ISA)
  Lees, 1981; Man in 't Veld and Schalekamp, 1984; Wilffert et al, 1984.

3.2.4 Localization and function of β_2-adrenoceptors.

Heart:
In heart tissue β_2-adrenoceptors have been demonstrated; frog hearts even contain predominantly β_2-adrenoceptors in contrast to rabbit, guinea pig and rat heart (Ariens and Simonis, 1983). After stimulation of coronary β_2-adrenoceptors vasodilatation occurs (Shanks, 1984).

Brain:
Beta$_2$-adrenoceptors have been demonstrated in several brain regions, the regional absolute concentrations varying to a lesser degree (up to three fold) than β_1-adrenoceptors (Minneman, 1979[a]).

Muscle:
In smooth muscle (uterus) relaxation mediated by β_2-adrenoceptors has been described (Lees, 1981; Wilffert et al, 1984).
Skeletal muscle also encompasses β_2-adrenoceptors. In quickly contracting muscle increased force and duration of contraction is observed after stimulation of β_2-adrenoceptors, in slow contracting muscle decreased force and duration of contraction has been observed (Lees, 1981).
Furthermore, in skeletal muscle β_2-adrenoceptors promote glycogenolysis. In blood vessels of this tissue vasodilatation occurs after β_2-adrenoceptor stimulation.

Lungs:
In trachea and bronchi predominantly β_2-adrenoceptors are recognized. These mediate bronchodilatation (Lees, 1981; Wilffert et al, 1984; Shanks, 1984). In human beings the existence of a homologous β_2-adrenoceptor population has been established (Ariens and Simonis, 1983).

Adipocytes:
Beta$_2$-adrenoceptors are recognized at adipocytes, after activation of these receptors lipolysis is stimulated (Motulsky and Insel, 1982).

Kidney:
This subject will be dicussed in Chapter 3.4.

Adrenergic nerve endings:

Tissues without sympathetic innervation contain almost exclusively β_2-adrenoceptors. By definition β_2-adrenoceptors on granulocytes and lymphocytes are located extrasynaptically. This applies also to striated and uterine smooth muscle (Ariens and Simonis, 1983). Bryan et al (1981) studied rat, guinea pig and cat atria. They presumed that β_2-adrenoceptors are hormonal receptors which are stimulated by circulating adrenaline. Hawthorn and Broadly (1982) also concluded to the existence of a non-innervated β_2-adrenoceptor in guinea pig cardiac and pulmonary preparations.
In pithed rats Wilffert et al (1982[a]) studied effects evoked by intravenously administered adrenaline or DMPP on noradrenaline and adrenaline release previously to and after bilateral adrenalectomy. In this study selective α_1-, α_2-, β_1- and β_2-antagonists were used. Catecholamines released after DMPP stimulated α_1- and β_1-adrenoceptors. At high doses β_2-adrenoceptors were also activated. These effects disappeared after removal of the adrenals. They concluded that the predominant natural stimulant for the β_2-adrenoceptor is adrenaline. This is in keeping with the view that β_2-adrenoceptors are not innervated and probably located extrasynaptically.

Most authors indeed seem to agree that the β_2-adrenoceptor is preponderantly exposed to the circulating hormone adrenaline and is not innervated (Ariens and Simonis, 1983; Man in 't Veld et al, 1983; Stiles and Lefkowitz, 1984; Shanks, 1984; Bolli et al, 1985; Schalekamp and Man in 't Veld, 1985), though in heart tissue this receptor type seems to be activated by neuronally released noradrenaline (Wilffert et al 1982[b]; Ariens and Simonis, 1983). A postsynaptic localization has been established, but, in addition a presynaptic localization is likely, where the receptor facilitates neuronal release of noradrenaline (Lees, 1981; Shepherd and Van Houtte, 1981; Ariens and Simonis, 1983; Lefkowitz et al, 1984; Davis et al, 1984; Schalekamp and Man in 't Veld, 1985).
This proposition was formulated after the following studies.
In 1975 Adler-Graskinsky and Langer established after isoprenaline infusion an enhanced neurotransmitter release as elicited by electrical stimulation. Stjärne and Brundin (1976) studied effects of field stimulation on ^{3}H-noradrenaline release in human omental blood vessels. Release was unaffected by a β_1-agonist, but increased after previous administration of β_2-agonists. Furthermore, the effects of isoprenaline on noradrenaline release were blunted or abolished by β_2-antagonists. In human omental arteries and veins increases in fractional secretion per shock of radioactive noradrenaline were obtained after adrenaline administration. At dosages of adrenaline exceeding 0.04 micromol, however, noradrenaline release was inhibited. The authors proposed a dual mediated control of noradrenaline release (Stjärne and Brundin, 1975).
In 1983 Vincent et al described plasma noradrenaline elevations after salbutamol and isoprenaline infusion, which were prevented by propranolol but not by atenolol. Furthermore, isometric exercise increased noradrenaline levels to a greater degree during adrenaline infusion than during control infusion. In another experiment, concomitant prenalterol appeared not to elevate plasma noradrenaline levels. These data are in favour of a facilitatory release mechanism via prejunctional β_2-adrenoceptors.

Anden (1964) presumed that circulating adrenaline may be taken up via an active process in the sympathetic nerve endings, following

which adrenaline would be co-released (with noradrenaline) during nerve stimulation. Neuronally released adrenaline may stimulate presynaptic β_2-adrenoceptors, which mediate a positive feedback system. This hypothesis was studied by Majewski et al (1980). In experiments in guinea pig atria, they incubated labelled noradrenaline and adrenaline. After field stimulation (5 Hz during 20 seconds) adrenaline and noradrenaline were released in equal amount.
Rand and Majewki (1984) supposed that facilitory effects of adrenaline on noradrenaline release are prolonged and reinforced when adrenaline is stored in the sympathetic nerve endings and thereafter released as co-transmitter. This mechanism may have a role in stress-induced hypertension. In 1986 Majewski et al published data which support this assumption. Adrenal medullectomized and stressed rats did not develop hypertension in contrast with stressed control rats. Propranolol and desipramine prevented stress-induced hypertension. Adrenaline levels in cardiac tissue were significantly higher in stress induced hypertensive rats in comparison with adrenal medullectomized rats. In rats treated with desipramine and propranolol hypertension was prevented and cardiac adrenaline levels were not elevated. Unfortunately, Eikenburgh and Schwartz (1986) could not confirm these data.

Except for the fact that adrenaline is related to a positive feedback mechanism, it is assumed that adrenaline per se acts as an α-agonist. Indeed, in processes in the synaptic cleft dynamic interactions occur between uptake processes, facilitatory release via β_2-adrenoceptors and (at higher adrenaline and noradrenaline concentrations) inhibitory actions via presynaptic α_2-adrenoceptors.
Majewski et al (1985) described the latter mechanism. In pithed rabbits adrenaline (0.06 microgram/kg/min) reduced sympathetic outflow, which was reversed by phenoxybenzamine, which eliminates α_2-adrenoceptor mediated effects on noradrenaline release.
Propranolol inhibited noradrenaline release slightly; this effect was more pronounced after pretreatment with both propranolol and desipramine. In rabbits pretreated with captopril and desipramine, propranolol did not affect noradrenaline release. The authors speculated on an inhibitory action on noradrenaline release evoked by propranolol via

blockade of presynaptic β_2-adrenoceptors. On the other hand a suppressive effect on renin and angiotensin II formation may also have a role, since angiotensin promotes noradrenaline release via presynaptic angiotensin II receptors.
To complicate the interpretation further, phenoxybenzamine inhibits uptake processes, which could have influenced the results.
In man, Goldstein et al (1986) described elevated plasma noradrenaline levels after isoprenaline infusion. Presumably this effect is mediated via presynaptic β_2-adrenoceptors. Plasma noradrenaline levels increased in a dose related manner with isoprenaline infusion, whereas no alterations in plasma adrenaline levels were obtained. The authors, therefore, provided neither evidence for adrenaline being co-released with noradrenaline from the sympathetic nerve endings nor for secretion from the adrenal medulla. Some years earlier Yamaguchi et al (1977) found comparable data.
Gutman et al (1979) suggested that β_2-adrenoceptors mediate adrenaline release from the adrenal medulla.
However, it should be taken into account that clearance of neurotransmitters is enhanced by theirselves, whilst β-blocking agents reduce clearance (Cryer, 1980; Clutter et al, 1980; Man in 't Veld and Schalekamp, 1984; Ziegler et al, 1986). This mechanism may be relevant to the interpretation of the results mentioned above.

To conclude: evidence is presented regarding the existence of an extrasynaptic β_2-adrenoceptor and a presynaptic β_2-adrenoceptor. The latter mediates a positive feedback-mechanism (see also Schmidt et al, 1984; Davies et al, 1984).
$Beta_2$-adrenoceptors are predominantly activated by adrenaline. It is suggested that adrenaline is co-released with noradrenaline from the sympathetic nerve endings after uptake from the extracellular fluid and/or β_2-adrenoceptors modulate adrenaline release from the adrenal medulla.

3.2.5 Beta$_2$-agonist and antagonists (incomplete list).

β_2-agonist:

- salbutamol ("selective" β_2-agonist): Stjärne et al, 1976; Salvetti et al, 1978; Minneman et al, 1979[a]; Altiere et al, 1981; Lees, 1981; Nakane et al, 1982; Man in 't Veld et al, 1983; Vincent et al, 1983.

β_2-antagonists:

- butoxamine ("selective" β_2-antagonist): Oates et al, 1978; Minneman, 1979[a,c]; Osborn et al, 1981; Lees, 1981; Schalekamp and Man in 't Veld, 1985.

- ICI 118.551 ("selective" β_2-antagonist): Bilski et al, 1980; Johns et al, 1981; Wilffert et al, 1982[b], 1983; Brown et al, 1983; Lefkowitz et al, 1984; Schmidt et al, 1984; Lees et al, 1985; Schalekamp and Man in 't Veld, 1985; Man in 't Veld et al, 1986.

3.3 Alpha-receptors.

3.3.1 Introduction:

In 1974 Langer proposed a subclassification of alpha-receptors in a presynaptic α_2-adrenoceptor and a postsynaptic α_1-adrenoceptor. On the basis of a rank order for potencies of different agonists and antagonists, they were, independently of their localization, divided in α_1- and α_2-receptors (Berthelsen and Pettinger, 1977; Wikberg, 1979).

Recently some investigators proposed the existence of a gamma-

receptor. This hypothetical new receptor type would be localized at the postsynaptic membrane (Hirst, 1980; Langer and Hicks, 1984; Bevan et al, 1985). The existence of such a receptor is, however, not uniformly accepted.

3.3.2 Types of α-adrenoceptors.

Originally, two subpopulations of alpha adrenergic receptors were assumed to exist with different localization (presynaptic-α_2 and postsynaptic-α_1). Later on evidence was presented as to the existence of a postsynaptic α_1-adrenoceptor and a pre- and postsynaptic α_2-adrenoceptor (Timmermans et al, 1980[a]; Timmermans and Van Zwieten, 1980[a]; Langer et al, 1980[a]; Timmermans and Van Zwieten, 1981[a]; Ruffolo et al, 1981; Hamilton and Reid, 1982; Ariens and Simonis, 1983; Shoji et al, 1983; Jie et al, 1984).
Recently Docherty et al (1984) and Story et al (1985) described a presynaptic α_1-adrenoceptor in rat vas deferens and heart tissue. This prejunctional α_1-adrenoceptor appears to represent a small proportion of the total presynaptic α-adrenoceptor population, mediating a negative feedback on noradrenaline release from the sympathetic nerve endings.

In addition, Drew (1985) proposed the existence of even more postsynaptic alpha-adrenoceptors after comparison of binding characteristics for prazosin and yohimbine against those for noradrenaline in isolated tissues of various species. These data are in agreement with the observations by Ruffolo et al (1982, 1985), in experiments performed in aortas from various mammmalian species. Beckeringh et al (1984) described differences in architecture between α_1-adrenoceptors of rat and guinea pig aorta. Thus, rat α-adrenoceptors are possibly different from rabbit, guinea pig and human α-adrenoceptors in their response to agonists and antagonists (McGrath and Reid, 1985). In addition, differences in potency of antagonists for various tissues have been noticed (Waterfall et al, 1985) and interindividual variations (in digital blood vessels) have also been recorded (Stevens and Moulds, 1985).

With respect to affinity and efficiency several reports have dealt with discrepancies between affinity and relative efficiency of imidazole derivatives. This resulted in diverging interpretations of the receptor type involved in various species (Ruffolo et al, 1979; Timmermans et al, 1980[c]). In addition, Langer et al (1985) mentioned differences at the recognition sites of α_2-receptors with regard to imidazolidines and phenylethylamines.

In most tissues a mixed population of α_1- and α_2-adrenoceptors is present, but marked species differences are assumed, and regional differences in distribution have been observed (de Mey and Van Houtte, 1981; Horn et al, 1982; Shoji et al, 1983; Langer and Hicks, (1984); Bevan et al (1985) described a variation of receptor density and sensitivity with vascular diameter.

In an attempt to estimate the distribution and function of α-adrenoceptors several agonists and antagonists have been developed. Responses are dependent on numerous factors which one should bear in mind, and these factors may have considerable influence on results. A survey of possible confounding factors is listed below.

- species (Ruffolo et al 1982; Beckeringh et al, 1984; Langer and Hicks, 1984; Drew, 1985).
- vascular diameter (Langer and Hicks, 1984, Bevan et al, 1985).
- arterial or venous vessels (De Mey and Van Houtte, 1981; Shoji, 1983).
- number of receptor subtypes (spare receptors; Ruffolo and Yaden, 1984; Hamilton et al, 1985[b]).
- affinity and relative efficiency of agonists, affinity of antagonists, sequence of administration of pharmaca and stereospecificity of pharmaca (McGrath and Reid, 1985; Langer et al, 1985; Grant et al, 1985).
- concentration of pharmaca which are studied.
- co-existence of receptortypes (e.g. β-receptors, angiotensine II-receptors) (Langer and Hicks, 1984; Strandhoy, 1985; Hamilton et al, 1986).

- respective number of receptors in high or low affinity state (Hoffman and Lefkowitz, 1980[a,b]; Hoffman et al, 1982).
- involvement (possibly) of endothelium derived relaxant factor (McGrath and Reid, 1985; Strandhoy, 1985; Godfraind et al, 1985).
- temperature, pH, pO_2, pCO_2 (Shepherd and Van Houtte, 1981; O'Brien et al, 1985; Van Houtte et al, 1985).
- frequency and duration of nerve stimulation (Rand et al, 1982; Hamilton et al, 1986).
- presence or absence of corticosteroids, calcium, angiotensine II and several other factors in in vitro experiments (Lees, 1981; Starke and Docherty, 1982; Schumann and Lues, 1983; Strandhoy, 1985).
- absence or presence of concomitant neuronal blockade (Iversen, 1973; Rand et al, 1982; Angus et al, 1984).

3.3.3 The alpha$_1$-adrenoceptor.

This receptor is considered to be localized mainly at the postsynaptic site of the junctional cleft, though also a presynaptic location has been presumed (Docherty, 1984; Story et al, 1985).
Functional existence of α_1-adrenoceptors, which mediate vasoconstriction, was established in the mesenteric, splenic, renal and skeletal muscle vascular beds of pithed rats (Hicks and Waldron, 1983), smooth muscle of rabbit pulmonary artery and aorta (Starke and Docherty, 1982), vascular beds of pithed rats (Timmermans et al, 1980[b]; Yamaguchi et al, 1980), rabbits (Hamilton and Reid, 1982) and dogs (Constantine et al, 1980). Furthermore α_1-adrenoceptors were demonstrated in femoral hindlimb vasculature of dogs, cats, rabbits and rats (Langer and Hicks, 1984), in fore-arm vasculature of man (Jie et al, 1984) and in the renal circulation of man (de Leeuw et al, 1986). In rat aorta, which is lacking sympathetic innervation, α_1-adrenoceptors have not been found (Ruffolo et al, 1981), although sometimes different characteristics of α-adrenoceptors in aortas of several species was assumed (Beckeringh et al, 1984). As far as the venous system is concerned, alpha$_1$-adrenoceptors have been recognized in rabbit portal vein (Starke and Docherty, 1982), rabbit

and canine saphenous veins (Langer and Hicks, 1984; Sullivan and Drew, 1980) and canine renal veins (Shoji et al 1983).

3.3.4 Localization (and function) of α_1-adrenoceptors:

Heart:
Müntz et al (1985) demonstrated in autoradiographic studies with ^{3}H-prazosin the presence of α_1-adrenoceptors in cardiac myocytes in both ventricles of rat hearts.
In heart tissue α_1-adrenoceptors mediate the inotropic action of several agonists (Schumann, 1983; Hamilton et al, 1986). Furthermore, chronotropic responses may be under the influence of these receptors (Flavahan and McGrath, 1981). Schumann (1983) found that the principal stimulants to the cardiac α_1-adrenoceptor are adrenaline and dopamine. In addition, the cardiac α_1-adrenoceptor is implicated in arrhythmogenesis (Hamilton et al, 1986), as has been demonstrated in experiments with myocardial reperfusion.

Brain:
Using radioligandbinding techniques U'Prichard and Snyder (1979) were able to describe α_1-adrenoceptors in rat and calf brain, though they used a weakly α_1-selective radioligand (Motulsky and Insel, 1982). Abrams (1984) described α_1-adrenoceptors in rat brain. In normotensive WKY-rats this receptor type number was more numerous compared with SHR-rats.
The function of α_1-adrenoceptors in the brain is controversial (Hamilton et al, 1986); possibly stimulation is mediated via this receptor (Langer et al, 1985); α_1-adrenoceptors may be involved in baroreceptor regulation (Van Zwieten, 1986).
Liver:
In the liver α_1-adrenoceptors are involved in activation of glycogen phosphorylase (Hoffman and Lefkowitz, 1982).

Kidney:
This will be discussed in Chapter 3.5.

Adrenergic nerve endings: Pre- and postsynaptic localization.

Peripheral α_1-adrenoceptors are located on the postsynaptic or postjunctional membrane (Langer, 1974; Berthelsen and Pettinger, 1977; Timmermans et al, 1980[a], 1980[b]; Lees, 1981; Schalekamp and Man in 't Veld, 1985), where they are apparently located near the adrenergic nerve endings (Langer et al, 1980[a], 1980[b]; Yamaguchi and Kopin, 1980). Therefore, they are believed to be exposed to a high concentration of noradrenaline, and a preferential noradrenergic innervation of this receptor subtype is assumed.

Zukowska-Grojec et al (1983) performed experiments in rats. After electrical stimulation (endogenously) released noradrenaline acted predominantly at the α_1-adrenoceptors which are situated within the synaptic cleft, whereas injected noradrenaline acted preferentially at the α_2-adrenoceptor. Wilffert et al (1982[a]) obtained similar results after experiments in pithed normotensive Wistar rats. Comparable differences in the effectivity of antagonists in antagonizing responses of neuronally released noradrenaline and exogenously provided noradrenaline were observed by Langer et al (1980[b]) and Yamaguchi and Kopin (1980). Langer et al (1980[b]) demonstrated in the hindlimb of the dog that prazosin inhibited markedly vasoconstriction after lumbar sympathetic stimulation, whereas responses to injected noradrenaline were unaffected. Otherwise, Osborn et al (1983) described a more efficient antagonistic effect of prazosin both after renal nerve stimulation and exogenously applied noradrenaline in comparison with yohimbine.

Elsner et al (1984) studied in anaesthetized, despinalized dogs (and after β-blockade) the effects of intra-arterial noradrenaline and lumbal sympathetic stimulation (0.1-0.3 Hz) on vasoconstrictory responses. Attenuation of such responses was as great after prazosin as after rauwolscine administration. Flavahan et al (1984) described (in canine saphenous veins) that contractile responses evoked by tyramine (an indirect sympathomimetic drug) were inhibited to a greater extent by rauwolscine than prazosin. This argues against a preferential noradrenergic innervation of α_1-adrenoceptors. Although Constantine et al (1982) did not demonstrate functional α_1-adrenoceptors in canine saphenous veins, others (Sullivan and Drew, 1980; De Mey and Van

Houtte, 1981; Shoji et al, 1983) described a mixed population of α-adrenoceptors in saphenous vein.

Langer and Shepperzon (1982) established (in the perfused cat spleen) that pressor responses evoked by nerve stimulation and phenylephrine were effectively antagonized by prazosin when compared to the antagonistic effect of prazosin on exogenously applied noradrenaline. In the presence of cocaine pressor responses were equally blocked by prazosin. Thus, during blockade of neuronal uptake, exogenously provided noradrenaline may reach the α_1-adrenoceptor from outside the synaptic cleft. They concluded that this is in favour of preferential noradrenergic innervation of α_1-adrenoceptors, which are located intrasynaptically, and that α_2-adrenoceptors have a predominant extrasynaptic localization.
This view is supported by Hirst and Neild (1981). They obtained, at portions close to those sympathetic nerves which innervated arterioles, depolarisation of the membranes. Most arterioles receive noradrenergic innervation in the neighbourhood of the adventitial surface. Therefore, it is possible that α_1-adrenoceptors predominate in the adventitial medial layer where the highest density of noradrenergic nerve terminals is found, whilst α_2-adrenoceptors can more comfortably be situated near the intima in the neighbourhood of circulating catecholamines (Langer and Shepperzon, 1982; Langer and Hicks, 1984; Langer et al, 1985).
This assumption might be a reasonable explanation for the observations that exogenously applied noradrenaline may only reach a sufficient gradient in the synaptic cleft in the presence of neuronal uptake inhibitors, in order to stimulate the α_1-adrenoceptor. Only under this restriction it favours a intrasynaptic location of the α_1-adrenoceptor. According to Docherty and McGrath(1980) and Starke and Docherty (1982) the actual difference is less clear than that. Wilffert et al (1983) described an intrasynaptic location of both α-adrenoceptor subtypes, but the α_2-adrenoceptor is also located extrasynaptically.

Besides a postsynaptic location of α_1-adrenoceptors Docherty et al (1984) recently proposed a presynaptically located α_1-adrenoceptor in

rat vas deferens. Story et al (1985) demonstrated in rat atria in vitro after electric stimulation (with trains of 4,8 and 16 pulses, 2Hz), an increased efflux of radiolabelled noradrenaline. Prazosin and idazoxan (α_2-antagonist) were equally effective, but phentolamine augmented noradrenaline efflux to a greater extent. Similar enhancement of nerve stimulated noradrenaline efflux was observed in experiments with simultaneous administration of prazosin and idazoxan, methoxamine diminished nerve stimulated noradrenaline efflux at a concentration of 10 micromol/l, which effect was antagonized by prazosin.
These experiments provide arguments in favour of an α_1-(and α_2)-adrenoceptor mediated negative feedback mechanism on noradrenaline release.
To conclude: the concept that α_1-adrenoceptors are innervated and located intrasynaptically appears to be justified, though they may be localized at a presynaptic site as well.

3.3.5 Natural stimulus on α_1-adrenoceptors.

The relative affinity of the natural agonists noradrenaline and adrenaline to α_1-adrenoceptors has been the subject of many investigations. It has become clear that noradrenaline released from the sympathetic nerve endings and the adrenomedullary hormone adrenaline do possess little selectivity to either α_1- or α_2-adrenoceptor (Berthelsen and Pettinger, 1977; Lees, 1981; Heinsimer and Lefkowitz, 1982; Ariens and Simonis, 1983; Lefkowitz et al, 1984; Langer et al, 1985). Noradrenaline appears to be the principal stimulant of α_1-adrenoceptors.

3.3.6 Alpha$_1$-adrenoceptor agonists and antagonists.

The subclassification of α-adrenoceptors was based on the relative order of selectivity of agonists and antagonists for these receptors. A general opinion on the various agonists and antagonists is difficult in view of the great variability of responses from one tissue to the other, and species differences. In addition, many of the agonists and antagonists display a varying degree of selectivity. Furthermore, the co-existence of other receptor types, anoxia, pH, etc. may influence

the conclusions. Nevertheless, the next (incomplete) list of α_1-adrenoceptor agonists and antagonists can be made:

α_1-agonists:

- phenylephrine (α_1 selective agonist): Timmermans et al, 1980[b]; Sullivan and Drew, 1980; Van Meel et al, 1980, 1981; Lees, 1981; Ruffolo et al, 1981; Wolff et al, 1984; Flavahan et al, 1984; Reid, 1984; Langer et al, 1985.

- methoxamine (α_1-selective agonist): Sullivan and Drew, 1980; Lees, 1981; Kobinger et al, 1981; Horn et al, 1982; Reid, 1984; Jie et al, 1984; Flavahan et al, 1984; Schalekamp and Man in 't Veld, 1985.

- cirazoline (α_1-selective agonist): Van Meel et al, 1980, 1981; Flavahan et al, 1984; Beckeringh et al, 1984; Langer et al, 1985; Timmermans et al, 1985; Wolff et al, 1985.

α_1-antagonists:

- phenoxybenzamine (non-competitive non-selective α-antagonist): Yamaguchi, 1977; Doxey et al, 1977; Lees, 1981; Constantin et al, 1982; Reid, 1984; Hamilton et al, 1985.

- phentolamine (non-selective α-antagonist): Drew, 1976; Doxey et al, 1977; Timmermans et al, 1980[b]; Lees, 1981; Langer et al, 1985; Story et al, 1985.

- prazosin (α_1-selective antagonist): Timmermans et al, 1980[b]; Langer et al, 1980[a]; Wilffert et al, 1982[a]; Flavahan, 1984; Bolli et al, 1985; Hamilton and Reid, 1981; Hamilton et al, 1985.

- doxazosin (α_1-selective antagonist): Vincent et al, 1983; Jie et al, 1984; Van Brummelen et al, 1983[a], 1985; De Leeuw et al, 1986[a,b].

3.3.7 The α_2-adrenoceptor.

This receptor was originally presumed to be located presynaptically (Langer, 1974). Later on a postsynaptic location of this receptor was established, both by means of a pharmacological approach and by using radioligand binding techniques.

Apparently functional postsynaptic α_2-adrenoceptors were established in various tissues: vascular smooth muscle in the anaesthetized cat (Drew and Whiting, 1979), canine saphenous vein (Shepperzon and Langer, 1981; Flavahan et al, 1984), canine hindlimb (Langer et al, 1980[a,b]), mesenterical and tail vascular bed of the pithed rat (Hicks et al, 1983), isolated canine veins (De Mey and Van Houtte, 1981), vascular smooth muscle of the pithed rat (Timmermans et al, 1980[b]; Yamaguchi et al, 1980; Van Meel et al, 1981[b]; Wilffert et al, 1982[b]), vascular smooth muscle of conscious rabbits (Hamilton and Reid, 1982), rat aorta (Ruffolo et al, 1981), human fore-arm (Van Brummelen et al, 1983[a], 1983[b]; Jie et al, 1984), human vasculature (Goldberg and Robertson, 1984) and human kidney (de Leeuw et al, 1987).

In the majority of studies a homologous population of this receptor type has not been demonstrated. The distribution of α_2-adrenoceptors (and α_1-adrenoceptors) on smooth muscle of the vascular system may vary according to the diameter of the vessel, the organ studied and animal species. For instance, canine saphenous vein appears to possess both α_1- and α_2-adrenoceptors (Constantine et al, 1982;

Sullivan and Drew, 1980; Shepperzon and Langer, 1981; De Mey and Van Houtte, 1981), whilst canine arterial smooth muscle as well as (isolated) rabbit aorta and pulmonary artery only possess α_1-adrenoceptors (De Mey and Van Houtte, 1981; Starke and Docherty, 1982).

With regard to rat aorta, Ruffolo et al (1981) established the presence of α_2-adrenoceptors. Beckeringh et al (1984) provided evidence for heterogenicity in postsynaptic α-adrenoceptors of various species, which was confirmed by Ruffolo et al (1982), Ruffolo (1985) and Drew (1985).

Again, in this field conflicting data might (in part) be explained by differences in relative efficiency and affinity, lack of specificity of pharmaca etc. Radioligands may have some advantages in this regard, although they are not able to discriminate between the relative biological efficiencies of agonists (Lees, 1981; Schalekamp and Man in 't Veld, 1985). Characterization, estimation of affinity and localization of receptors is possible with this technique (Hoffman and Lefkowitz, 1980[a]; Motulsky and Insel, 1982).

3.3.8 Localization and function of α_2-adrenoceptors.

Brain:

U'Prichard and Snyder (1979) and Timmermans et al (1981[b]) described α_2-adrenoceptors in vitro (by using radioligand techniques); after intraventricular administration of 6-OH-dopamine the total number of receptors increased, which is an argument in favour of a postsynaptic localization (U'Prichard and Snyder, 1979) and non-innervation of α_2-receptors. Other explanations might be: few presynaptic α_2-adrenoceptors or upregulation in response to denervation (Timmermans and Van Zwieten, 1981[a]). Nevertheless, in brain, a predominant postsynaptic localization is assumed (Van Zwieten, 1984). Central α_2-adrenoceptors, when stimulated, may enhance inhibition of neuron activity and thereby decrease the peripheral sympathetic tone. In addition, vagally mediated bradycardia is enhanced by central α_2-adrenoceptors (Van Zwieten, 1984; Schmitt et al, 1984; Reid, 1984).

Pancreas:
α_2-adrenoceptor stimulation causes inhibition of insulin-release. Idazoxan stimulates insulin-release (Roach et al, 1985), whilst glucagon-release raises after guanfacine (Brown et al, 1985).

Adenohypophysis:
(extraneuronally localized) α_2-adrenoceptors, when stimulated, evoked growth hormone release (Brown et al, 1985), but in hypertensives α_2-agonists did not elicit growth hormone release (Abrams, 1984).

Fat cells:
α_2-adrenoceptors were identified postsynaptically at fat cells (Tharp et al, 1981; Lafontan and Berlan, 1982), the major stimulant being adrenaline (in comparison with noradrenaline), resulting in inhibition of lipolysis.

Platelets:
α_2-adrenoceptors have been demonstrated on platelets (Markwardt, 1980; Motulsky and Insel, 1982; Hoffman et al, 1982; Motulsky et al, 1983; Wahrenberg et al, 1986).
Adrenaline at physiological concentrations of 10 Nm potentiates platelet aggregation induced by ADP.

Kidney:
This subject will be discussed in a separate chapter 3.5.

Adrenergic nerve endings: Pre- and postsynaptic localization.

The presynaptic and postsynaptic localization of α_2-adrenoceptors has been established as a fact (Langer, 1974; Berthelsen and Pettinger, 1977; Starke, 1977; Timmermans and Van Zwieten, 1981[a]; Hamilton and Reid, 1982; Ariens and Simonis, 1983).

In classical pharmacological experiments prazosin (a selective α_1-antagonist) was more effective in antagonizing the response to stimulation-evoked neuronally released noradrenaline than in antagonizing exogenously applied noradrenaline (Drew and Whiting,

1979; Yamaguchi et al, 1980; Langer et al, 1980[a], 1980[b]; Zukowska-Grojec et al, 1983), whereas α_2-antagonists more effectively antagonized vasoconstriction induced by exogenously applied noradrenaline. After inhibition of neuronal uptake by cocaine the differences disappeared in vitro (Langer and Shepperzon, 1982). Similar results were obtained in pithed rats (Zukowska-Grojec et al, 1983; Langer et al, 1985). It was assumed that neuronal uptake attenuated the degree of vasoconstriction induced by α_1-adrenoceptors when exogenous noradrenaline was applied. After uptake inhibition a larger proportion of exogenous noradrenaline was supposed to reach the α_1-adrenoceptor. This view is consistent with an extrasynaptic localization of this receptor subtype. However, the data of Docherty and McGrath (1980) and Robie (1980) contrast to the observations by Langer and Shepperzon.

Docherty and Hyland (1985) observed (in human saphenous vein) a greater inhibition of stimulation-evoked contractions by yohimbine compared with prazosin.

Starke and Docherty (1982) established α_2-adrenoceptor mediated vasoconstriction after sympathetic stimulation as did Hamilton and Reid (1982) and Hesse and Johns (1984).

Elsner et al (1984) and Flavahan et al (1984) described comparable vasoconstrictory responses after low frequency nerve stimulation or intra-arterially infused noradrenaline. A comparable inhibition of vasoconstriction after prazosin or rauwolscine was observed.

Wilffert et al (1982[a,b]) proposed that postsynaptic α_2-adrenoceptors are located in the proximity of ganglionic muscarinic receptors. In a subsequent study (Wilffert et al, 1983) an intra- and extrasynaptic localization of α_2-adrenoceptors was established.

Langer and Shepperzon (1982), Langer and Hicks (1984) and Langer et al (1985) assumed the α_2-adrenoceptor to be present close to the intima of the vessel wall, in close contact with blood borne substances, whilst α_1-adrenoceptors were located in the richly innervated advential layer. This was not fully supported by Summers et al (1985). They studied in canine kidney slices (using an autoradiographical technique) the localization of α_2-adrenoceptors. This receptor type was apparently associated with both the intimal and the

adventitial surface of bloodvessels.

In conclusion: the postsynaptic α_2-adrenoceptor may be localized extrasynaptically and is probably not innervated (Langer et al, 1980[b]; Timmermans and Van Zwieten, 1981[a]; Wilffert et al, 1982[a]; Langer and Shepperzon, 1982; Ariens and Simonis, 1983; Man in 't Veld et al, 1983; Zukowska-Grojec et al, 1983), though several data do not support this assumption. The proposed anatomical distribution may represent an oversimplification (Lees, 1981) and some authors regard this distinction as to be not helpful in explaining all findings (McGrath and Reid, 1985).

Besides a postsynaptic a presynaptically localized α_2-adrenoceptor is assumed to exist.
In 1972 Enero et al published data on the effects of phenoxybenzamine on noradrenaline overflow in experiments in an isolated cat nictic membrane preparation. Phenoxybenzamine at doses of 10^{-8}-10^{-7} g/ml enhanced noradrenaline-overflow evoked by nerve stimulation at 10Hz, whilst higher concentrations (10^{-6}-10^{-5} g/ml) caused neuronal uptake inhibition. The authors presumed that a presynaptic α-receptor mediated negative feedback mechanism might be involved, in other words, noradrenaline release evoked by nerve stimulation might inhibit further release of neurotransmitter once a threshold-concentration of the transmitter has been reached in the synaptic cleft in the neighbourhood of this presynaptic receptor. Thereafter, many studies have confirmed this hypothesis (Westfall, 1977; Starke, 1977). The importance of this negative feedback-mechanism might be greater in tissues with a relatively narrow synaptic cleft (Shepherd and Van Houtte, 1981).
When high noradrenaline concentrations are present in the synaptic cleft subsequent nerve impulses release less noradrenaline (Stjärne and Brundin, 1975), but after high frequency stimulation apparently presynaptic α_2-adrenoceptors are maximally activated, and, under those circumstances the negative feedback mechanisms is overruled (Westfall, 1977).

Considerable controversy exists with regard to the physiological

significance of this negative feedback-mechanism. (Angus, 1980; Hoffman and Lefkowitz, 1980[a]; Kalsner, 1982; Rand et al, 1982; Schalekamp and Man in 't Veld, 1985; Hamilton et al, 1986).

By the use of pharmacological tools presynaptic α_2-adrenoceptors were established in vivo and in vitro in various organs of different species; in isolated guinea pig atria (Langer et al, 1977), isolated vas deferens of the rat (Doxey et al, 1977; Shepperzon and Langer, 1981), canine saphenous vein (Sullivan and Drew, 1980), pithed rats (Drew, 1976; De Jonge et al, 1983; Baker et al, 1983), conscious rat (Graham and Pettinger, 1979; Graham et al, 1980), pithed and conscious rabbits (Hedler et al, 1983), and canine and rabbit pulmonary artery (Yamaguchi et al, 1977 resp. Starke et al, 1975).

Yamaguchi et al (1977) collected evidence as to both a negative and a positive feedback-mechanism on noradrenaline release after nerve stimulation up to 30 Hz. Noradrenaline release was measured as catecholamine-levels in the coronary sinus in these experiments. In the presence of desmethylimipramine (which blocks neuronal uptake) at stimulation frequencies of 1-4 Hz, clonidine reduced noradrenaline release, phenoxybenzamine and isoproterenol elevated noradrenaline release, whereas sotalol was without effect. In addition, a correlation was established between alterations in noradrenaline release and heart rate. Dubocovich et al (1980) found no apparent correlation, but they studied cirazoline which was later found to act as a α_1-agonist (Van Meel et al, 1981[a]).

In pithed rats Yamaguchi and Kopin (1980), after low doses of α_2-antagonists, observed increments in noradrenaline release at stimulation frequencies of 3 Hz; this was confirmed by Zukowska-Grojec et al (1981), who studied the effects of yohimbine at similar stimulation frequencies.

Low doses of phentolamine enhanced chronotropic responses in guinea pig atria after nerve stimulation, at higher doses non-significant results were obtained, probably because of the blocking properties of this non-selective antagonist (Langer et al, 1977).

De Jonge et al (1983) described an apparently functional feedback-mechanism of presynaptic α_2-adrenoceptors. Nerve stimulation at 0.1-10 Hz in the presence of rauwolscine enhanced heart rate as compared to nerve stimulation without rauwolscine. This feedback-

mechanism was more pronounced in adult and SHR rats than in young and WKY rats. Stjärne and Brundin (1975) observed in human omental artery and vein preparations after low doses of adrenaline a clear stimulation of the fractional secretion per shock of labelled noradrenaline. Higher doses of adrenaline caused a reduction in released noradrenaline. These data were interpreted as an α-adrenoceptor mediated negative feedback-mechanism. Doxey et al (1985) studied the vas deferens of the pithed rat. After neuronal blockade with desipramine, idazoxan potentiated electrically induced contraction; in the absence of neuronal blockade attenuation of this effect occurred. Jie et al (1986) described functional α_2-adrenoceptors in the human forearm. Tyramine stimulated noradrenaline overflow, which was enhanced during yohimbine (dose: 1.0 microgram/kg/min) and unaffected by doxazosin.

On the other hand Kalsner and Chi (1979) observed after neuronal uptake blockade (cocaine) in isolated cattle radial artery strips decrements in stimulation-induced transmitter overflow after yohimbine. Oxymetazoline (an α_2-agonist) inhibited efflux in the radial but not in the renal artery. A feedback-mechanism could not be denied, but results were inconsistent and not reproducible in all tissues tested. This obscured the functional significance. Likewise Robie (1980) failed to identify a physiological significant α_2-adrenoceptor mediated negative feedback-mechanism in canine vascular beds. Bolli et al (1983) obtained results from experiments with clonidine performed in the forearm of normotensives, but could not support an auto-inhibitory feedback mechanism. In conscious rabbits Hamilton at al (1982) observed an increase of blood pressure and noradrenaline overflow in the presence of yohimbine, but in these experiments central effects could not be ruled out.

In view of these data many discrepancies appear to exist. One possible explanation might be found in differences in frequency and duration of nerve stimulation (Rand et al, 1982), whether or not concomitant uptake blockade (Angus et al, 1984) and in tissue and species differences. In addition, concentrations of agonists and

antagonists differ markedly in these studies. Furthermore, baroreceptor reflex mechanisms may obscure some effects (Hamilton et al, 1982).
Despite a clear demonstration of the auto-inhibitory response of presynaptic α_2-adrenoceptors their physiological role is still controversial (Langer and Hicks, 1984; Hamilton et al, 1986).

3.3.9 Natural stimulus on α_2-adrenoceptors.

As mentioned in the previous chapter, the principal stimulant of α_1-adrenoceptors is noradrenaline. The principal natural agonist on presynaptic α_2-adrenoceptors might also be noradrenaline, but controversial data have been obtained. The EC 20% value (agonist-concentration which reduces stimulation-evoked overflow by 20%) in rabbit pulmonary artery for adrenaline was 1.9×10^{-9} M and 1.2×10^{-8} M for noradrenaline.
Abrams (1984) supposed that α_2-adrenoceptors in brainstem and hypothalamus are more responsive to adrenaline than to noradrenaline.
Wilffert et al (1982[a]) observed only α_2-adrenoceptor-mediated pressor effects after high doses of DMPP with the adrenals in situ.

Lafontan et al (1982) described that adrenaline is the major stimulant on α_2-adrenoceptors in isolated human fat cells; this confirmed data by Tharp et al (1981) in adipocyte membrane preparations. The intrinsic activity of adrenaline, when measured as the inhibition of adenylate-cyclase activity in platelets, is higher than that of noradrenaline (Hoffman et al, 1982). In addition, near physiological concentrations of adrenaline potentiated ADP stimulated aggregation. This might serve as an argument in favour of adrenaline being the major stimulant of α_2-adrenoceptors (Clutter et al, 1980; Motulsky and Insel, 1982).

In the human fore-arm Bolli et al (1985) performed the following experiments. With simultaneous α_1-adrenoceptor blockade (prazosin) and β-adrenoceptor blockade (propranolol) adrenaline caused a decrease in fore-arm bloodflow. Since yohimbine prevented this vasoconstriction, the investigators proposed an α_2-adrenoceptor-mediated

vasoconstriction. Van Brummelen et al (1985) found a similar degree of vasoconstriction due to infusion of noradrenaline and to adrenaline in human fore-arm, and this was equally attenuated by yohimbine and doxazosin. Both studies are in favour of the view that adrenaline is at least capable of stimulating postsynaptic α_2-adrenoceptors, though Grant et al (1985) and McGrath and Reid (1985) mentioned that results might have been different when both antagonists would have been given in different sequences. Majewki et al (1985) demonstrated that adrenaline can be taken up into the adrenergic nerve terminals (in pithed rabbits) and after nerve stimulation adrenaline may be (co-)released with noradrenaline and may activate presynaptic α_2-adrenoceptors (Majewski et al, 1984).
Ariens and Simonis (1983) proposed that regulation of (presynaptic) noradrenaline-release is controlled both by noradrenaline itself and by adrenaline. Similar propositions were offered by Bolli et al (1984), Schalekamp and Man in 't Veld (1985) and Langer et al (1985).

In conclusion: both noradrenaline and adrenaline can stimulate α_2-adrenoceptors; in some tissues or cell types adrenaline acts as a pure agonist at the α_2-adrenoceptor with a higher measured intrinsic activity than noradrenaline.

3.3.10 Alpha$_2$-adrenoceptor-agonists and antagonists.

The following (incomplete) list can be composed with regard to α_2-agonists and antagonists.

α_2-agonists:

- guanabenz (α_2-"selective" agonist): Langer et al, 1980; Hamilton et al, 1982; Langer et al, 1982; Hesse and Johns, 1985; Wolff et al, 1984; Gutkind and Enero, 1986.

- guanfacin (α_2-"selective" agonist):
  Man in 't Veld et al, 1983; Jie et al, 1984; Flavahan et al, 1984; Godfraind et al, 1985.

- clonidine (α_2-agonist, partial α_1-agonist):
  Sullivan and Drew, 1980; Van Meel et al, 1981; Hamilton et al, 1982; Hoffman et al, 1982; Man in 't Veld et al, 1983; Beckeringh et al, 1984; Godfraind et al, 1985; Hamilton et al, 1985.

- B HT 920 (α_2-"selective" agonist):
  Van Meel et al, 1980; Van Meel et al, 1981[a]; Kobinger et al, 1981; Flavahan et al, 1984; Van Zwieten, 1984; Timmermans et al, 1985.

- B HT 933 (α_2-"selective" agonist):
  Timmermans et al, 1980[b]; Van Meel et al, 1981[a]; Van Zwieten, 1984; Jie et al, 1984; Timmermans et al, 1985; Waterfall, 1986.

- UK 14303 (α_2-"selective" agonist):
  Van Meel et al, 1981[a]; Flavahan et al, 1984; Doxey et al, 1985; Thom et al, 1985; Timmermans et al, 1985; Godfraind et al, 1985; Hesse and Johns, 1985.

α_2-antagonists:

- rauwolscine (α_2-"selective" antagonist):
  Timmermans et al, 1980[b]; Shepperzon et al, 1981; Flavahan et al, 1984; Van Zwieten, 1984; Elsner et al, 1984.

- yohimbine (α_2-"selective" antagonist): Timmermans et al, 1980[b]; Hamilton and Reid, 1982; Hamilton et al, 1983; Goldberg, 1984; Waterfall, 1985; Drew, 1985; Van Brummelen et al, 1985; Bolli et al, 1985.

- idazoxan (α_2-"selective" antagonist): Langer and Hicks, 1984; Roach et al, 1985; Doxey et al, 1985; Story et al, 1985.

Table 3.3.1

Rank order of selectivity of $\alpha_{1,2}$-agonists and antagonists (according to Timmermans et al, 1980[b]; Langer et al, 1985).

Agonists:	Antagonists
α_1	α_1
methoxamine	prazosin
cirazoline	doxazosin
phenylephrine	
norepinephrine	phenoxybenzamine
epinephrine	phentolamine
α-CH_3 norepinephrine	
dopamine	
clonidine	
B HT 920	yohimbine
B HT 933	rauwolscine
guanafacine	idazoxan
guanabenz	
UK 14304	
α_2	α_2

3.4 Beta-adrenoceptors in the kidney

3.4.1 Distribution of beta-adrenoceptors in the kidney.

Classical pharmacological methods, radioligand binding techniques and autoradiography have been used to identify β-adrenoceptors.
Summers (1985) demonstrated in canine kidneys β_1-adrenoceptors associated with the juxtaglomerular apparatus. High concentrations of β_2-adrenoceptors were demonstrated in cortical sections. In this study, as in experiments by Münzel et al (1984), radioligands and autoradiographical techniques were used. Münzel et al (1984) studied rat kidney slices. Their preparations were pretreated with ascorbic acid (in order to prevent oxidation of catecholamines) and phentolamine, which diminishes non-specific trapping of the radioligand. Competition curves with adrenaline and noradrenaline demonstrated equipotency in competing for radioligand (^{125}I-iodopindolol) binding sites; therefore, the β-adrenoceptor in this preparation appears to be predominantly of the β_1-adrenoceptor type. Autoradiography showed pronounced binding in cortex and outer medulla, more specifically in glomeruli and thick ascending limbs of Henle's loop and other tubular structures. There was no obvious association with vascular elements. Morel (1981) studied microdissected rat tubules and described an identical localization of β_1-adrenoceptors. A distinct localization was established in rabbits. Gavendo et al (1980) supported these findings as did Summers et al (1983). Struyker Boudier et al (1986) identified in rat kidney relatively high amounts of β-adrenoceptors along the distal part of the tubulus and glomeruli. In guinea pig kidney β-adrenoceptors were mainly associated with the proximal part of tubules; as in the previous studies β-adrenoceptors were not found to be associated with bloodvessels.
Snavely et al (1982[a]) performed studies with radioligands in rat cortical membranes. They demonstrated the existence of both β-adrenoceptor subtypes, β_2-adrenoceptors representing 30% of the total β-adrenoceptor population. After chemical sympathectomy no alterations in receptor number were found which is an argument in favour of β-adrenoceptors being localized mainly postsynaptically.

To conclude: the kidney predominantly contains β_1-adrenoceptors, which are probably associated with tubules. β_2-adrenoceptors represent 30% of total β-adrenoceptor population and these receptors are presumed to be localized at the postsynaptic site.

3.4.2 Functions of beta-adrenoceptors in the kidney.

These can be summarized as follows:

1. modulation of salt and water reabsorption.

2. modulation of vascular resistance with alterations in glomerular filtration rate and renal blood flow.

3. modulation of renin secretion.

3.4.2.1 Modulation of vascular resistance and salt and water reabsorption.

Johnson et al (1976), after intrarenal infusion of 0.1. microgram kg^{-1} min^{-1} isoproterenol in normal dogs, observed increments in $U_{Na}V$ and RBF with simultaneous slight (but significant) decrements in mean arterial pressure. Propranolol infusion (0.05 $mg/kg^{-1}/hour^{-1}$) into the renal artery of sodium depleted dogs raised RBF without concomitant alterations in sodium excretion (in these experiments again a slight, but significantly lower MAP was established). During intravenous isoproterenol infusion (0.1 microgram kg^{-1} min^{-1}) sodium excretion decreased, like MAP, but RBF increased. In subsequent experiments, at a dose of 0.018 microgram kg^{-1} min^{-1} isoproterenol, no haemodynamic effects were observed. Seymour et al (1981) studied conscious sodium depleted dogs. After i.v. isoproterenol administration (0.4 microgram kg^{-1} min^{-1}) GFR and sodium excretion decreased significantly (renal plasma flow fell not significantly), whereas MAP remained unaltered. After 0.2 microgram kg^{-1} min^{-1} isoproterenol i.v. no haemodynamic alterations were documented.
Osborn et al (1981) also published on experiments in dogs. Isoproterenol (2 microgram infused into the renal artery) increased RBF,

this response was abolished by propranolol and moderately reduced by atenolol. Butoxamine (a selective β_2-antagonist) caused a significant reduction in RBF.
Isoproterenol infusion (doses 0.006 microgram/min and 0.03 nmol/min) into the isolated perfused rat kidney did not elevate RBF in comparison with control experiments (Vandongen, 1973; Strang, 1978).
Kopp et al (1981[a]) observed in dogs after i.v. administration of 20 microgram kg^{-1} prenalterol elevated heart rate and decreased urinary flow, whereas GFR, RBF and sodium excretion were not influenced. After denervation no alterations in these variables were observed.
Thames and Di Bona (1979), Holdaas et al (1981) and Osborn et al (1981, 1982[a], 1982[b], 1984) during renal nerve stimulation at frequencies between 0,25 and 0.7 Hz found no significant alterations in renal haemodynamics and sodium excretion. These experiments were performed at normal perfusion pressure in dogs. At a stimulation frequency of 1.0 Hz sodium excretion diminished, whereas RBF and GFR were unaffected. This response was abolished during phentolamine and prazosin infusion whilst rauwolscine, yohimbine and atenolol infusion did not induce sodium excretion (Osborn, 1983).

To conclude: these data indicate that intrarenally infused isoproterenol initiated vasodilatation in most experiments. Since propranolol and butoxamine reduced isoproterenol-induced renal vasodilatation a β_2-adrenoceptor mediated effect is likely. In absence of haemodynamic alterations sodium excretion is unaltered by β-agonists. After renal nerve stimulation without affecting RBF or GFR antinatriuresis is mediated by α_1-adrenoceptors and is not dependent on β-adrenoceptors.

3.4.2.2 Modulation of renin secretion.

Isoproterenol in kidney slices stimulated renin secretion (Nolly et al, 1974; Weinberger et al, 1975; Capponi and Valloton, 1976; Desaulles et al, 1978; Richards, 1981; Naftilan and Oparil, 1982). In glomeruli

isolated from rat kidney, incubated in a medium containing isoprenaline, adrenaline and noradrenaline, enhanced renin release was recorded (Morris et al, 1976). Adrenaline, noradrenaline, salbutamol and isoprenaline stimulated renin release in isolated renal cortical cells. Salbutamol appeared to be as about one third as potent as isoproterenol (Johns et al, 1975). On the other hand, Wilton and Joshi (1977) obtained no stimulatory effect of salbutamol on renin release (doses were not mentioned).

Vandongen et al (1973) found elevated renin concentrations in effluent fluid from the isolated perfused rat kidneys after isoproterenol infusion. This effect was attenuated by DL-propanolol but unaffected by phenoxybenzamine. A subsequent study (Vandongen, 1974) revealed that the isoproterenol-stimulated renin secretion was blunted by simultaneous methoxamine infusion. In these experiments perfusion pressure rose markedly. Isoproterenol-induced stimulatory effects on renin release were supported by several other papers (Johnson, 1976; Vandongen and Greenwood, 1975; Vandongen, 1975; Strang, 1978; Vandongen et al, 1979).
Beta-agonistic pharmaca stimulated renin secretion in man (Leenen et al, 1975; Johnson et al, 1976). In addition, inhibitory actions of β-antagonists (without intrinsic sympathetic activity) on renin secretion were observed (Man in 't Veld and Schalekamp, 1984). Small or non-significant alterations in plasma renin concentrations were observed after β_1-selective or non-selective β-antagonists (Birkenhäger et al, 1971; Amery et al, 1974), though methodological problems were encountered in the sense that total renin concentration was measured in these studies. Derkx et al (1976) demonstrated that propranolol depressed active renin with concomitant increments in inactive renin; total renin was unaffected.

Controversy exists as to whether β_1- or rather β_2-adrenoceptors mediate renin secretion.
Davies et al (1975) and Johnson et al (1976) concluded after experiments in human beings that the β_1-adrenoceptors are mainly responsible for alterations in plasma renin activity. This was supported by Oates et al (1978) and Johns et al (1981). They infused

i.v. β_1- and β_2-antagonists in anaesthetized rats. Atenolol and propranolol (at a high dose) depressed PRA in contrast to butoxamine (a selective β_2-antagonist) and low doses of propranolol. Johns et al (1981) studied atenolol and ICI 118.551. Dose-related inhibition of isoprenaline-induced vasodepression, without affecting PRA, was observed during ICI 118.551, whereas atenolol also attenuated isoprenaline- or renal nerve stimulated renin secretion. Van Hees et al (1985) obtained similar data. Lijnen et al (1979) demonstrated in hypertensives (at rest and during exercise) that metoprolol caused decrements in active renin without affecting total renin.

Blair et al (1981, 1983) initially found that α-adrenoceptors mediated renin release in experiments at low perfusion pressure. Later on, the authors (Blair et al, 1985) published data on experiments at low as compared to normal perfusion pressure. After low grade renal nerve stimulation (0.6-1.2 Hz) at normal perfusion pressure propranolol prevented increments in RSR; by contrast, at low perfusion pressure both α- and β-blockade were necessary in order to prevent an increase in RSR.

Kopp et al (1981[a]) performed experiments in innervated and denervated kidneys of anaesthetized dogs. Prenalterol i.v. stimulated renin secretion (in innervated and denervated kidneys); this effect was abolished after pretreatment with metoprolol i.v.. In these experiments renal haemodynamics remained constant.

In subsequent experiments (Kopp et al, 1981[b], 1983) a predominant role for β_1-adrenoceptors in mediating renin release was further established.

Thames et al (1979), Holdaas et al (1981) and Osborn et al (1981) described that low frequency renal nerve stimulation elicited an elevated RSR in absence of alterations in renal haemodynamics or sodium excretion.

Intravenously or intrarenally administered atenolol or propranolol abolished these effects, whereas butoxamine (a selective β_2-antagonist) did not. In 1983 Osborn et al demonstrated that renal nerve stimulation at 1.0 Hz diminished sodium excretion and stimulated RSR. With simultaneous administration of α_1-antagonistic pharmaca antinatriuresis was no longer observed, but RSR remained elevated. After atenolol (0.5 microgram kg^{-1} min^{-1} resp. 5.0 microgram kg^{-1}

min^{-1} intrarenally) renin secretion was significantly depressed without any influence on sodium excretion. The authors concluded that β_1-adrenoceptors on the juxtaglomerular cells mediate renin secretion when haemodynamics were stable (Ammons et al, 1982; Di Bona, 1985). At higher stimulation frequencies, with concomitant alterations in GFR and RBF, renin secretion is controlled both by β_1-and α-adrenoceptors.

The preceding data suggest that the β_1-adrenoceptors mediate renin release. An other series of experiments seems to imply that β_2- rather than β_1-adrenoceptors are the main mediators in renin secretion.
Meurer et al (1976) described in normal volunteers elevated PRA during i.v. orciprenaline infusion; this was counteracted by propranolol, but not by metoprolol. In 1980 Meurer et al infused prenalterol in human subjects. Systolic blood pressure increased as well as heart rate. Renal haemodynamics, PRA and plasma noradrenaline concentration were unchanged. They concluded that prenalterol does not affect the renal circulation, and that renin secretion is predominantly mediated via β_2-adrenoceptors.
Salvetti et al (1978) found, again in man, that salbutamol stimulated renin secretion. In patients with essential hypertension with normal as well as high renin levels similar data were recorded, whilst in low renin hypertensives renin release was not promoted.
Brown et al (1983) studied in normal volunteers the effects of a β_2-antagonist (ICI 118.551) on adrenaline-stimulated PRA. ICI 118.551 inhibited PRA to a modest degree. Vincent et al (1985) and Man in 't Veld et al (1986) studied in hypertensives the effects of the same drug. Blood pressure as well as renin levels were reduced.
Weber et al (1974) recorded in rabbits prominent reductions of PRA after propranolol in comparison with several β_1-antagonists (some with ISA). They proposed that renin release is mainly mediated through β_2-adrenoceptors. Lefkowitz et al (1984) also stated that β_2-adrenoceptors are responsible for mediating renin secretion.
Nakane et al (1980) infused isoproterenol and salbutamol into the isolated perfused rat kidney. Both drugs stimulated RSR in a dose dependent fashion, though isoproterenol was more potent. In these experiments perfusion pressure (85 mmHg) and renal blood flow

remained constant. Acebutolol and propranolol inhibited renin secretion to an equal degree. They concluded that the β-adrenoceptors which mediate renin secretion do not fall in two distinct subtypes.

In conclusion: renin release is mediated via β_1-adrenoceptors in absence of alterations in renal haemodynamics. Some data favour a role for both β-adrenoceptor subtypes. Indirectly presynaptic β_2-adrenoceptors (which facilitate noradrenaline release) may also be involved.

3.5 Alpha-adrenoceptors in the kidney

3.5.1 Distribution of alpha-adrenoceptors in the kidney.

The innervation of the renal vessels is predominantly of the sympathetic type. Adrenoceptor subtypes modulate the responses due to alterations in sympathetic activity and alterations in circulating catecholamines. Sympathetic nerve endings are situated in the proximity of the juxtaglomerular cells (Barajas, 1964; Hartroft, 1966; Wagermark, 1968) and the macula densa (Hartroft 1966; Barajas, 1979).
In cortical tubules Barajas et al (1984) obtained evidence for noradrenergic innervation of proximal tubules, ascending thick limb of Henle's loop, distal convoluted tubules and collecting ducts.

U'Prichard and Snyder (1979) demonstrated (by using the radioligands ^{3}H-clonidine, ^{3}H-WB 4101 and ^{3}H-DHE) α_1- and α_2-adrenoceptors in rat kidney. The receptor number did not decrease after chemical sympathectomy with 6-OH-dopamine, suggesting a postsynaptic localization of α-adrenoceptors in the kidney. The α_2-adrenoceptors population accounted for 25% of the total receptor population. Insel et al (1985) demonstrated α_2-adrenoceptors in rat renal cortical membranes. Chemical sympathectomy (6-OH-dopamine) resulted in a depletion of the renal noradrenaline contents by 80%, but, again, the receptor number was left unchanged. In this study "selective" radioligands (rauwolscine and yohimbine) were used in

comparison with U'Prichards study. In 1982 Snavely and Insel studied rat renal cortical membranes with selective radioligands, their results indicated a threefold preponderance of α_2-adrenoceptors. Similar data were described by Schmitz et al (1981).

McPherson and Summers (1981) likewise used radioligands (^{3}H-prazosin and ^{3}H-clonidine), both of which were bound with high affinity. ^{3}H-prazosin binding was mainly localized in rat renal cortex, with fewer binding sites in the medulla. ^{3}H-clonidine was bound both in the cortex and the medulla of rat kidneys, in contrast with guinea pig kidneys where only binding to the renal cortex could be established. Umemura et al (1986), using human renal plasma membranes from cortex and medulla, described binding to sites with α_1- and α_2-specifity. Binding was proved to be rapid, saturable, reversible and specific with regard to the radioligand, and competitive displacement studies with various adrenergic agents indeed suggested binding to both α_1- and α_2-adrenoceptors.

Insel et al (1985) used in rat kidneys autoradiography and localized α_2-adrenoceptors predominantly on proximal tubular cells. Identical findings were described by Young and Kuhar (1980). In 1984 Summers showed in the rat kidney that few α_1-adrenoceptors were associated with glomeruli, most α_1-adrenoceptors being present on the proximal tubules. α_2-Adrenoceptors were also detectable in small amounts in the glomeruli; these receptors, too, were predominantly associated with proximal tubules. In other species the density of α_2-adrenoceptors varied widely. Furthermore, it was found that prazosin in rat kidney exhibited high affinity to α_2-adrenoceptors.
With regard to other species, Summers et al (1985) studied canine kidneys in vitro by using autoradiography and ^{3}H-rauwolscine radioligands. Alpha$_2$-adrenoceptors were recognized in glomeruli and in lesser concentrations on the intimal and adventitial surfaces of arcuate bloodvessels. In the renal cortex the small blood vessels were not found to be associated with α_2-adrenoceptors. In the inner medulla these receptors were found in bundles of structures running from the cortico medullary junction towards the papilla.
Muntz et al (1985) studied rat kidney by using autoradiography and

^{3}H-prazosin. The highest density of α_1-adrenoceptors was found in renal cortical tubules, less quantities were recognized in medullary tubules or glomeruli. Struyker Boudier et al (1986) counted a relatively high number of α_1- and α_2-adrenoceptors in renal blood vessels (by using autoradiography and immunohistochemical techniques).

By means of agonist and/or antagonist dose response studies, both α-adrenoceptors were demonstrated in the renal vasculature of rats (Schmitz et al, 1981), rabbits (Hesse and Johns, 1984[a]), dogs (Horn et al, 1982; Wolff et al, 1984; Duval et al, 1985) and human subjects (de Leeuw et al 1986[a,b]). In the vascular bed of rats and dogs α_1-adrenoceptors predominate.

To conclude: with varying methods such as classical pharmacological studies, radioligand bindings techniques and autoradiography considerable variations in distribution of α-adrenoceptors in the kidney have been found, as well as species differences.
α_1-Adrenoceptors are associated with blood vessels (Muntz et al, 1984; Struyker Boudier et al, 1986) and tubules (Summers, 1984; Muntz et al, 1985; Insel et al, 1985). The number of α_2-adrenoceptors appears to be higher than that of α_1-adrenoceptors (Schmitz et al, 1981; Snavely and Insel, 1982; Di Bona, 1985; Insel et al, 1985; Strandhoy, 1985). These α_2-receptors are probably located post-synaptically in view of the findings that binding sites are unaltered after chemical sympathectomy. This phenomenon may also might be explained by a paucity of presynaptic α_2-adrenoceptors which loss is not detected or by upregulation of postsynaptic adrenoceptors which masquerades decrements in presynaptic adrenoceptors.

3.5.2 Functions of alpha-adrenoceptors in the kidney, as far as they are relevant to the present investigations.

These can be summarized as follows:

1. modulation of vascular resistance, thereby altering glomerular filtration rate and renal blood flow (and the latter's distribution

within the kidney).

2. modulation of tubular functions.

3. influence on secretory patterns of hormones and enzymes, e.g. renin.

3.5.2.1 Modulation of renal vascular resistance.

Renal vascular resistance generally increases during α-adrenoceptor stimulation. Vasoconstrictory responses are predominantly mediated by α_1-adrenoceptors in rats, cats and dogs (Horn et al, 1982; Osborn et al, 1983; Wolff et al, 1984; Wolff et al, 1985; Strandhoy, 1985; Di Bona, 1985; Duval et al, 1985), whereas in rabbit kidney α_2-adrenoceptors were shown to mediate this response (Hesse and Johns, 1984[a], 1985).

Wolff et al (1984), after infusion of phenylephrine, clonidine and guanabenz into the canine renal artery, observed a pronounced vasoconstriction elicited by phenylephrine, which was almost abolished by prazosin. The latter also reduced clonidine induced vasoconstriction. The vasoconstrictory response evoked by guanabenz was weakly attenuated, and only at the highest dose of guanabenz a significant reduction in vasoconstriction was achieved through prazosin.

The vasoconstrictory response due to phenylephrine was not inhibited by yohimbine, whilst this substance reduced significantly the vasoconstriction evoked by clonidine or guanabenz. Furthermore, it appeared that clonidine was more effective in reducing renal blood flow than guanabenz. Renal denervation did not influence the results. The authors concluded that both α_1- and α_2-agonists are capable of reducing renal blood flow, but α_1-agonists are more potent (the rank order of selectivity to the α_1-adrenoceptor was: phenylephrine>clonidine>guanabenz). In a subsequent study, Wolff et al (1985) described in rats an even more predominantly α_1-adrenergic type of mediated sympathetic vasoconstriction whereas α_2-adrenoceptors were either inactive or relatively sparse. The latter hypothesis is in conflict with other studies (Schmitz et al, 1981; Snavely and Insel, 1982). Thus, apparently, the α_2-receptor subtype

is less attained to vascular contractile elements. Horn et al (1982) studied several α-agonists and antagonists on the canine renal and femoral vasculature (after pretreatment with β-antagonists and ganglionic blockade). They, too, assumed a predominant role for α_1-adrenoceptors in the renal vasculature, whilst in the femoral bed rather α_2-adrenoceptors appear to mediate vasoconstriction. With regard to the (canine) renal vasculature Osborn et al (1983) and Di Bona (1985) found that prazosin reversed the vasoconstriction induced by renal nerve stimulation and noradrenaline infusion, whilst neither yohimbine nor rauwolscine attenuated vasoconstriction after these stimuli. Robie (1980) obtained comparable data with the exception of yohimbine; in this study yohimbine attenuated noradrenaline-evoked decrements in renal blood flow.
De Leeuw et al (1986[a,b]) infused doxazosin and yohimbine into the human renal artery. Without altering mean arterial pressure both pharmaca raised renal bloodflow, but (at doses up to 1-3 microgram kg^{-} min^{-1}) yohimbine caused greater increments in renal blood flow than doxazosin did. They presumed that for a given level of blood pressure the α_2-adrenoceptors are the predominant ones to constrict the renal vasculature.

With regard to influence on GFR and RBF the following studies have been carried out.
At low doses intrarenally infused methoxamine did not alter GFR, RBF and urinary sodium excretion. At high doses GFR and RBF decreased significantly without changes in arterial pressure (Osborn et al, 1982[b]). Hesse and Johns (1985) confirmed these results in rabbits, though in their experiments mean blood pressure rose. Furthermore, the authors studied guanabenz and UK 14303. Both α_2-agonists caused significant decrements in RBF and GFR at high doses, mean blood pressure being significantly lower during UK 14303.
Strandhoy et al (1982) and Strandhoy (1985) described increments in GFR after intrarenal clonidine and guanabenz infusion in dogs. Kauker (1982) confirmed these results in rats. The rise in GFR after guanabenz was not counteracted by yohimbine.

Renal nerve stimulation can induce vasoconstriction, which is mediated via α-adrenoceptors. After low frequency (0.25-1.0 Hz) renal nerve stimulation GFR and RBF remained unaffected (Thames and Di Bona, 1979; Holdaas et al, 1981; Osborn et al, 1982[b]; Ammons et al, 1982). Beyond stimulation frequencies of 2.0 Hz at normal perfusion pressure, both GFR and RBF decrease (Ammons et al, 1982; Di Bona, 1985; Blair et al, 1985).

To summarize these data: it is proposed that renal vasoconstriction is mainly mediated via α_1-adrenoceptors, except possibly for rabbits and humans.
In addition, some precautions have to be taken into account: α_2-adrenoceptors require co-factors to come to full expression (Lees, 1981; Langer and Hicks, 1984; Strandhoy, 1985; Hamilton et al, 1986). In particular experiments performed in vitro could be influenced in this manner. Furthermore, Flavahan et al (1984) postulated that α_2-agonists are partial agonists which only produce partial activation of vascular smooth muscle. Finally, Summers (1985), Godfraind (1985), and McGrath and Reid (1985) mentioned an α_2-adrenoceptor-mediated role in releasing endothelium derived relaxant factor (EDRF), which may obscure vasoconstrictory properties of α_2-agonists.

3.5.2.2 Modulation of tubular function.

Osborn et al (1982[b]) studied effects of intrarenally administered methoxamine in anaethetized dogs. At doses not affecting GFR and RBF a decrease in urinary sodium excretion was recorded. At higher doses, which lowered GFR and RBF, further decrements in $U_{Na}V$ were observed.
Hesse and Johns (1985) infused phenylephrine and methoxamine in rabbits. At doses not altering RBF and GFR, sodium retention ensued. After guanabenz and UK 14304 $U_{Na}V$ remained unaltered or rose. With high doses of α_1-agonists and UK 14304, which lowered GFR and RBF, $U_{Na}V$ fell by 50%. Guanabenz , which attenuated RBF only slightly, did not significantly alter $U_{Na}V$. Intrarenal infusion of noradrenaline, at doses not altering GFR or RBF, reduced urine flow

and $U_{Na}V$. This effect was abolished by prazosin but not by idazoxan.
Kauker (1982) demonstrated in hypertensive rats that guanabenz promoted natriuresis while mean arterial pressure decreased. Fildes et al (1985) found after yohimbine infusion in dogs dose-dependent increases in urinary sodium excretion occuring without changes in renal haemodynamics. They used doses up to 100 microgram kg^{-1} min^{-1}, but it was questioned whether α_2-"selective" doses were used (Di Bona, 1985).

After low grade renal nerve stimulation (0.25-0.7 Hz) water and sodium excretion are unaffected (Di Bona, 1985). Kopp et al (1981^b) observed declinations in water and sodium excretion after intensified renal nerve stimulation. The antinatriuresis caused by RNS at 1.0 Hz was abolished by phentolamine and prazosin, but was unaffected by yohimbine and rauwolscine (Osborn et al, 1983; Di Bona, 1985). Hesse and Johns (1984) obtained comparable data in rabbits with regard to prazosin. Yohimbine augmented antinatriuresis slightly. These data indicate that tubular function in dogs and rabbits is mainly mediated by α_1-adrenoceptors.

Pettinger et al (1985) and Smyth et al (1986) demonstrated antinatriuresis after RNS in isolated rat kidneys, which was counteracted by prazosin and not attenuated by yohimbine. After chronic pretreatment with prazosin antinatriuresis became in part dependent on α_2-adrenoceptors. The most likely explanation is that newly formed α_2-adrenoceptors migrate to the postsynaptic sites.

In conclusion: after α_1-agonists or renal nerve stimulation (at 1.0 Hz or above) α_1-adrenoceptor mediated increases in tubular water and sodium reabsorption occurs in dog, rats and rabbits, after chronic treatment α_2-adrenoceptors may occupy the postsynaptic α_1-adrenoceptor site.

3.5.2.3 Influence on renin secretion.

Conflicting results have been obtained with regard to the α-adrenoceptor type which are involved in mediating renin release. Graham and Pettinger (1979), Colluci (1982) and Nicholson et al (1985) described after prazosin a rise in PRA.
Massingham and Hayden (1975) and Hamilton and Reid (1981) found that prazosin left PRA unaltered. Leenen et al (1975) observed increased PRA-levels after methoxamine infusion at vasoactive dosages.
Clonidine has been observed to reduce PRA (Onesti et al, 1971; Reid et al, 1975; Pettinger et al, 1976; Chevillard et al, 1978; Nolan and Reid, 1978), Kho et al (1976) did not observe any change. All these studies are difficult to interprete because of marked differences in routes of administration and other variables.

Michelakis and McAllister (1972) and Johnson et al (1976) obtained no evidence for an α-adrenoceptor mediated influence on renin release. The latter infused phentolamine into the renal artery of sodium depleted dogs; at a low dose of phentolamine, without decrement in mean arterial pressure, renin release was unaffected. Ayers et al (1981) studied α- and β-agonists in uninephrectomized conscious dogs. Methoxamine administered into the renal artery increased MAP and PRA; phentolamine reduced MAP and PRA towards control levels. Leenen et al (1975), using methoxamine, demonstrated that propranolol prevented PRA increments after this α_1-agonist. They favoured the concept of a combined (α- and β-adrenoceptor) influence on renin release, which was in agreement with the findings of Winer et al (1969).
Osborn et al (1982[b]) infused a low dose of methoxamine intrarenally in anaesthetized dogs. Renin secretion rate was unchanged as like renal haemodynamics and MAP. At a higher dose with concomitant decrements in RBF and sodium excretion, renin secretion rate (RSR) increased. After renal nerve stimulation (0.5 Hz) RSR rose without alterations in renal haemodynamics. Prazosin nor phentolamine attenuated this increase, but phenoxybenzamine abolished the increment. At higher stimulation frequencies RBF declined; this effect

was attenuated by all three α-antagonists. The authors concluded that α-adrenoceptors do not have a role in renin secretion elicited by direct neural activation. At higher stimulation frequencies, which stimulate the vascular baroreceptor and macula densa receptor, α-adrenoceptors may influence renin secretion.

In subsequent experiments (Osborn et al, 1983), effects of both α_1- and α_2-antagonists on the results of renal nerve stimulation (1.0 Hz) were studied. At this frequency RSR was stimulated and was not attenuated during prazosin or yohimbine infusion. RNS-induced antinatriuresis was antagonized by prazosin, but not affected by yohimbine.

Kopp et al (1981[b]) studied anaesthetized dogs. After high level RNS (mean 2.6 Hz) RSR increased which declined to 50% during intrarenally infused phenoxybenzamine. During simultaneous infusion of phenoxybenzamine and metoprolol RNS-stimulated renin secretion was abolished. In these experiments RBF decreased and antinatriuresis occurred. The authors suggested that at high level renal nerve stimulation renin release is dependent on both α-adrenoceptor mediated renal vasoconstriction and β_1-adrenoceptor stimulation.

Ammons et al (1982) presented experiments in anaesthetized cats whose kidney were perfused with a constant perfusion pressure. Renal nerve stimulation frequencies beyond 2 Hz reduced RBF and GFR and raised RSR, when phentolamine was administered the alterations in renal haemodynamics were abolished, but RSR rose further. The latter rise was prevented by propranolol. Papaverine caused similar effects on GFR, RBF and RSR; it was concluded that increments in RSR were not mediated by α-adrenoceptor activity.

In rabbits Hesse and Johns (1985) found that intrarenal infusion of phenylephrine and UK 14304 stimulated RSR only at such a dose which decreased renal blood flow and glomerular filtration rate, Batenburg (1983) observed (in the isolated perfused rat kidney) vasodilatation after prazosin without significant alterations in RSR.

Blair et al (1979) studied dogs pretreated with propranolol. After infusion of phenoxybenzamine PRA declined. MAP decreased in this experiments, the perfusion pressure was 80 mmHg. In subsequent studies (Blair, 1981) phenoxybenzamine did not affect RSR. After

pretreatment with propranolol RSR decreased. In another series phenoxybenzamine was infused either intrarenally or intravenously. During intrarenal infusion RSR decreased and RBF increased, whilst RSR increased and RBF remained constant during intravenous infusion. In 1983 Blair et al described increments in RSR during intrarenal infusion of methoxamine and phenylephrine in denervated kidneys. Renal bloodflow remained stable in these experiments. Prazosin counteracted RSR increments, whereas propranolol exhibited no effect on renin secretion. These experiments were carried out at a constant perfusion pressure (90 mmHg).

In a fourth study Blair et al (1985) conducted experiments at low and normal perfusion pressures. Low frequency renal nerve stimulation (without inducing vasoconstriction in experiments at normal perfusion pressure) raised RSR, which rise was prevented by propranolol. In similar experiments at low perfusion pressure, both prazosin and propranolol were necessary to antagonize increments in renin secretion rate.

Di Bona (1985) commented on these studies and stated that reduced renal perfusion pressure causes autoregulatory vasodilatation which augments renin secretion after various stimuli. In this connection Kirchheim et al (1986) studied in conscious dogs relations between blood pressure levels and renin secretion. They demonstrated that below a certain minimum perfusion pressure renin secretion was stimulated markedly; this threshold pressure point is raised by methoxamine. Reversal to the initial point is caused by prazosin whilst β-antagonists did not affect this threshold point.

To summarize: these results are rather difficult to interprete because of a vast variety in methods, pharmaca, species etc. Most data indicate that α-adrenoceptor activation stimulates renin secrection, when simultaneous vasoconstriction is evoked. It appears to be unlikely that this effect is directly dependent on α_1-receptors since prazosin did not attenuate RSR.

Another series of experiments rather appears to indicate that alpha receptor stimulation supresses renin release. Pettinger et al (1976) performed experiments in conscious rats. After intraperitoneal administration of clonidine renin activity decreased. This effect was

not prevented by propranolol or cholinergic or ganglionic blockade. Methoxamine also depressed PRA. Clozapine (a weak α_1-antagonist), phenoxybenzamine and phentolamine attenuated clonidine-evoked suppressive effects on renin release. These data suggest an intrarenal α-adrenoceptor mediated suppressive effect on renin release elicited by clonidine. In later experiments (Graham and Pettinger, 1979), the effects of prazosin and phentolamine were studied. Dose related decrements in MAP coincided with increases in PRA. A given reduction in MAP was associated with lesser increments in PRA after prazosin than after phentolamine. The authors speculated that phentolamine would enhance (via presynaptic α_2-adrenoceptors) noradrenaline release, thereby stimulating β-adrenoceptors. This view was supported by further experiments by Keeton and Pettinger (1979). They observed that propranolol prevented phentolamine induced PRA elevations. They concluded, therefore, that after vasodepressor doses of an α-antagonist renin release is due to reflex activation of renal sympathetic nerves with minor contributions from alterations in perfusion pressure.

Keeton et al (1985) recorded in chronically cannulated conscious dogs yohimbine- and rauwolscine-induced elevations in PRA. Increments were postulated to be stronger related to the ability of α_2-antagonists to raise plasma noradrenaline concentrations than to blood pressure or heart rate alterations.

Pedrinelli et al (1981) and Morganti et al (1981) observed an elevation in PRA after prazosin and phentolamine without alterations in haemodynamics. De Leeuw et al (1986[a,b]) infused yohimbine and doxazosin into the renal artery of untreated hypertensives. Both drugs raised renal bloodflow. Renin secretion rate was more markedly augmented by yohimbine than by doxazosin. In these experiments mean arterial pressure remained unaffected.

Seymour et al (1981) demonstrated during phentolamine infusion significant increments in plasma renin activity in sodium repleted dogs, MAP was unchanged. In subsequent experiments in rats PRA only increased during phentolamine infusion after pretreatment with indomethacine.

In the isolated perfused rat kidney Vandongen et al (1973) demonstrated that phenoxybenzamine infusion prior to noradrenaline or isoproterenol did not prevent renin release. In 1974 Vandongen et al found that noradrenaline could stimulate renin secretion when phenoxybenzamine was administered simultaneously; this response was blunted by propranolol. Methoxamine did not alter RSR, whilst isoproterenol-induced RSR was attenuated by methoxamine. In experiments with simultaneous infusion of methoxamine and phenoxybenzamine RSR rose. They assumed a predominantly stimulatory role of β-adrenoceptors and an inhibitory action of α-adrenoceptors. This was confirmed in other experiments with clonidine (Vandongen and Greenwood, 1975).

In subsequent experiments (Strang, 1978; Vandongen et al, 1979), it was established that phenylephrine infusion prior to isoproterenol inhibited isoproterenol-stimulated renin secretion (perfusion pressure elevated during phenylephrine infusion). Simultaneous infusion of phenoxybenzamine abolished the vasoconstrictory response. Renin secretion rate was unaffected in comparison with isoproterenol infusion. Simultaneous infusion of phenylephrine and dihydralazine prior to isoproterenol infusion (to prevent alterations in perfusion pressure) attenuated RSR significantly. The authors concluded, therefore, to the existence of a distinct non-vascular α-adrenoceptor with an inhibitory action on the juxtaglomerular cell.

In studies conducted in the isolated perfused rat kidney Batenburg (1983) observed vasodilatation after prazosin infusion, but alterations in renin secretion rate were not obtained in comparison with control experiments. Mizoguchi et al (1983) infused phentolamine into the renal artery of conscious dogs. Renal bloodflow and renin secretion rate remained unaffected.

It should be concluded that contradictory data have been obtained: α-adrenoceptors are associated with stimulatory effects on renin secretion when vasoconstriction occurs. On the other hand, some experiments point to an inhibitory action of α-adrenoceptors on renin secretion. In species other than rabbits, suppressive effects on RSR were observed after α_2-adrenoceptor-stimulation and enhanced renin secretion occurred after the application of α_2-antagonists with simultaneous vasodilatation.

CHAPTER 4

Methods

In our study we used the model of the perfused isolated rat kidney, this technique was described by Vandongen (1973) and Strang (1978) and modified by Batenburg (1983). In these preceeding studies the reliability of the model was assessed.

4.1 Perfusion technique.

Male Wistar rats (300-400 g), maintained on a standard diet (Hope Farms AM-2) with water supply ad libitum, were anaesthetized with sodium pentobarbital (60 mg/kg) intraperitoneally and heparinized (80-100 units intra-arterially) via a polypropylene cannula inserted in the right carotid artery. Through a midline incision the abdominal vessels and the left kidney were exposed. The ureter was cut (since ureteral occlusion increases reninsecretion (Cooke et al, 1970; Freeman et al, 1974)). The adrenal vein was ligated tightly as well as the iliac artery and vein, the spermatic artery and vein and the lumbar arteries and veins. Next, the vena cava and aorta above the left renal artery and vein were carefully separated from each other and a ligature was placed without tightening; caudally the aorta and vena cava at the level of the bifurcation were tightly ligated. Polypropylene cannules were inserted into the aorta and vena cava to the junction of the left renal vein and artery respectively. With perfusion fluid flowing, the ligatures above the left renal vessels were tightened, thereby allowing selective perfusion of the left kidney in situ without previous interruption of the blood flow. Perfusate was collected via the vena caval cannula. Thereafter the rat was sacrified by an overdosis sodium pentobarbital via the carotid cannula.

This perfusion model was allowed to stabilize during the next 10 minutes (stabilization period); next the investigation period in which the pharmaca were administered was started. The duration of this period was at most 16 minutes. Light microscopy after this period showed normal architecture and histology of the kidneys.

Reasons for discarding experiments:

- anoxia of the anaesthetized rat during the surgical procedure.
- more than one renal artery supplying the left kidney.
- haemorrhage and hypotension during the cannulation procedure.
- inadequate flow after 3 minutes in the stabilization period.
- contamination with blood of the effluent collected from the vena caval cannula.

The perfusion pressure was kept constant during the entire perfusion period, including the stabilization period, and was adjusted at 110 mmHg mean pressure; this was made possible by introducing a device allowing return of the perfusion fluid towards the reservoir. Perfusion pressure was kept constant to avoid secretory alterations in renin which would be attributable to changes in renal haemodynamics and autoregulatory mechanisms (Kopp and Di Bona, 1984; Blair et al, 1985; Di Bona, 1985; Kirchheim et al, 1986).

Initially perfusion pressure was monitored with an analogue device, later on experiments were carried out with a digital perfusion measurement device. Only the first salbutamol dose-response curves were performed with the old device.

Perfusion fluid was constantly delivered as pulsatile flow by a roller pump (Watson Marlow MHRE 100) from a reservoir (Pyrex) adjusted at a temperature of 37°C and a pH between 7.35 and 7.45 controlled by a pH electrode (Sensorix Radio, Copenhagen) connected with a pH device (Philips PW 9410). Air bubbles were caught and removed. Propulsion of perfusion fluid after filtering occurred through silicone conducts. A secondary heating device neutralized shifts in temperature and adjusted temperature at a level between 36.5 and 37.5°C at a distance of 6 cm from the insertion of the aorta cannula.

Since March 1985 a new heating device was used, adjustment of temperaturethen becoming possible at 0.1°C. The temperature sensor was localized at 7 cm from the insertion of the aorta cannula; with this

device a second retour conduit was introduced.

- Perfusion pressure was monitored by Thomson Telcotranducers and adjusted at 110 mmHg as electronic mean pressure.
- Flow was measured as the amount of effluent collected from the vena caval cannula.
- Renal vascular resistance was derived from the quotient of perfusion pressure and flow.

4.2 Perfusion fluid.

This fluid contained a modified Krebs-Ringer solution (macrodex 6% - NaCl 0.9%) oxygenated with carbogene (95% O_2 and 5% CO_2). This solution contains dextran (mol. weight 70.000 Pharmacia), sodium bicarbonate and HEPES.
This fluid was dissolved in distilled water, potassiumchloride, calciumchloride etc.
The exact composition was:

Na^+	145	mmol/l
K^+	4.8	mmol/l
Ca^{2+}	2.60	mmol/l
Mg^{2+}	1.1	mmol/l
Cl^-	105	mmol/l
SO_4^{2-}	1.1	mmol/l
H_2PO_4	1.1	mmol/l
HCO_3	39.1	mmol/l
D-glucose	9.6	mmol/l
pyruvate	5.0	mmol/l
L-glutamine	4.9	mmol/l
HEPES	15.0	mmol/l
dextran	0.53	mmol/l
osmolarity	314	mosmol/l
viscosity	1.42	centistokes

4.3 Renin assay.

The collected renal effluent was dialyzed at pH 4.5 and temperature 4°C over 18-24 hours against buffers containing sodium chloride, EDTA, citric acid, sodium dihydrogen phosphate and neomycine (modification of method described by Skinner, 1967). Subsequently samples were dialyzed at pH 7.5 at 32°C over 60 minutes, thereafter samples were dialyzed at pH 7.5 at 4°C over 18-24 hours in a buffer containing sodium dihydrogen phosphate, disodium hydrogen phosphate, sodiumchloride, EDTA and neomycine. Polyvinylpyrralidone and trasylol were added to this buffer, volume depletion was restored with buffer pH 5.5.
Samples were then added at temperature 4°C to plasma obtained from nefrectomized rats, which serves as the renin-substrate, together with buffer pH 7.5 and human albumin.
Incubation was performed at 37°C over 60 minutes. Generated angiotensin I was measured by radioimmunoassay (New England Nuclear). Renin activity was expressed as nanogram equivalents of angiotensin I generated per milliliter of perfusate per hour of incubation.
All samples from each series of experiments were processed and assayed at the same time. Assays were performed in duplo. The perfusion fluid did not possess any renin-activity before passage through the kidney, and recirculation is abandoned in our model.

4.4 Pharmacological experiments.

All pharmacological agents were constantly infused by an infusion pump (Precidor 5003), volumes were adjusted at levels not causing alterations in outflow volume (maximum 0.04 ml/min.). In control experiments equal volumes of isotonic saline were infused. The outflow volumes were collected in calibrated glass tubes (Haak) and immediately stored in ice. After each experiment two samples from the effluent were drawn after shaking, and stored at -20°C.
All agonists and antagonists (table 4.1), except yohimbine and ICI 118.551, were dissolved in isotonic saline. Yohimbine was dissolved in glucose 5%, ICI 118.551 in distilled water. Control experiments were performed with equal volumes of isotonic saline.

Table 4.1: Agonists and antagonists used in this study.

prenalterol	(β_1-agonist)	atenolol	(β_1-antagonist)
salbutamol	(β_2-agonist)	ICI 118.551	(β_2-antagonist)
cirazoline	(α_1-agonist)	doxazosin	(α_1-antagonist)
clonidine	(α_2-agonist)	yohimbine	(α_2-antagonist)

First of all dose response curves were obtained in anaesthetized but otherwise intact rats. Mean arterial pressure was determined by a Thomson Telcotransducer that was connected with the cannula inserted in the carotid artery. Pharmaca were infused via a cannula in the jugular vein. In that way it was possible to establish which doses would have systemic effects. Subsequently, dose-response curves were determined in the isolated perfused rat kidney to identify doses which had vasoactive effects and those that had not. On the basis of these data the doses were chosen for the experiments proper.
In the first part of the study fixed doses of agonists and antagonists were infused. In the second part dose-response relationships for the various agonists were studied in the presence of an antagonist.
All dose response curves in the isolated perfused rat kidney were obtained with the active pharmacon or isotonic saline alternating in random order. In combination experiments infusions of pharmaca or isotonic saline was applied according to a randomly assigned protocol.

4.5 Statistical analysis.

Group data will be presented as mean ± SEM (standard error of the mean). The paired Student t-test was applied to assess intragroup differences, for comparisons of data between groups the unpaired Student t-test was used.
Two way analysis of variance was applied for comparisons of data between groups at various measure points (Snedecor and Cochran, 1967).
Differences were considered to be significant when the t-values

exceeded those given for the 5% level of probability.

4.6 Explanation of data in figures.

Flow: amount of effluent from the vena caval cannula, expressed in milliliters min^{-1}.

Renal vascular resistance (RVR): quotient of perfusion pressure and flow, expressed in units (U).

Renin: renin concentration (ng $A_1.ml^{-1}.hour^{-1}$).

Renin secretion rate: calculated by multiplication of flow times renin concentration, expressed as standard units (SU).

% RVR: percentual changes with time in RVR with t=0 minutes as zero point.

% RSR: percentual changes with time in RSR with t=0 minutes as zero point.

4.7 Assessment of reliability of the "new" perfusion pressure measurement device in comparison with the "old" device

As mentioned before, initial experiments were carried out with an analogue device, thereafter a digital device was used. A separate series of experiments was performed in order to assess variations in data which could be ascribed to this change in methodology. These experiments with saline revealed that mean renin secretion decreased to -20.0 ± 7.5% with the new device versus -2.1 ± 4.4% in the series with the old device.

CHAPTER 5

Influence of beta-adrenoceptor agonists and antagonists on renal flow and renin secretion.

5.1 Introduction.

In Chapter 3 it was shown that there is still controversy on which β-adrenoceptor is involved in renin release and which interrelation exists between haemodynamic changes and renin release. Therefore experiments were performed with selective β_1- and β_2-agonists at doses which either produced non-vasoactive or vasoactive responses. In addition, dose response curves with selective β_1- and β_2-antagonists were established. Thereafter experiments were performed in which prior to and simultaneous with incremental doses of β-agonists a β-antagonist was infused. All experiments were carried out at a constant perfusion pressure approximating normal blood pressure levels. Dose-response curves obtained for the "active" pharmacon were alternated with control experiments with isotonic saline.

5.2 Experiments with beta$_1$-adrenoceptor agonist.

As outlined in Chapter 4 (table 4.1), we performed experiments with prenalterol as a β_1-adrenoceptor agonist.
This compound is regarded as to be a rather selective β_1-agonist (Meurer et al, 1980; Altiere et al, 1981; Kopp et al, 1981[a]; Man in 't Veld et al, 1983; Williams et al, 1983; Staessen et al, 1983; Vincent et al, 1983; Wilffert et al, 1984).

At first dose-response curves were established. In these experiments prenalterol was infused at various doses into the jugular vein of the anaesthetized rat. At a dose of 10 microgram kg^{-1} min^{-1} a modest increase in heart rate was demonstrated without a pressor response. Subsequently dose-response curves were established in the isolated perfused rat kidney. Incremental doses (between 3 and 30 microgram kg^{-1} min^{-1}) of prenalterol were infused, but alterations in RVR were not encountered as compared with saline infusion (fig. 5.2.1). Evalua-

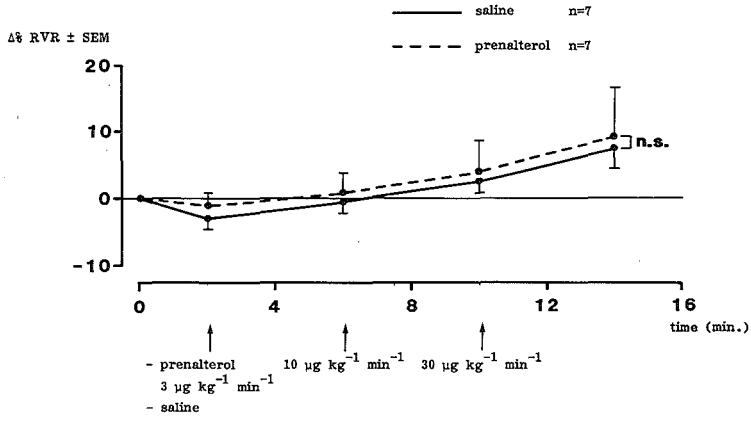


Fig. 5.2.1: The effects of infusion of incremental doses of prenalterol or saline on percentual changes in RVR.

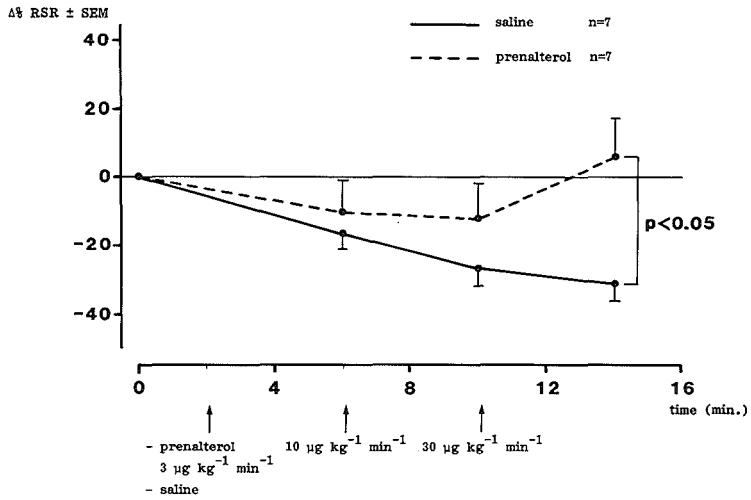


Fig. 5.2.2: The effects of infusion of incremental doses of prenalterol or saline percentual on changes in RSR.

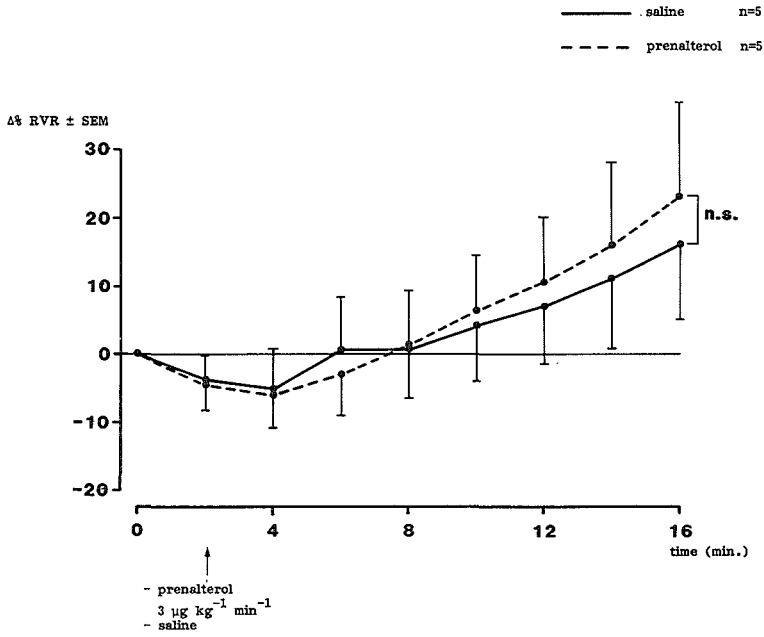


Fig. 5.2.3: The effects of infusion of prenalterol or saline on percentual changes in RVR.

tion of the percentual changes in RSR revealed a significant difference (fig. 5.2.2).
Thereafter, to explore possible changes in renin secretion and flow during the entire duration of the perfusion period, experiments were performed at a dose of 3 microgram kg^{-1} min^{-1} (n=5). The results are shown in figure 5.2.3 and 5.2.4.
Renal flow at the starting point was 9.7 ± 0.7 ml min^{-1}, after 16 min. 8.4 ± 1.2 ml min^{-1} (N.S.), in alternately performed control experiments (n=5) with isotonic saline (in equal volume as the active pharmacon) flow decreased from 10.0 ± 0.5 ml min^{-1} to 9.0 ± 1.0 ml min^{-1} (N.S.). Comparison of changes in renal vascular resistance during prenalterol or saline infusion revealed no statistical significant differences (fig. 5.2.3).
Renin secretion decreased in both experiments. During prenalterol infusion renin secretion was reduced from 1455 ± 237 SU to 878 ± 51 SU ($p<0.05$), during saline infusion a fall in renin secretion rate from 1286 ± 122 SU to 849 ± 63 SU ($p<0.05$) was observed. Percentual alterations in renin secretion rates are depicted in figure 5.2.4. Comparison of these data revealed no significant difference.

5.3 Experiments with beta$_2$-adrenoceptor agonist.

Salbutamol was chosen as the β_2-adrenoceptor agonist. In the literature this drug is accepted as a relatively selective β_2-agonist (Stjärne et al, 1976; Minneman et al, 1979[a]; Lees, 1981; Man in 't Veld et al, 1983; Wilffert et al, 1984; Schalekamp and Man in 't Veld, 1985). After a similar procedure as previously mentioned three doses were chosen (0.6, 1.8 and 6 microgram kg^{-1} min^{-1}). In figure 5.3.1 and 5.3.2 data are shown after infusion of the incremental doses of salbutamol. Vasodilatation is observed as compared with saline infusion. Comparison of these data revealed just unsignificant differences, but comparison of RSR data expressed as percentual changes in RSR (fig. 5.3.2) did show significant differences.
Thereafter, in experiments in which salbutamol was infused during the entire perfusion period (n=5), a dose of 1.8 microgram kg^{-1} min^{-1} was chosen. At t=0 min. flow was 10.6 ± 0.7 ml min^{-1} and fell to 9.9 ± 0.5 ml min^{-1} (N.S.). In control experiments (n=5) flow

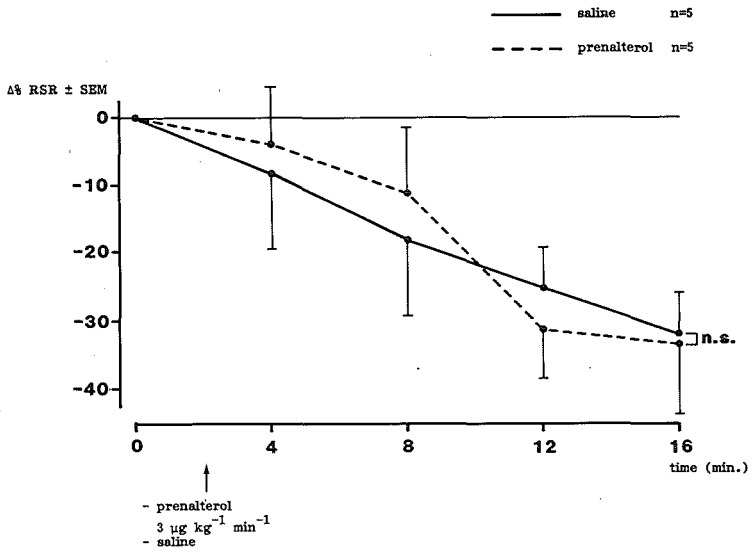


Fig. 5.2.4: The effects of infusion of prenalterol or saline on percentual changes in RSR.

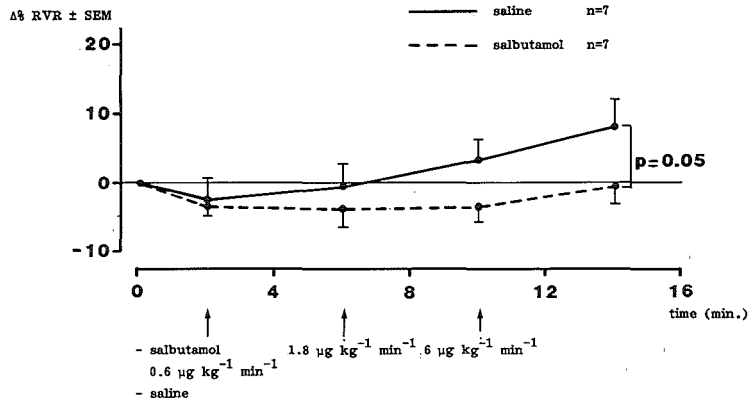


Fig. 5.3.1: The effects of infusion of incremental doses of salbutamol or saline on percentual changes in RVR.

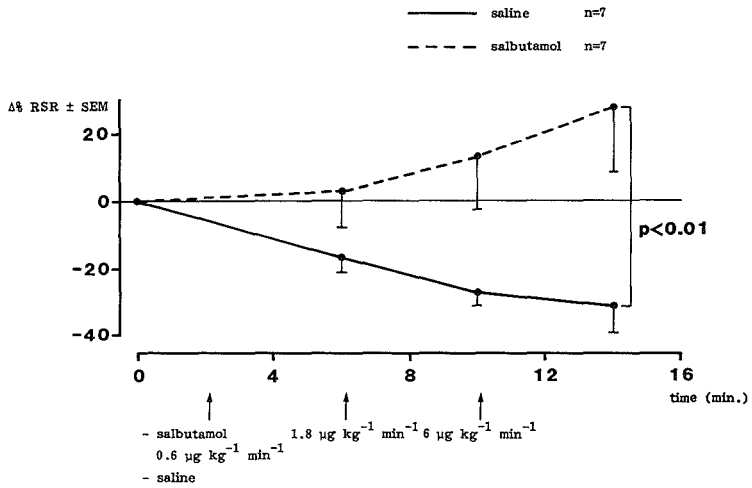


Fig. 5.3.2: The effects of infusion of incremental doses of salbutamol or saline on percentual changes in RSR.

decreased from 10.8 ± 0.4 ml min^{-1} to 9.5 ± 0.5 ml min^{-1} (N.S.). Comparison of percentual changes in renal vascular resistance revealed a significant difference between the groups (fig. 5.3.3). Thus slight, but significant vasodilatation was observed at a dose of 1.8 microgram kg^{-1} min^{-1} salbutamol in comparison with saline infusion.

Salbutamol elicited increased renin secretion between 2 and 6 minutes after starting infusion of salbutamol. Renin secretion at t=0 min. was 1178 ± 122 SU, after 16 minutes 1815 ± 148 SU ($p<0.05$). Control experiments revealed unaltered renin secretion (946 ± 56 SU vs 920 ± 148 SU, N.S.). As depicted in figure 5.3.4 percentual alterations in renin secretion during salbutamol infusion at this dose are significantly different from saline infusion.

Since we intended to study renin secretion patterns during non-vasoactive and vasoactive doses of drugs, a separate series at a lower dosage of salbutamol was carried out (n=5). Data are depicted in figure 5.3.5 and 5.3.6. The dose of salbutamol chosen was 0.6 microgram kg^{-1} min^{-1}. Flow fell from 9.3 ± 0.2 ml min^{-1} to 8.9 ± 0.3 ml min^{-1} (N.S.), control studies likewise revealed no significant differences (9.7 ± 0.2 ml min^{-1} vs 9.0 ± 0.6 ml min^{-1}). Percentual changes in RVR during salbutamol or saline are not significantly different. During salbutamol infusion renin secretion was reduced (1257 ± 282 SU vs 974 ± 160 SU, at t=8 min. RSR was 684 ± 124 SU, not significantly different from t=0 min. or t=16 min.). Thus, a tendency downwards is noticed initially, thereafter a reversed pattern is observed. Control experiments showed decrements from 1008 ± 106 SU to 763 ± 111 SU (N.S.). Percentual changes are depicted in figure 5.3.6. Significant differences between salbutamol and saline infusion with respect to renin secretion patterns were not obtained.

5.4 Experiments with beta$_1$-adrenoceptor antagonist.

Atenolol was chosen as the β_1-adrenoceptor antagonist. This drug is well recognized and accepted as a rather selective β_1-antagonist without intrinsic sympathetic activity (Oates et al, 1978; Lees, 1981; Wilffert et al, 1982[a]; Osborn et al, 1983; Vincent et al, 1983; Wilffert

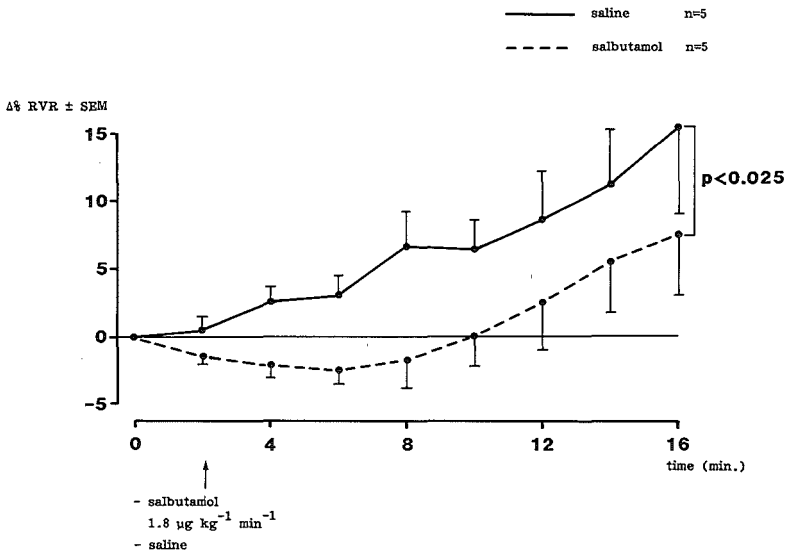


Fig. 5.3.3: The effects of infusion of salbutamol or saline on percentual changes in RVR.

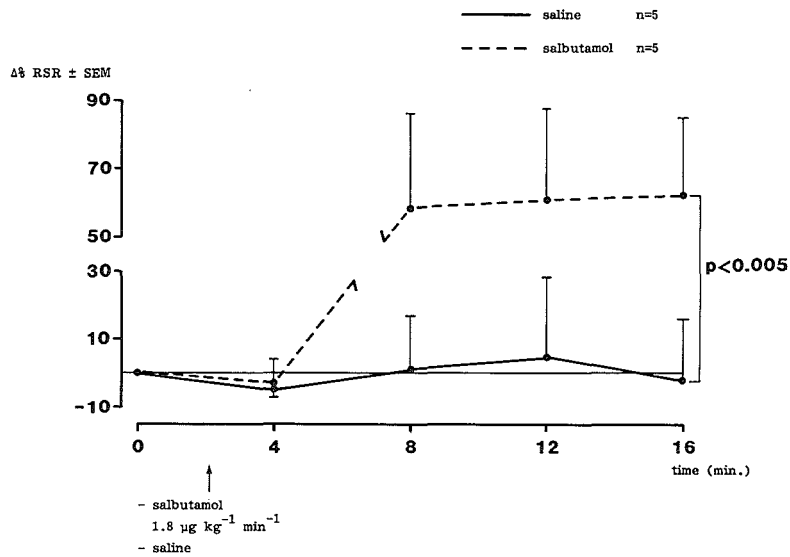


Fig. 5.3.4: The effects of infusion of salbutamol or saline on percentual changes in RSR.

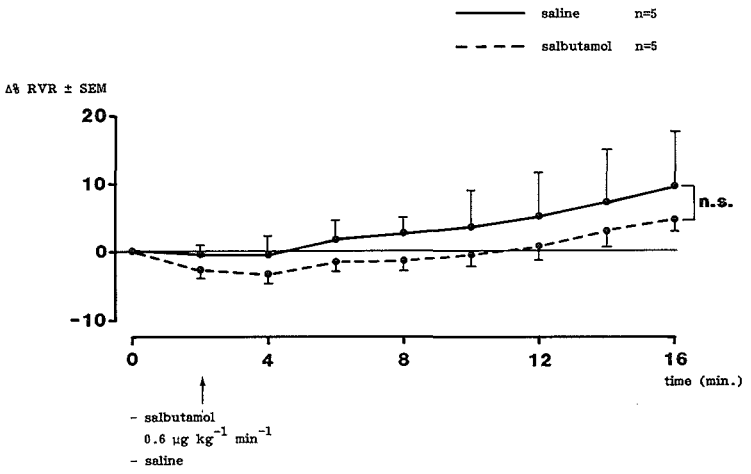


Fig. 5.3.5: The effects of infusion of salbutamol or saline on percentual changes in RVR.

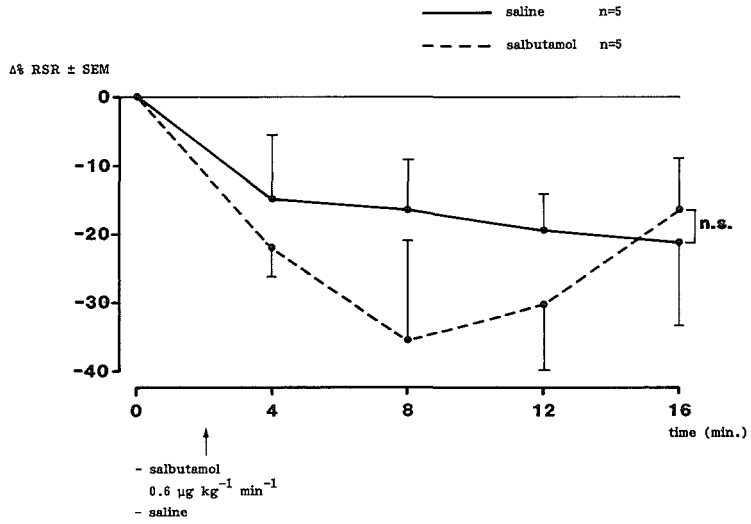


Fig. 5.3.6: The effects of infusion of salbutamol or saline on percentual changes in RSR.

et al, 1984; Schalekamp and Man in 't Veld, 1985).

Dose response curves in the intact rat revealed a negative chronotropic effect at a dose of 0.03 milligram kg^{-1} min^{-1}. Dose response curves were thereafter established in the isolated perfused rat kidney (dose 0.001, 0.003, 0.01 and 0.03 mg kg^{-1} min^{-1}). Since none of these doses elicited alterations in flow 0.03 mg kg^{-1} min^{-1} atenolol was infused during the entire perfusion period. Data are presented in figure 5.4.1 and 5.4.2. In these experiments (n=5) flow was unchanged, 11.3 ± 0.4 ml min^{-1} and 11.5 ± 0.6 ml min^{-1} (N.S.); as in the control experiments (n=5); 12.3 ± 0.4 ml min^{-1} and 11.5 ± 0.3 ml min^{-1} (N.S.). Percentual alterations in renal vascular resistence are presented in figure 5.4.1. Comparison of data during atenolol and saline infusion revealed a significant difference ($p<0.005$). Renin secretion was reduced in both groups, changes between t=0 min. and t=16 min. in these groups did not differ significantly (atenolol: 1333 ± 224 SU resp. 920 ± 174 SU vs saline 1353 ± 263 SU resp. 981 ± 128 SU).
When changes in reninsecretion are evaluated from an equal starting point (fig. 5.4.2), a significant difference between both groups is noticed. In the experiments with atenolol RSR was reduced by -30 ± 12%, in experiments with saline RSR was lowered by -25 ± 5%. The decrement in the atenolol curve is steeper; after two-way variance analysis a modestly significant difference is found ($p<0.05$).

5.5 Experiments with $beta_2$-adrenoceptor antagonist.

ICI 118.551 is accepted as a rather selective β_2-adrenoceptor antagonist (Bilski et al, 1980; Johns et al, 1981; Wilffert et al, 1982[b]; Brown et al, 1983; Wilffert et al, 1984; Schalekamp and Man in 't Veld, 1985).

At first dose response curves in the intact rat were obtained. It proved to be difficult to obtain any effect on blood pressure or heart rate. Doses varied from 1.0 to 300 microgram kg^{-1} min^{-1}. Thereafter experiments were performed in the isolated rat kidney; eventually we decided to infuse 30 microgram kg^{-1} min^{-1} (n=5). Data are depicted

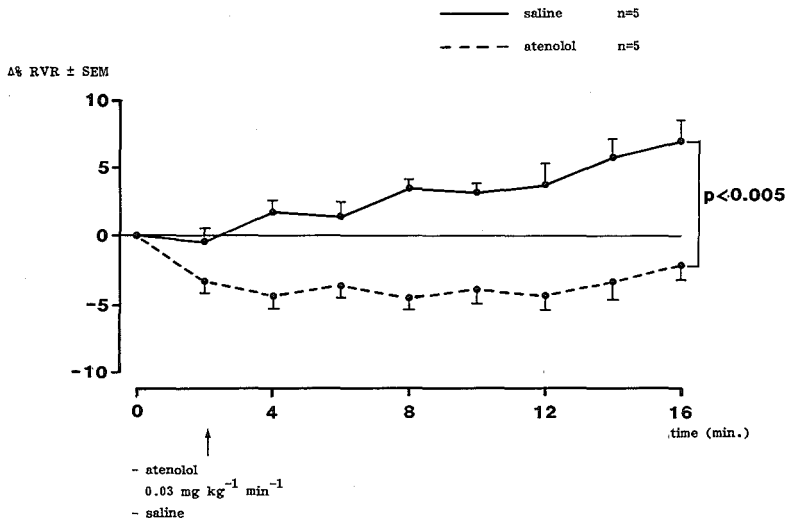


Fig. 5.4.1: The effects of infusion of atenolol or saline on percentual changes in RVR.

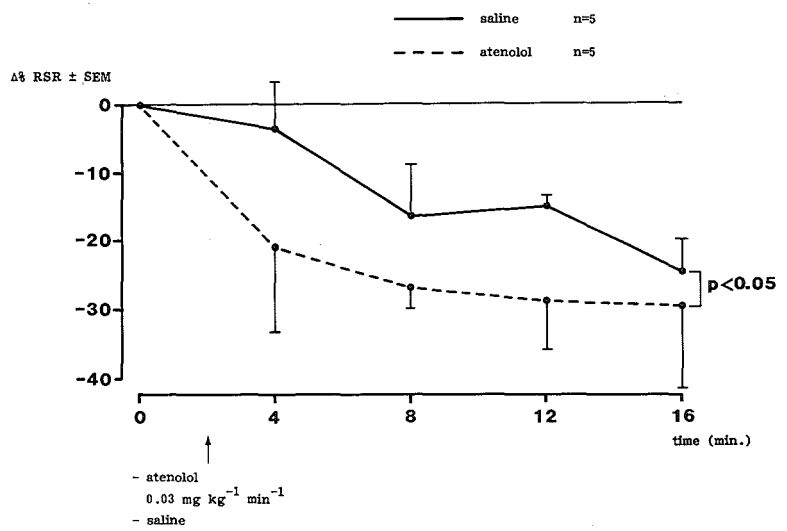


Fig. 5.4.2: The effects of infusion of atenolol or saline on percentual changes in RSR.

in figure 5.5.1 and 5.5.2. Flow did not alter (10.6 ± 0.8 ml min^{-1} vs 10.5 ± 1.0 ml min^{-1} (N.S.)), though, after 8 minutes some increase in flow was attained (11.6 ± 0.54 ml min^{-1}). In control experiments (n=5) flow decreased from 11.2 ± 0.6 ml min^{-1} to 9.0 ± 0.6 ml min^{-1} ($p<0.01$).

Percentual alterations in RSR showed significant differences between the active pharmacon and saline infusion ($p<0.005$, fig. 5.5.1).

Renin secretion rate fell from 1028 ± 109 SU to 711 ± 134 SU at the end of the perfusion period during ICI 118.551 infusion ($p<0.05$); control studies showed a similar pattern (755 ± 97 SU resp. 474 ± 21 SU, $p<0.05$); however, initially, simultaneously with slight vasodilatation during infusion of the β_2-antagonist renin secretion remained constant (t=0 min: 1028 ± 109 SU; t=8 min: 1037 ± 181 SU). Statistical significance between t=8 min. and t=16 min. was not achieved due to large variability in the results.

Percentual changes in renin secretion (fig. 5.5.2) showed no statistical significant difference.

These experiments initially revealed some relative increase in renin secretion, but thereafter a marked decrease was observed. These curves are the mirror image of the salbutamol experiments (fig. 5.3.6).

5.6 Experiments with beta$_1$-agonist versus beta$_{1,2}$-antagonists.

After establishing the effects of prenalterol, atenolol and ICI 118.551 we carried out a series of experiments with combined infusion of these pharmaca. In these series an antagonist or saline was infused just before t=0 min. at a constant dose, as outlined in Chapter 5.4 and 5.5. By contrast, the agonist was infused in incremental dosages with increases at t=2 min., t=6 min. and t=10 min. All groups comprised 7 animals. The various pharmaca as well as saline were infused according to a random order. Volumes of infusion fluid were equally and did not exceed the volumes mentioned in Chapter 4.

In figure 5.6.1, 5.6.2 and 5.6.3 data are depicted with respect to our results obtained by infusing the β_1-adrenoceptor agonist prenalterol (dose 3, 10 and 30 microgram kg^{-1} min^{-1}) in combination with

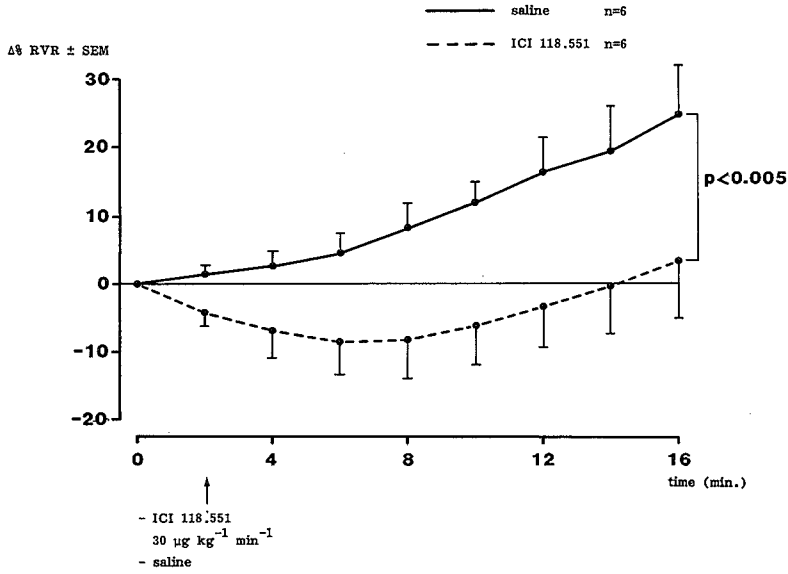


Fig. 5.5.1: The effects of infusion of ICI 118.551 or saline on percentual changes in RVR.

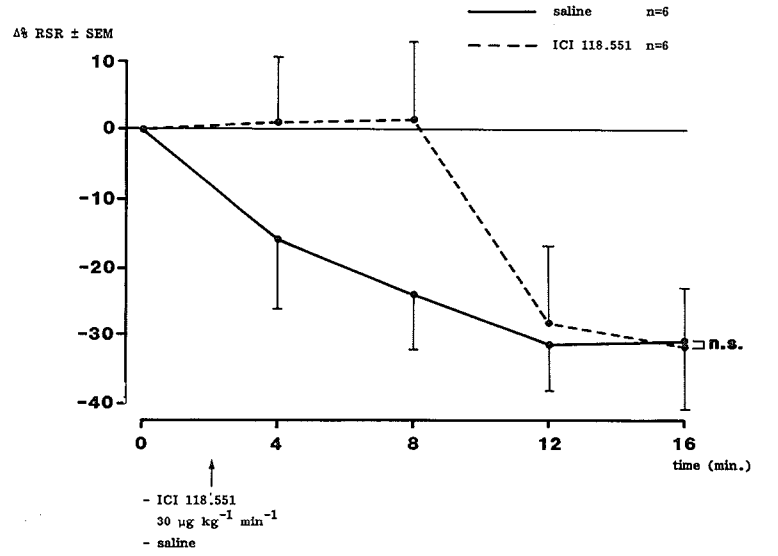


Fig. 5.5.2: The effects of infusion of ICI 118.551 or saline on percentual changes in RSR.

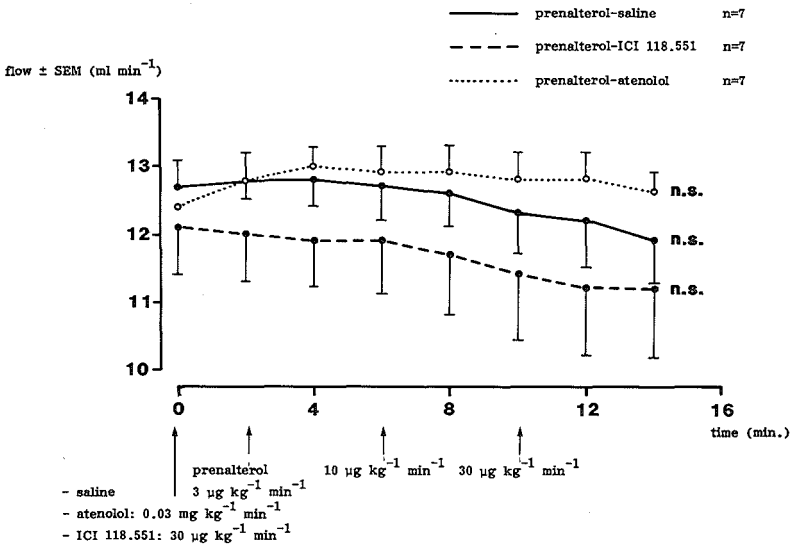


Fig. 5.6.1: The effects of infusion of incremental doses of prenalterol on flow. Prior to prenalterol infusion $\beta_{1,2}$-antagonist- or saline infusion was started.

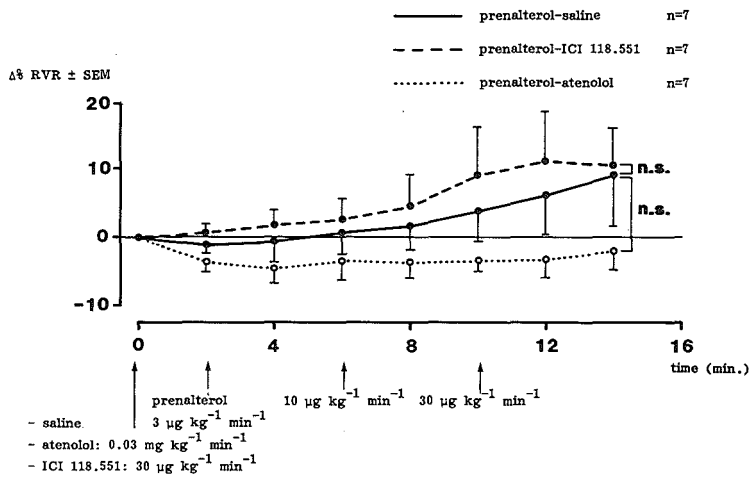


Fig. 5.6.2: The effects of infusion of incremental doses of prenalterol on percentual changes in RVR. Prior to infusion of prenalterol $\beta_{1,2}$-antagonist- or saline infusion was started.

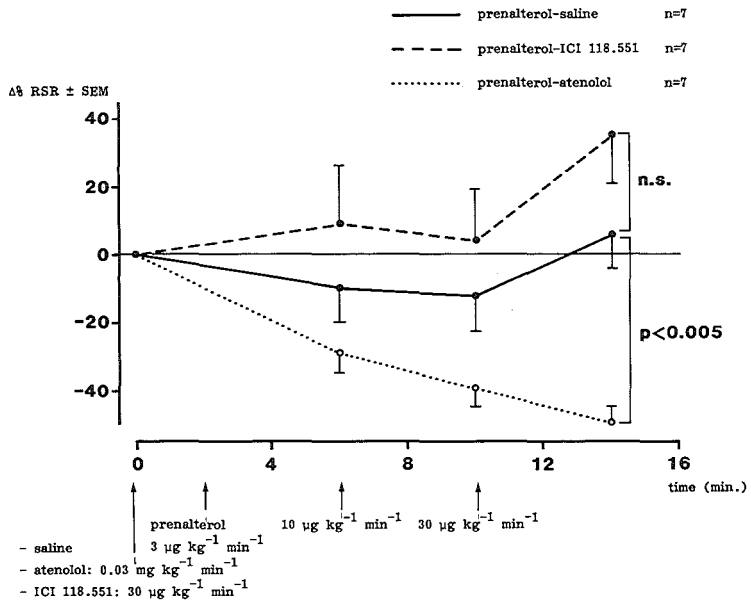


Fig. 5.6.3: The effects of infusion of incremental doses of prenalterol on percentual changes in RSR. Prior to infusion of prenalterol $\beta_{1,2}$-antagonist- or saline infusion was started.

saline, atenolol or ICI 118.551.

Prenalterol at incremental doses did not significantly alter flow (12.7 ± 0.4 ml min^{-1} vs 11.9 ± 0.8 ml min^{-1}, N.S.) in experiments with simultaneous infusion of saline, nor when combined infusion of prenalterol and ICI 118.551 was applied (12.1 ± 0.73 ml min^{-1} vs 11.2 ± 1.0 ml min^{-1}, N.S.). Infusion of atenolol and prenalterol showed a slightly distinct pattern, that is, atenolol prevented the common decline in flow (12.4 ± 0.4 ml min^{-1} vs 12.6 ± 0.4 ml min^{-1}, N.S.). This is clarified in the evaluation of percentual changes in renal vascular resistance (fig. 5.6.2).

Statistical analyses revealed no significant differences between prenalterol versus saline and prenalterol versus ICI 118.551, but comparison of prenalterol versus ICI 118.551 and prenalterol versus atenolol provided a significant difference ($p<0.005$). Analysis of renin secretion rates revealed in the prenalterol-saline experiments an initial transient decrease from 2270 ± 356 SU (t=0 min.) to 1953 ± 283 SU (t=6 min.), thereafter RSR remained constant, but after 10 min. RSR increased to 2483 ± 450 SU (N.S.). Prenalterol with simultaneous infusion of ICI 118.551 provided a comparable pattern, that is to say: RSR remained stable during 10 minutes, (2394 ± 399 SU at t=0 min., 2397 ± 358 SU at t=6 min.), thereafter RSR raised to 3238 ± 599 SU (t=14 min.).

During prenalterol infusion preceded by atenolol infusion, renin secretion was reduced significantly (1628 ± 193 SU at t=0 min. vs 801 ± 92 SU at t=14 min., $p<0.001$). Thus, previous administration of atenolol prevented prenalterol induced increments in renin secretion rate (as demonstrated in the prenalterol-saline and prenalterol-ICI 118.551 series at a prenalterol dose of 30 microgram kg^{-1} min^{-1}) concomitantly with non-significant effects on flow.

Percentual changes in renin secretion are depicted in fig 5.6.3. In the prenalterol-saline experiments a transient decrement was observed, infusion of prenalterol preceded by ICI 118.551 caused a comparable pattern at a higher level. The series with combined infusion of prenalterol and atenolol elicited a decrement in renin secretion rate. Two-way analyses of variance on these data clarified that the differences between prenalterol-saline and prenalterol-ICI

118.551 experiments were not significant, but alterations in patterns obtained in experiments with prenalterol-saline and prenalterol-atenolol differed significantly ($p<0.005$). Similar differences were observed between prenalterol-ICI 118.551 and prenalterol-atenolol.

5.7 Experiments with beta$_2$-agonist versus beta$_{1,2}$-antagonists.

In these series we performed combined infusions of salbutamol with saline, atenolol or ICI 118.551. As mentioned in Chapter 5.6 an antagonist or saline was infused just prior to t=0 min. at a constant dosage. The agonist was infused in incremental dosages. The various pharmaca and saline were infused according to a randomly assigned protocol. The volumes infused did not exceed the volumes mentioned in Chapter 4.

In figure 5.7.1, 5.7.2 and 5.7.3 results are shown which were obtained during infusion of the β_2-adrenoceptor agonist salbutamol (dose 0.6, 1.8 and 6.0 microgram kg^{-1} min^{-1}) in combination with saline, atenolol or ICI 118.551.
In the experiments with simultaneous infusion of salbutamol and saline flow did not alter (12.9 ± 0.6 ml min^{-1} resp. 12.9 ± 0.4 ml min^{-1}, N.S.). In the salbutamol-atenolol series flow fell significantly from 12.6 ± 0.6 ml min^{-1} to 10.6 ± 0.6 ml min^{-1} ($p<0.02$). Salbutamol infusion preceded by ICI 118.551 affected flow not significantly (12.5 ± 1.0 ml min^{-1} resp. 11.8 ± 1.0 ml min^{-1}, N.S.) (fig. 5.7.1).
Evaluation by analysis of variance of percentual changes in renal vascular resistance (fig. 5.7.2) demonstrated that differences between salbutamol-saline and salbutamol-atenolol are significant ($p<0.005$) as well as differences between salbutamol-saline and salbutamol-ICI 118.551 ($p<0.05$). Furthermore, significance is obtained when results of salbutamol-atenolol and salbutamol-ICI 118 551 infusions are compared ($p<0.01$).
Analysis of renin secretion rate patterns revealed that salbutamol-saline infusion causes a dose dependent tendency to raise renin secretion (t=0 min.: 2330 ± 359 SU; t=6 min.: 2310 ± 295 SU; t=10 min.: 2584 ± 416 SU; t=14 min.: 3046 ± 655 SU) without significant differences between t=0 min. and t=14 min.

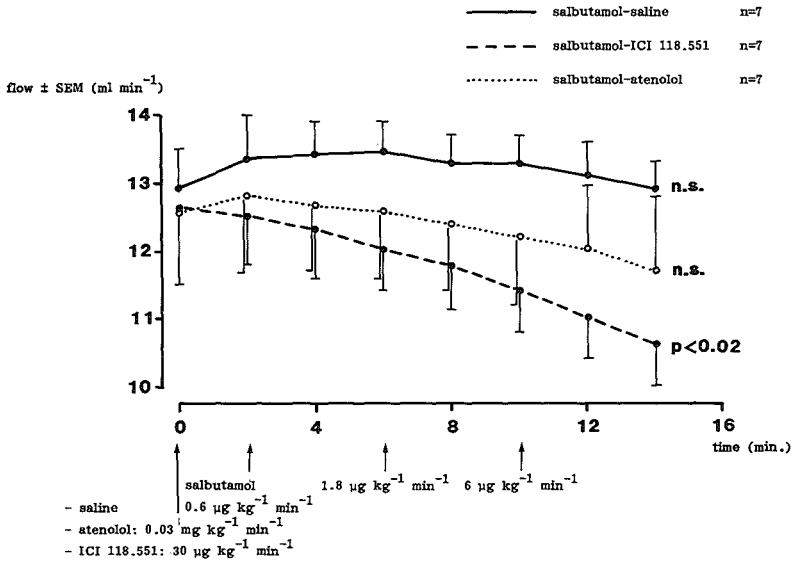


Fig. 5.7.1: The effects of infusion of incremental doses of salbutamol on flow. Prior to infusion of salbutamol $\beta_{1,2}$-antagonist- or saline infusion was started.

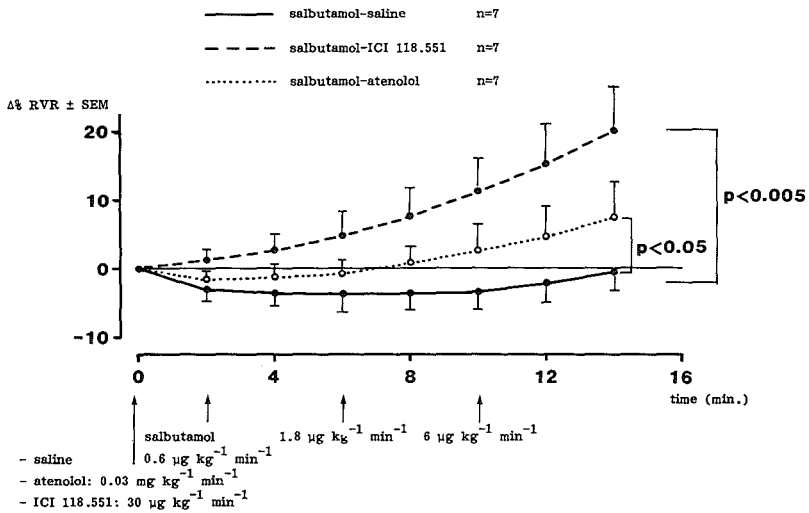


Fig. 5.7.2: The effects of infusion of incremental doses of salbutamol on percentual changes in RVR. Prior to infusion of salbutamol $\beta_{1,2}$-antagonist- or saline infusion was started.

Salbutamol infusion preceded by atenolol caused a relatively constant renin secretion at lower levels (t=0 min.: 1856 ± 210 SU; t=14 min.: 1734 ± 298 SU, N.S.) in comparison with both salbutamol-saline and salbutamol-ICI 118.551.

The latter combination raised RSR from 2537 ± 362 SU to 3280 ± 587 SU (N.S.), although the differences after t=10 min. and t=14 min. being significantly ($p<0.05$).

Two-way analysis of variance (fig. 5.7.3) showed that percentual alterations in renin secretion rate between salbutamol-saline and salbutamol-atenolol were just significantly different ($p<0.05$), similar findings are obtained when salbutamol-ICI 118.551 and salbutamol-atenolol are analysed ($p<0.05$).

Non-significant differences in patterns during salbutamol-saline and salbutamol-ICI 118.551 infusion are observed.

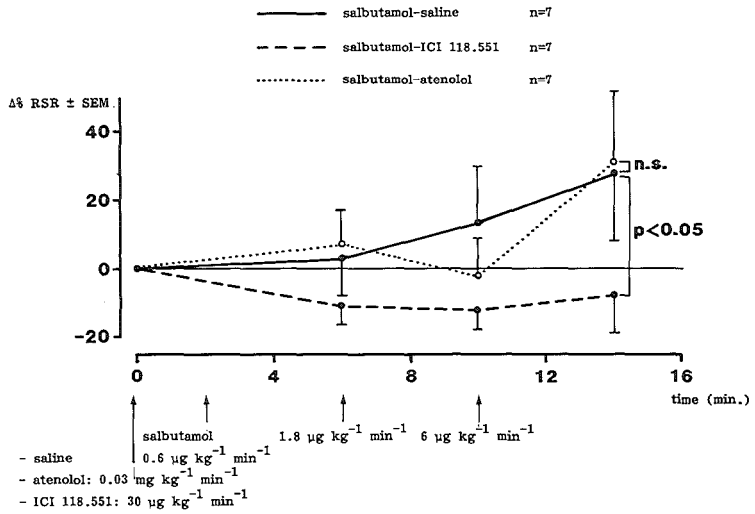


Fig. 5.7.3: The effects of infusion of incremental doses of salbutamol on percentual changes in RSR. Prior to infusion of salbutamol $\beta_{1,2}$-antagonist- or saline infusion was started.

CHAPTER 6

Discussion.

6.1 Discussion with respect to dose-response curves of $beta_{1,2}$-agonists and $beta_{1,2}$-antogonists.

As outlined in Chapter 5.2 prenalterol (dose 3 microgram kg^{-1} min^{-1}) does not influence renal flow or renin secretion when compared with saline infusion. The possibility remains that the dose used was not appropriate and/or access to the receptor was prohibited. Comparison of these results with data obtained by others is difficult because those were not based on prenalterol being infused into the renal artery.

Meurer et al (1980) infused prenalterol intravenously and found that heart rate and blood pressure increased, while renin secretion remained constant. These data were supported by Staessen et al (1983). In anaesthetized dogs with innervated or denervated kidneys Kopp et al (1981[a]) demonstrated that prenalterol (20 microgram kg^{-1} min^{-1} i.v.) stimulated renin secretion without affecting renal haemodynamics or mean arterial pressure.

Salbutamol, infused at the rate of 0.6 microgram kg^{-1} min^{-1} into the renal artery, exhibited no effect on renal flow. A short lasting (non-significant) decrease in renin secretion was observed. At a dose of 1.8 microgram kg^{-1} min^{-1} a slight but in comparison with control experiments significantly different behaviour in RVR pattern was observed. In addition, renin secretion was raised during the infusion although with some delay. These data point to a dose related stimulatory effect of salbutamol on renin secretion which is perhaps dependent on vasodilatation.

Davis et al (1975) presented data in which they achieved after salbutamol no increments in renin release, Wilton and Joshi (1977) were unable in rat kidney slices to stimulate renin secretion after adding salbutamol to the medium. Johns et al (1975) described opposite results in isolated rat cortical cells.

Johnson et al (1976), Meurer et al (1976) and Salvetti et al (1978)

obtained evidence that salbutamol and fenoterol could stimulate renin secretion after intravenous administration in human beings. Johnson et al observed a dose related increase in systolic blood pressure and decrease in diastolic blood pressure; Salvetti et al observed decreased diastolic blood pressure and unaltered systolic blood pressure. Nakane et al (1980) described after salbutamol infusion increments in renin secretion in the isolated perfused rat kidney.

Atenolol (0.03 mg kg^{-1} min^{-1}) caused a modest but significant vasodilatation in comparison with saline infusion. In addition, renin secretion rate was significantly reduced. This supports the presumption that in the isolated rat kidney adrenergic activity influences vascular tone even under basal conditions. After inhibition of β_1-adrenoceptor activity renin secretion is suppressed and vasodilatation ensues. In the literature abundant data are presented in that atenolol and other β_1-antagonists suppress renin secretion (Buhler et al, 1975; Johnson et al, 1976; Meurer et al, 1976; Desaulles et al, 1978; Salvetti et al, 1978; Distler et al, 1978; Winer et al, 1979; Nakane et al, 1980; Johns et al, 1985; Man in 't Veld and Schalekamp, 1984; Vanhees et al, 1985).

ICI 118.551 (30 microgram kg^{-1} min^{-1}) is 200 times more potent at vascular β_2-adrenoceptors than cardiac β_1-adrenoceptors (Bilski et al, 1980). In our experiments vasodilatation was observed in comparison with saline infusion. A concomitant transient tendency towards increased renin secretion was noticed, but this effect was not significant. Different explanations can be thought off. For instance, preferential presynaptic β_2-adrenoceptor blockade with inhibition of spontaneous noradrenaline release might be expected, eventhough insufficient selectivity towards both β-adrenoceptors at this dose might be present. In the light of our results a satisfactory explanation is, however, lacking.
Johns et al (1981) described that ICI 118.551 did not prevent renal nerve-stimulated and isoprenaline-induced renin secretion, whereas ICI 118.551 antagonized vascular responses.

6.2 Discussion with respect to combination experiments with beta$_1$-agonist and $\beta_{1,2}$-antagonists.

In Chapter 5.6 data have been presented with respect to simultaneous infusion of prenalterol with saline, atenolol or ICI 118.551.
These data again provide evidence that in rats β_1-adrenoceptors mediate renin secretion. Infusion of atenolol prevented the renin secretion provoked by the highest dose of prenalterol. Since no significant alterations in flow and resistance were found, renin suppression probably occurred at the level of the β_1-adrenoceptor at the juxtaglomerular cell. Although distinct inhibitory effects of atenolol on the resting β_1-adrenoceptor are conceivable, it seems likely that atenolol exhibits a more marked influence on the activated β_1-adrenoceptors.
Whereas the absolute differences in renin secretion rate did not attain significance during incremental doses of prenalterol, it can be noticed that after 30 microgram kg^{-1} min^{-1} increased renin secretion is obtained. When we bear in mind the usually observed decrease in renin secretion of approximately -25% in control experiments and we compare this with + 6.9 ± 11.7% at t=14 min., this suggests that prenalterol at a dose of 30 microgram kg^{-1} min^{-1} stimulates the β_1-adrenoceptor at the juxtaglomerular cell. ICI 118.551 did neither alter renin secretion nor flow in comparison with the prenalterol-saline experiments. Our data are in agreement with Kopp et al (1981). They described in anaesthetized dogs, that prenalterol increased RSR at a dose of 20 microgram kg^{-1} min^{-1} intravenously without alterations in renal haemodynamics and mean arterial pressure. The changes in renin secretion rate were not dependent on renal innervation.
Osborn et al (1983) demonstrated that atenolol 0.5 microgram kg^{-1} min^{-1} attenuated renin secretion during 1.0 Hz renal nerve stimulation, whereas 5.0 microgram kg^{-1} min^{-1} atenolol abolished RNS renin secretion. In 1981 Osborn et al published similar data (2.0 microgram kg^{-1} min^{-1} inhibited 0.5 Hz renal nerve stimulated renin secretion).
Di Bona (1985) summarized that the direct neural stimulus to release renine from juxtaglomerular cells is dependent on renal β_1-adrenoceptors, at least at normal perfusion pressure.

6.3 Discussion with respect to combination experiments with beta$_2$-agonist and beta$_{1,2}$-antagonists

In Chapter 5.7 results have been presented with respect to simultaneous infusion of salbutamol with saline, atenolol or ICI 118.551. In these experiments salbutamol stimulated renin secretion to a lesser degree in comparison with our earlier experiments. Atenolol infusion prior to and simultaneously with salbutamol prevented increments in RSR elicited by salbutamol. After 14 min. renin secretion even tended to rise in these series. Vasodilatation was not observed.
In our hands ICI 118.551 slightly reduced vasodilatation in comparison with salbutamol-NaCl infusion, but no significant influence on renin secretion was observed.

How could these data be explained? In the salbutamol-saline series salbutamol at a low dose (0.6 microgram kg^{-1} min^{-1}) provoked no vasoactive effect. At higher dosages modest vasodilatation was observed with a delayed increase in renin secretion rate. Thus, renin secretion may be dependent on vasodilatation. This view would be supported by salbutamol-atenolol experiments in which vasodilatation was attenuated together with renin secretion. It remains obscure why in the latter experiments vasodilatation was inhibited. This might be explained with the assumption that presynaptic β_2-adrenoceptors are involved. When we assume that salbutamol facilitates neurotransmitter release from the renal sympathetic nerve endings renin secretion might be stimulated by activation of β_1-adrenoceptors at the juxtaglomerular cells. In salbutamol-atenolol experiments this stimulation of β_1-adrenoceptors is prevented by atenolol. In the latter experiments activation of postsynaptic α-adrenoceptors may occur which counterbalances β_2-adrenoceptor mediated vasodilatation though insufficient selectivity to either β-receptor subtype is not excluded. In experiments with salbutamol-ICI 118.551 a slight, but significant reduction in vasodilatation was obtained; this could be the result of antagonizing effects at the vascular β_2-adrenoceptor. In this series renin secretion was comparable with salbutamol-saline infusions. When the presumption is warranted that salbutamol provokes renin secretion via presynaptic β_2-adrenoceptors, then this implies that ICI 118.551

at the dose range we used, does not provide sufficient antagonistic activities at the presynaptic β_2-adrenoceptor; otherwise, these results would support the view that β_1-adrenoceptors are preferentially involved in renin secretion.

In view of these data it remains questionable whether vasodilatation or the presumed release of noradrenaline via stimulated presynaptic β_2-adrenoceptors, or both, causes renin release.

The literature is far from unanimous. Johns et al (1975) described in isolated renal cortical cells that salbutamol stimulated renin release, salbutamol being about one third as potent as isoprenaline. Meurer et al (1976), Johnson et al (1976) and Salvetti et al (1978) found that fenoterol or salbutamol enhanced RSR, which phenomenon was inhibited by β_1-adrenoceptor antagonists.

Nakane et al (1980) demonstrated in the isolated rat kidney that salbutamol stimulated renin secretion without consistent changes in haemodynamics. Renin secretion was suppressed during propranolol and acebutolol. These data support our results in the sense that salbutamol stimulates renin secretion, but others obtained opposite results (Davis et al, 1975; Wilton and Joshi, 1977).

Our presumption that salbutamol would stimulate presynaptic β_2-adrenoceptors thereby enhancing noradrenaline release, is based on the findings of Vincent et al (1983) and Schalekamp et al (1984). In their experiments salbutamol increased plasma noradrenaline levels whilst mean arterial pressure decreased modestly (-4 to -8 mmHg). Whether such mechanism is involved in our results remains unproven, in our experiments we could not prevent salbutamol induced renin secretion by infusing ICI 118.551, although vasodilatation was attenuated as described by Johns et al (1981). In their experiments renin secretion induced by isoprenaline was not counteracted by ICI 118.551, whereas the vascular effects were antagonized. Otherwise, Brown et al (1983) decribed in man that adrenaline infusion enhanced renin secretion and this effect was partially inhibited by ICI 118.551. In this study plasma noradrenaline was slightly reduced in comparison with controls, which is in favour of a presynaptic effect. Furthermore, Vincent et al (1985) demonstrated in a placebo controlled cross-over trial in 9 hypertensives that isoprenaline-induced noradrenaline release was abolished by ICI 118.551. In a long term study

ICI 118.551 reduced heart rate, systolic and diastolic blood pressure, and plasma renin activity. Thus, in the literature some evidence is obtained that ICI 118.551 indeed can diminish presynaptic β_2-adrenoceptor mediated noradrenaline release (Schmidt et al, 1984).
As mentioned before, we were unable to detect evidence for this attractive hypothesis. The reason is unclear. It may be possible that the dose range chosen was not appropriate or that access to the receptor was prohibited. In addition, differences in affinity to pre- and postsynaptic receptors and species differences may be involved.
Morphological data in the literature do not support our speculations since β_2-adrenoceptors were not found to be associated with blood vessels (Summers and Kular, 1983; Summers et al, 1983; Struyker-Boudier et al, 1986), whilst the number of presynaptic β_2-adrenoceptors appears to be low (Snavely et al, 1982; Munzel et al, 1984).

6.4 Conclusions.

1. Our data again indicate that in the isolated perfused rat kidney prenalterol stimulates renin secretion by a direct action on the β_1-adrenoceptor at the juxtaglomerular cells without vascular effects.

2. Salbutamol causes a dose-related increase in renin secretion concomitant with slight vasodilatation. Possibly vasodilatation is achieved by activation of postsynaptic β_2-adrenoceptors, since ICI 118.551 attenuated this effect, but renin secretion is not affected by ICI 118-551. This implies that an additional mechanism, e.g. presynaptic β_2-adrenoceptor mediated noradrenaline release, may be involved. This mediator may then stimulate postsynaptic β_1-adrenoceptors and thus renin secretion. ICI 118.551 did not prohibit this presumably presynaptic effect.

CHAPTER 7

Influence of alpha-adrenoceptor agonists and antagonists on renal flow and renin secretion.

7.1 Introduction

In Chapter 3 it was described that in rat kidney both α_1- and α_2-adrenoceptor subtypes have been demonstrated. There exists still controversy on which subtype of α-adrenoceptor is involved in renin secretion, with or without simultaneous vasoactive responses. Therefore experiments were carried out with relatively selective α_1- and α_2-agonists and antagonists in order to clarify this issue. Dose-response curves were obtained with all drugs in order to establish that dose of an agonist or antagonist which produced a non-vasoactive versus a vasoactive response. Thereafter, a separate series was carried out with all pharmaca at the non-vasoactive dose during the entire perfusion period, because than the impact of vasoactive responses in the other series could be elucidated.
Next, experiments were carried out with α-agonists at incremental doses combined with previously started infusion of either of the α-antagonists. Because of the variability of our model control experiments with saline were performed. In the combination experiments saline or α-antagonist infusion was given according to a randomly assigned protocol.

7.2 Experiments with alpha$_1$-adrenoceptor agonist.

Initially methoxamine was chosen as α_1-agonist, but at a dose up to 1000 ug kg^{-1} min^{-1} no vasoconstrictory response could be established. We decided to continue our experiments with cirazoline, a potent α_1-agonist (Van Meel et al, 1980, 1981; Flavahan et al, 1984; Beckeringh et al, 1984; Ruffolo et al, 1984; Langer et al, 1985; Timmermans et al, 1985; Wolff et al, 1985). In pithed rats Van Meel et al (1981) estimated cirazoline to be 6 times more potent than phenylephrine and 38 times more effective than methoxamine with respect to increases in diastolic blood pressure after intravenous administration.

At first dose response curves were established in the anaesthetized rat during infusion into the jugular vein. At a dose of 30 ugr kg^{-1} min^{-1} a fair vasoconstrictor response was established. Next dose-response curves in the isolated perfused rat kidney were carried out. Dosages varied from 30 to 300 ugr kg^{-1} min^{-1}; in the majority at a dose of 100 ugr kg^{-1} min^{-1} an appreciable decrease in flow was recorded. Data are shown in figure 7.2.1 and 7.2.2. The changes in RVR produced by cirazoline infusion are significantly different as compared with saline infusion. Eventhough percentual changes in RSR did show a significant difference between the groups. Thereafter, perfusion experiments during the entire perfusion period were done at a dose of 30 ugr kg^{-1} min^{-1} (n=5). Date are depicted in figure 7.2.3 and 7.2.4.

Flow was unchanged from 11.0 ± 0.5 ml min^{-1} to 11.0 ± 0.6 ml min^{-1} (N.S.), control experiments with saline (n=5) revealed a comparable curve (10.8 ± 0.8 ml min^{-1} vs 10.9 ± 0.8 ml min^{-1} (N.S.). Changes in renal vascular resistance are shown in figure 7.2.3; a significant difference was not obtained. Renin secretion rate declined with time in both groups; during cirazoline infusion RSR fell from 2137 ± 280 SU (t=0 min) to 792 ± 122 SU (t=16 min) ($p<0.01$), in saline experiments RSR decreased from 2287 ± 265 SU to 940 ± 201 SU ($p<0.01$). Percentual alterations in RSR are depicted in figure 7.2.4. Two way variance analysis assessed no significant difference. Obviously, a more prominent decrease in RSR in the control experiments was obtained as compared with previous experiments.

7.3 Experiments with alpha$_2$-adrenoceptor agonist.

Clonidine is well known as an α_2-adrenoceptor agonist, but with partial α_1-agonism (Sullivan and Drew, 1980; Schmitz et al, 1981; Hoffman et al, 1982; Hamilton et al, 1982; Man in 't Veld et al, 1983; Wolff et al, 1984; Beckeringh et al, 1984; Jie et al, 1984; Godfraind et al, 1985; Hamilton et al, 1985). We intended, by using clonidine, to clarify the by α_2-adrenoceptors mediated vasoconstrictory and renin releasing properties.

Initially dose response curves were determined via infusion into the jugular vein. At a dose of 3 ugr kg^{-1} min^{-1} blood pressure fell; at a

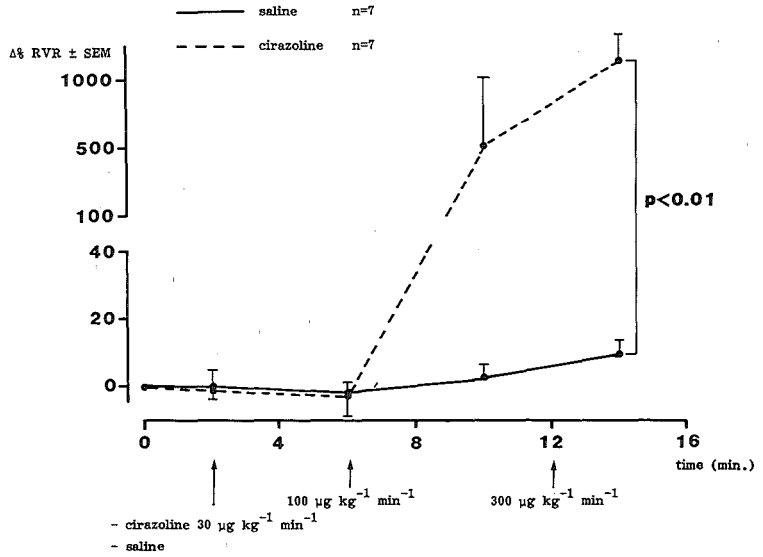


Fig. 7.2.1: The effects of infusion of incremental doses of cirazoline or saline on percentual changes in RVR.

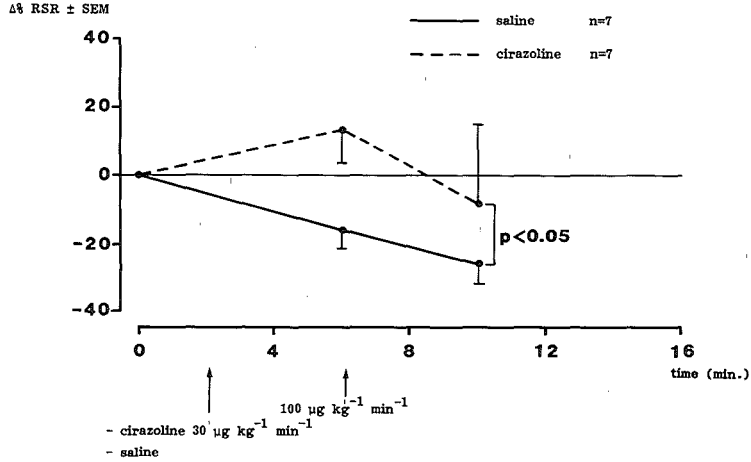


Fig. 7.2.2: The effects of infusion of incremental doses of cirazoline or saline on percentual changes in RSR.

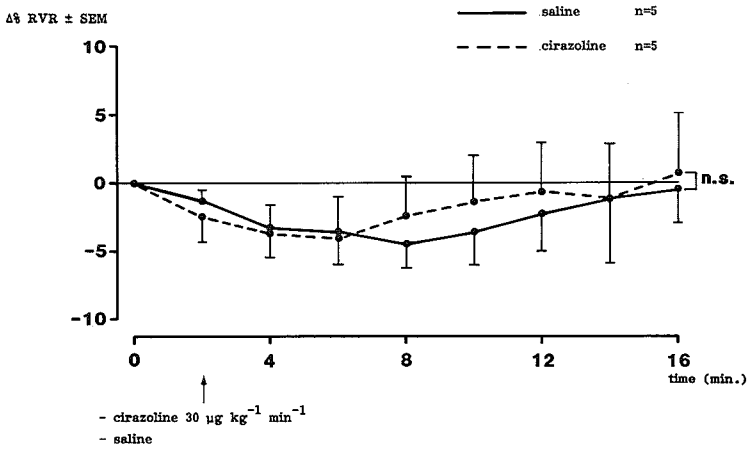


Fig. 7.2.3: The effects of infusion of cirazoline or saline on percentual changes in RVR.

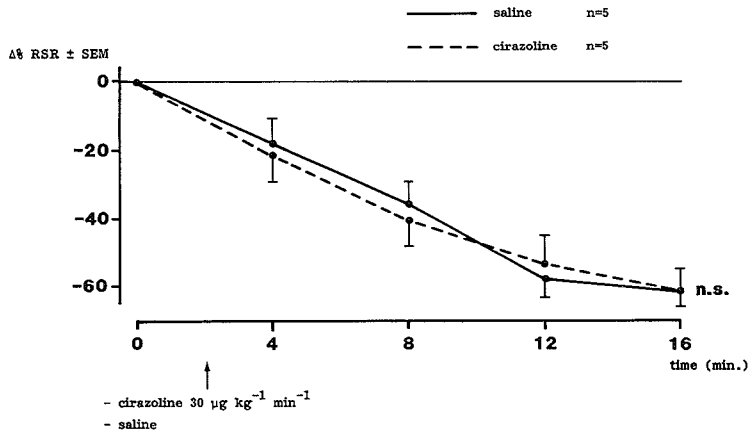


Fig. 7.2.4: The effects of infusion of cirazoline or saline on percentual changes in RSR.

dose of 30 ugr kg^{-1} min^{-1} blood pressure rose. Intrarenal infusion of clonidine was carried out with dosages ranging between 1.0 and 10 ugr kg^{-1} min^{-1}. Data are shown in figure 7.3.1 and 7.3.2. The changes in RVR produced by clonidine infusion are significantly different as compared with saline infusion. Evaluation of the percentual changes in RSR revealed a significant difference between the groups. Next experiments were performed with an apparently non vasoactive dose, which dose was assessed at 1.0 ugr kg^{-1} min^{-1} (n=5). Data are presented in figure 7.3.3 and 7.3.4.

Flow slightly increased from 10.3 ± 1.3 ml min^{-1} to 11.4 ± 1.3 ml min^{-1} (N.S.), in control experiments (n=5) a decrease in flow was obtained (11.2 ± 0.2 ml min^{-1} vs 10.6 ± 1.0 ml min^{-1} N.S.).

Percentual changes in renal vascular resistance are presented in figure 7.3.3. Significant differences were noticed ($p<0.005$), marking a fall in RVR during clonidine.

Renin secretion rate fell during clonidine from 956 ± 177 SU to 870 ± 218 SU. In control experiments comparable changes were obtained (from 1155 ± 177 SU to 1051 ± 57 SU, N.S.). Percentual alterations in RSR are depicted in figure 7.3.4, the difference was not significant.

A further series of experiments was carried out at a higher dose (10 ugr kg^{-1} min^{-1}, n=4). Data are shown in figure 7.3.5 and 7.3.6. An obvious vasoconstriction was now observed. Flow decreased from 11.3 ± 0.2 ml min^{-1} to 5.7 ± 0.5 ml min^{-1} ($p<0.001$). Saline infusion (n=4) caused a decrease from 11.4 ± 1.1 ml min^{-1} to 10.2 ± 1.0 ml min^{-1} (N.S.). Percentual changes in RVR are presented in figure 7.3.5, and exhibited a significant difference ($p<0.005$). Data on RSR show that during clonidine infusion RSR increased (517 ± 20 SU vs 1124 ± 346 SU, N.S.), in control experiments RSR declined from 723 ± 219 SU to 543 ± 83 SU (N.S.). Because of marked vasoconstriction RSR increments are partly tapered. Absolute renin values were in clonidine experiments 46 ± 3 ngA_I, ml^{-1} $hour^{-1}$ at t=0 min and 189 ± 59 ngA_I ml^{-1} $hour^{-1}$ at t=16 min., in comparison with 66 ± 24 ngA_I ml^{-1} $hour^{-1}$ resp. 55 ± 11 ngA_I ml^{-1} $hour^{-1}$ during saline infusion. Percentual changes in RSR are shown in figure 7.3.6. During clonidine infusion significant different values were obtained ($p<0.05$).

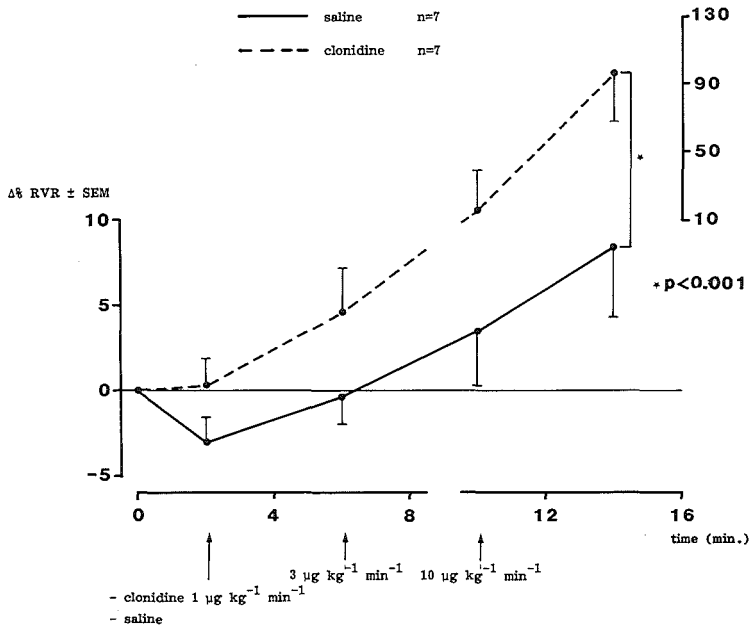


Fig. 7.3.1: The effects of infusion of incremental doses of clonidine or saline on percentual changes in RVR.

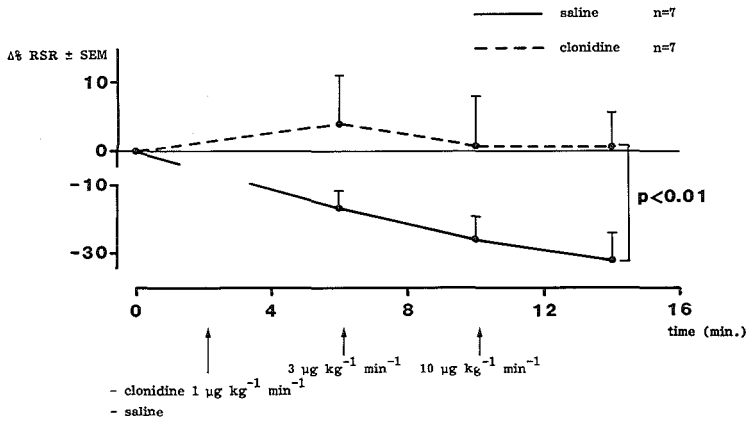


Fig. 7.3.2: The effects of infusion of incremental doses of clonidine or saline on percentual changes in RSR.

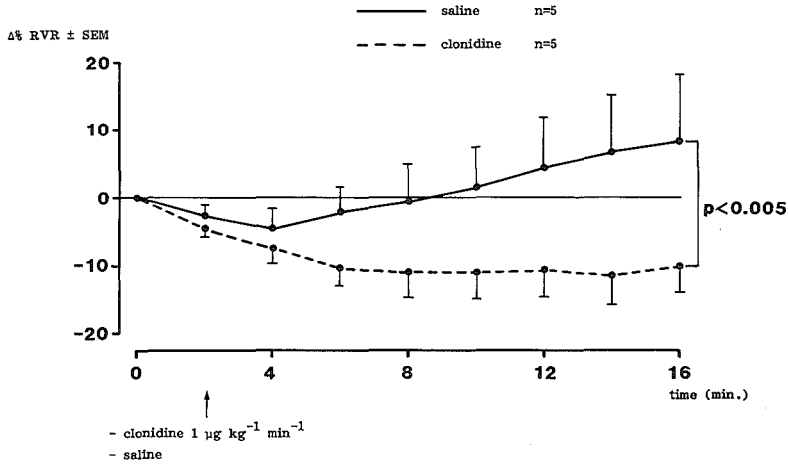


Fig. 7.3.3: The effects of infusion of clonidine or saline on percentual changes in RVR.

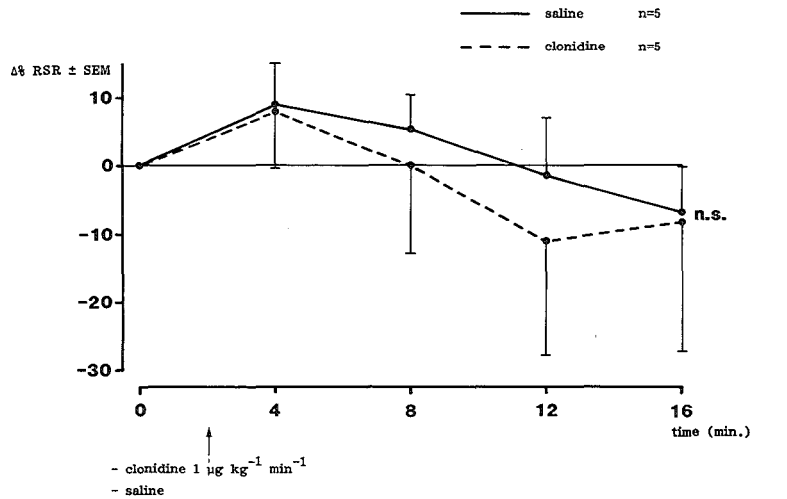


Fig. 7.3.4: The effects of infusion of clonidine or saline on percentual changes in RSR.

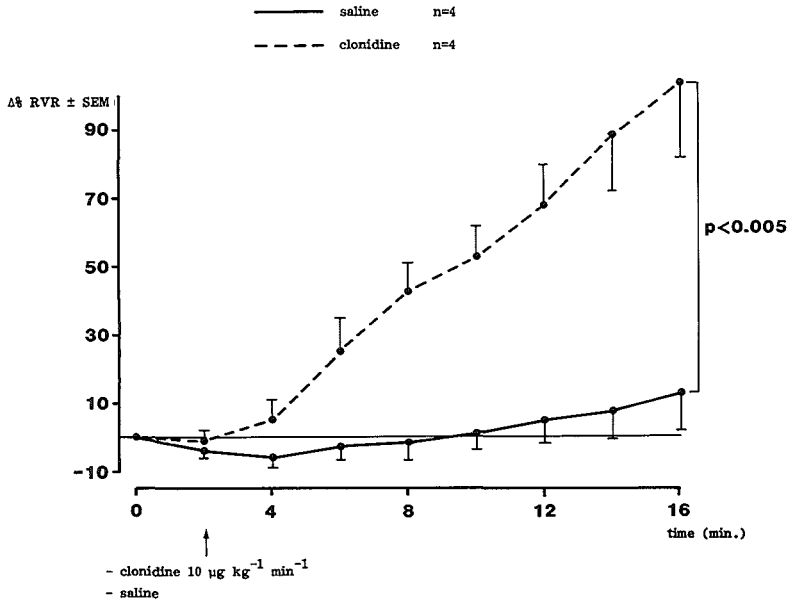


Fig. 7.3.5: The effects of infusion of clonidine or saline on percentual changes in RVR.

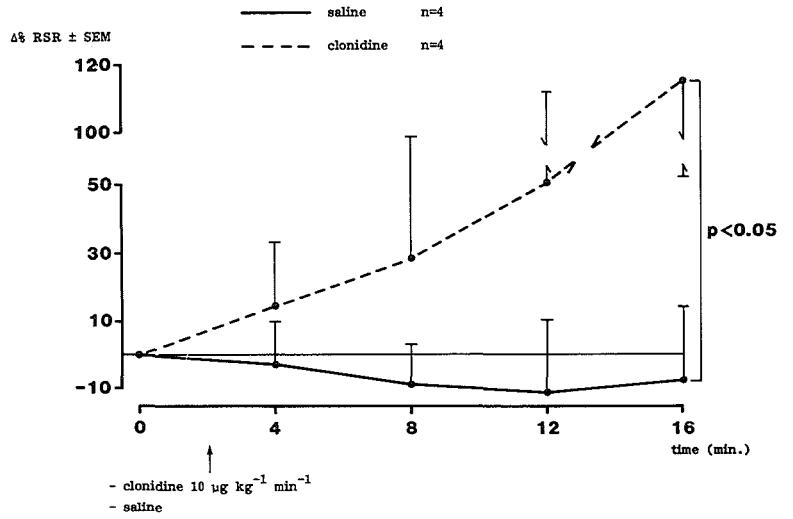


Fig. 7.3.6: The effects of infusion of clonidine or saline on percentual changes in RSR.

7.4 Experiments with alpha$_1$-adrenoceptor antagonist.

The next series of experiments was performed using doxazosin. Doxazosin, a quinazoline derivate, is accepted as a relatively selective α_1-antagonist (de Leeuw et al, 1982, 1986[a,b]; Vincent et al, 1983; Van Brummelen et al, 1983[a]; Meredith et al, 1985).
As in previous experiments, dose response curves were obtained in the anaesthetized rat. Doses examined were 0.03, 0.1, 0.3 and 1.0 ugr kg^{-1} min^{-1}. At the highest dose a slight reduction in MAP was observed. In experiments performed in the isolated rat kidney vasodilatation was not established after infusing doses ranging from 0.1 to 1.0 ugr kg^{-1} min^{-1}.
We decided to carry out experiments with doxazosin at a dose of 1.0 ugr kg^{-1} min^{-1} (n=5).
Results of these experiments are presented in figure 7.4.1 and 7.4.2. During doxazosin infusion flow decreased from 11.0 ± 0.3 ml min^{-1} to 9.8 ± 9.3 1.0 ml min^{-1} (N.S.) as in the saline experiments (n=5) (11.0 ± 0.4 ml min^{-1} vs 9.3 ± 1.0 ml min^{-1}, N.S.).
Changes in RVR were non-significant (fig. 7.4.1). Renin secretion rate declined after doxazosin from 1547 ± 179 SU to 1317 ± 144 SU (N.S.), after saline infusion RSR decreased from 1441 ± 211 SU to 944 ± 92 SU ($p<0.05$). Comparison of percentual changes in RSR revealed non-significant differences (fig. 7.4.2).

7.5 Experiments with alpha$_2$-adrenoceptor antagonist.

Yohimbine is regarded as a relatively selective α_2-antagonist, although like in other α_2-antagonistic drugs dose-dependent α_1-antagonism is observed (Timmermans et al, 1980[b], 1981[b]; Sullivan and Drew, 1980; Graham et al, 1980; Shepperzon et al, 1981; Ruffolo et al, 1981; Kobinger and Pichler, 1981; Van Meel et al, 1981; Hamilton and Reid, 1982; Hamilton et al, 1982; Constantine et al, 1982; Hoffman and Lefkowitz, 1982; Ruffolo and Yadin, 1984; Goldberg and Robertson, 1984; Beckeringh et al, 1984; Jie et al, 1984, 1986). During infusion into the jugular vein at a dose of 1.0 mg kg^{-1} min^{-1} a vasoconstrictory response was obtained. Thereafter dose response curves were assessed in the isolated rat kidney, dosages ranging between 0.03 mg

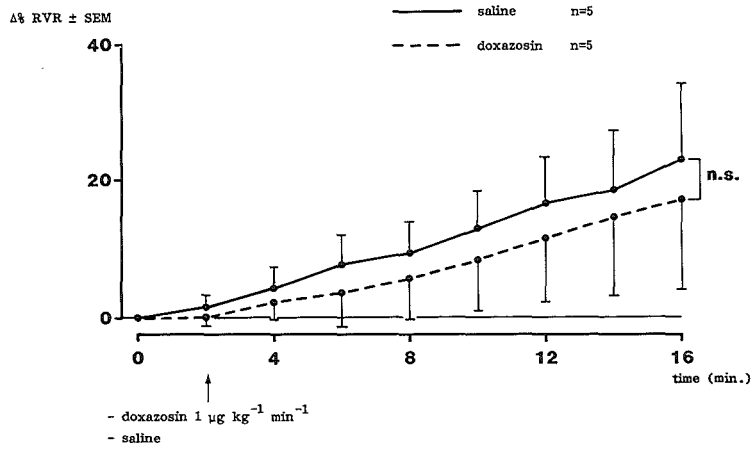


Fig. 7.4.1: The effects of infusion of doxazosin or saline on percentual changes in RVR.

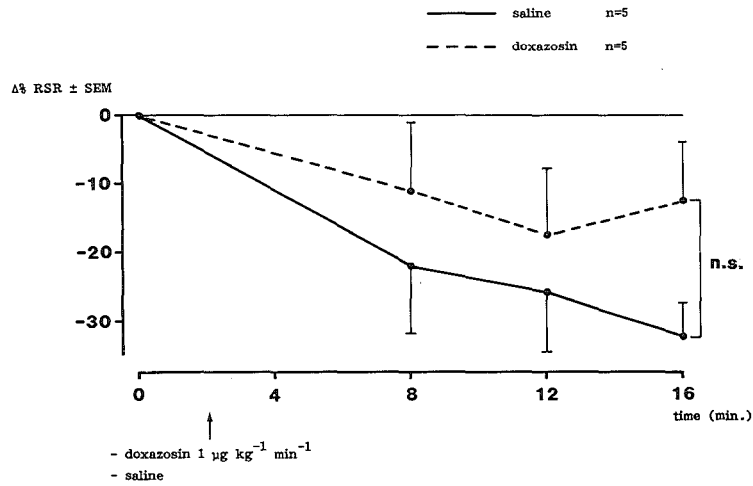


Fig. 7.4.2: The effects of infusion of doxazosin or saline on percentual changes in RSR.

kg^{-1} min^{-1} and 1.0 mg kg^{-1} min^{-1}. With the use of doses up to 0.3 mg kg^{-1} min^{-1} no vasoactive response was observed. Experiments (n=6) were carried out with the latter dose. Results are presented in figure 7.5.1 and 7.5.2. Flow increased from 10.7 ± 0.8 ml min^{-1} to 11.7 ± 0.4 ml min^{-1} (N.S.), as in control experiments (11.2 ± 0.5 ml min^{-1} resp. 12.1 ± 0.4 ml min^{-1}, N.S., n=6). Percentual changes in RVR are depicted in figure 7.5.1, two-way variance analyses did not reveal a significant difference. Renin secretion rate exhibited in both groups a comparable pattern; yohimbine series: 1107 ± 104 SU resp. 815 ± 66 SU, N.S., saline series: 1361 ± 104 SU resp. 1035 ± 184 SU, N.S.

Comparison of percentual alterations in RSR showed no statistically significant difference (fig. 7.5.2).

7.6 Experiments with alpha$_1$-agonist versus alpha$_{1,2}$-antagonists.

After establishing the effects of cirazoline, doxazosin and yohimbine in the isolated kidney, we performed a series of experiments with combined infusion of these pharmaca. In these experiments either an antagonist or saline was infused just before t=0 min. at a constant dose as mentioned before (Chapter 7.4 and 7.5). By contrast, the α_1-agonist was infused in incremental dosages with increases at 2, 6 and 12 minutes. All groups comprised 7 animals, the various pharmaca as well as the saline were infused according to a random order. The volumes of infused fluids were equal and did not exceed the volume mentioned in Chapter 4.

In experiments performed with cirazoline, this drug was infused at doses of 30, 100 and 300 ugr kg^{-1} min^{-1}. Data are shown in figure 7.6.1, 7.6.2 and 7.6.3.

In experiments with administration of cirazoline and saline, flow decreased from 11.1 ± 0.8 ml min^{-1} to 1.7 ± 0.7 ml min^{-1} ($p<0.001$). Cirazoline proved to be very potent at higher doses since in some experiments flow almost ceased.

After preceding infusion of doxazosin cirazoline likewise elicited a clear vasoconstrictory response (11.84 ± 0.59 ml min^{-1} resp. 3.0 ± 1.0 ml min^{-1}, $p<0.001$). When yohimbine was administered previously

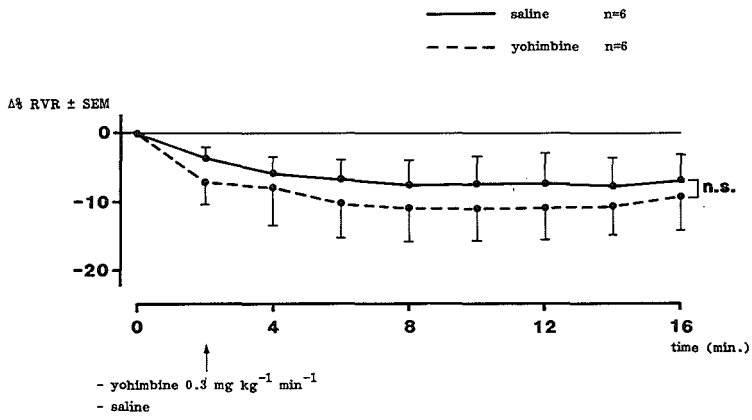


Fig. 7.5.1: The effects of infusion of yohimbine or saline on percentual changes in RVR.

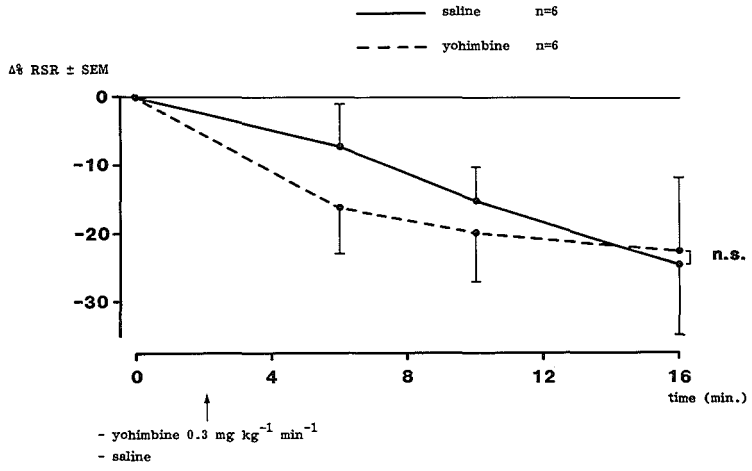


Fig. 7.5.2: The effects of infusion of yohimbine or saline on percentual changes in RSR.

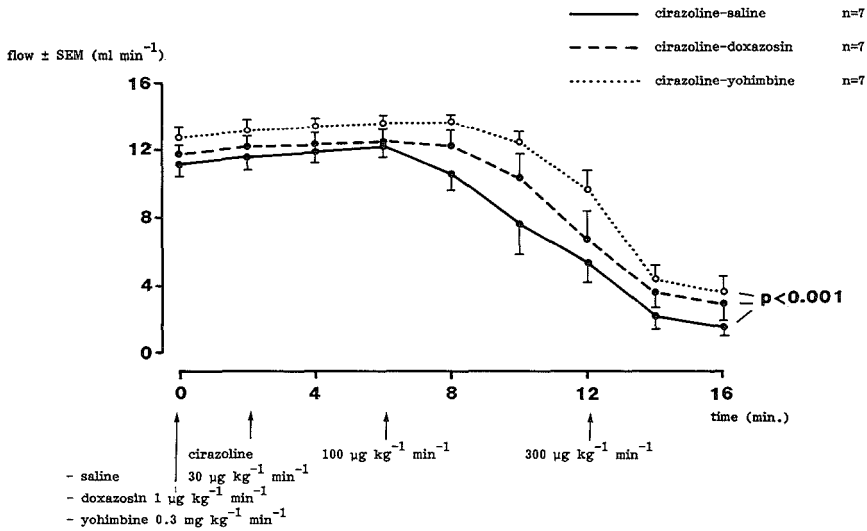


Fig. 7.6.1: The effects of infusion of incremental doses of cirazoline on flow. Prior to infusion of cirazoline $\alpha_{1,2}$-antagonist- or saline infusion was started.

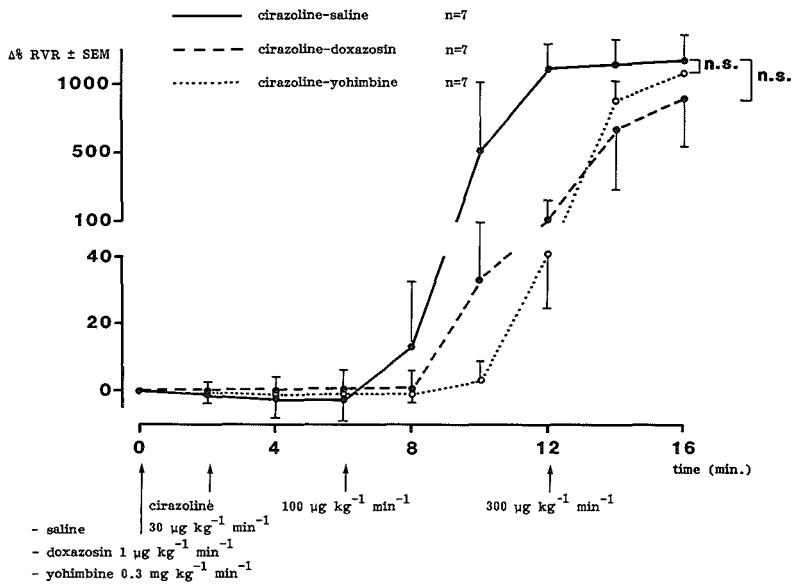


Fig. 7.6.2: The effects of infusion of incremental doses of cirazoline on percentual changes in RVR. Prior to infusion of cirazoline $\alpha_{1,2}$-antagonist- or saline infusion was started.

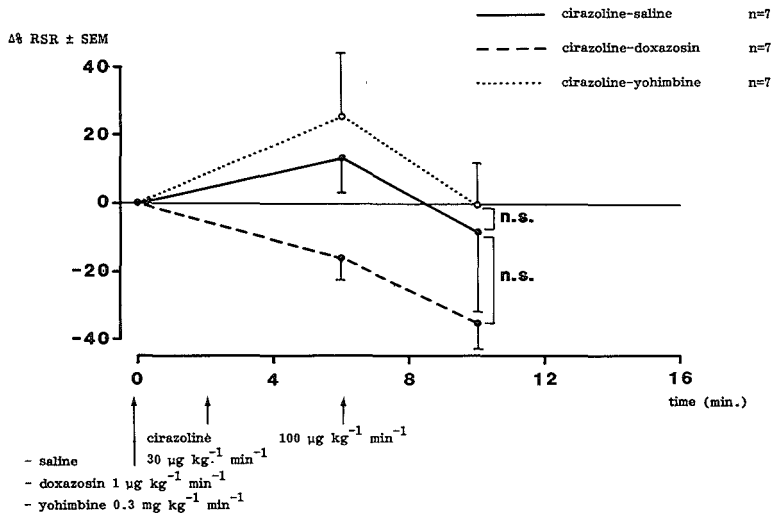


Fig. 7.6.3: The effects of infusion of incremental doses of cirazoline on percentual changes in RSR. Prior to infusion of cirazoline $\alpha_{1,2}$-antagonist- or saline infusion was started.

the flow curve shifted slightly rightwards, flow declining from 13.1 ± 0.5 ml min^{-1} to 3.6 ± 1.0 ml min^{-1} ($p<0.001$).

Although during doxazosin and yohimbine flow curves were displaced rightwards, percentual changes in RVR, while showing the expected findings, were not statistically significant different from each other (fig. 7.6.2).

Because of the substantial vasoconstrictory response during high-dose cirazoline infusion absolute renin patterns are given. These were as follows: during cirazoline-saline and cirazoline-yohimbine a non-significant difference between t=0 min. and t=10 min. was established, during cirazoline-doxazosin renin decreased significantly (171 ± 20 ngA ml^{-1} $hour^{-1}$ resp. 134 ± 22 ngA ml^{-1} $hour^{-1}$, $p<0.01$), renin values after 16 minutes could not be estimated because of inadequate perfusion fluid volumes in several experiments.

Renin secretion rates were as follows: in the cirazoline-saline series RSR was unchanged: 2191 ± 297 SU resp. 2155 ± 601 SU (N.S.), during cirazoline and doxazosin RSR fell from 1998 ± 240 SU to 1320 ± 263 SU ($p<0.01$), in the cirazoline-yohimbine experiments non-significant differences were observed (t=0 min: 1770 ± 191 SU vs 1693 ± 221 SU after 10 minutes).

Percentual changes in RSR were as follows (figure 7.6.3): cirazoline on top of doxazosin administration reduced percentual changes in RSR to -35.0 ± 8.5%; in the cirazoline-saline experiments a slight increase after 6 min. was established, whilst after 10 minutes RSR declined to 9.2 ± 22.8%. Statistical evaluation revealed non-significant differences between the groups.

Previous and simultaneous infusion of yohimbine induced a transient rise in Δ % RSR after 6 minutes, at t=10 minutes RSR decreased to -0.1 ± 11.9%. Analysis of percentual RSR alterations during cirazoline-yohimbine and cirazoline-doxazosin revealed a statistical difference ($p<0.05$).

7.7 Experiments with $alpha_2$-agonist versus $alpha_{1,2}$-antagonists.

In these series of experiments combined infusions of clonidine with saline, doxazosin or yohimbine were carried out. The protocol was identical as mentioned before in Chapter 7.6, except for the highest

dose clonidine, which was infused just after 10 minutes. After clonidine-saline infusion flow decreased from 12.2 ± 0.4 ml min^{-1} to 6.7 ± 0.8 ml min^{-1} ($p<0.001$), between 2 and 6 minutes flow was reduced modestly ($p<0.05$), clonidine preceded by doxazosin infusion caused a reduction in flow from 13.4 ± 0.4 ml min^{-1} to 9.1 ± 0.4 ml min^{-1} ($p<0.01$), whereas simultaneous infusion of clonidine and yohimbine decreased flow from 12.6 ± 0.4 ml min^{-1} to 9.3 ± 0.7 ml min^{-1} ($p<0.001$) (fig. 7.7.1).

Percentual alterations in RVR during clonidine-NaCl or clonidine-doxazosin administration were not significantly different and the same applied when the effects of clonidine-doxazosin and clonidine-yohimbine were compared. The difference between clonidine-yohimbine and clonidine-saline infusions was, however, significant ($p<0.025$) (fig. 7.7.2).

Comparison of absolute renin and renin secretion data showed that especially at the highest dosage of clonidine (10 ugr kg^{-1} min^{-1}) absolute renin values increased in all three groups, renin secretion rates showed a distinct behaviour because of different flow rates at higher doses of clonidine. In the clonidine-yohimbine and clonidine-doxazosin experiments a significant increase in RSR was obtained between t=10 minutes and t=14 minutes ($p<0.02$ resp. $p<0.005$). During clonidine-saline infusion RSR remained nearly constant.

Percentual changes in RSR were not observed at the various dosages of clonidine. However, when combined with saline infusion, in perfusions in which clonidine was preceded by yohimbine a different pattern was obtained; statistical analysis of the figures revealed modest, but significant differences between both groups ($p<0.05$) (fig. 7.7.3). Clonidine, infused after doxazosin caused a curious pattern: initially RSR tended to decrease, but at the highest dosage clonidine (10 ugr kg^{-1} min^{-1}) Δ % RSR increased. The differences between clonidine-saline and clonidine-doxazosin experiments were non-significant. The difference between data obtained during clonine-doxazosin and clonidine-yohimbine infusion were, however, significantly different ($p<0.05$).

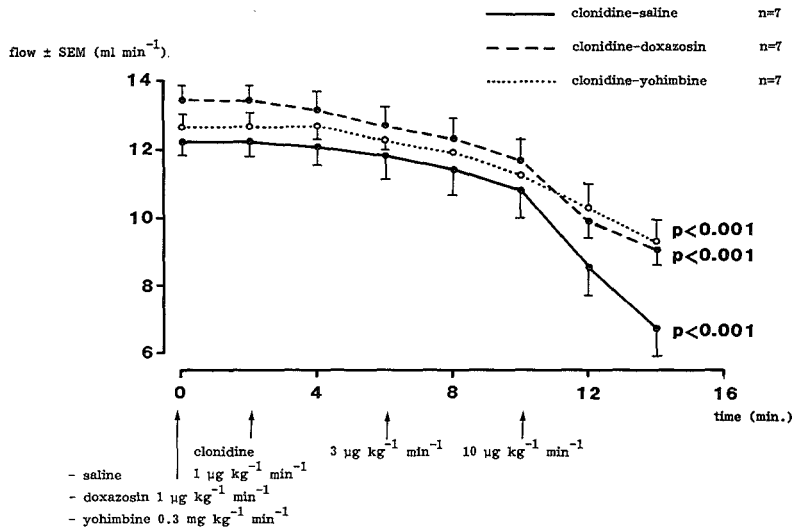


Fig. 7.7.1: The effects of infusion of incremental doses of clonidine on flow. Prior to infusion of clonidine $\alpha_{1,2}$-antagonist- or saline infusion was started.

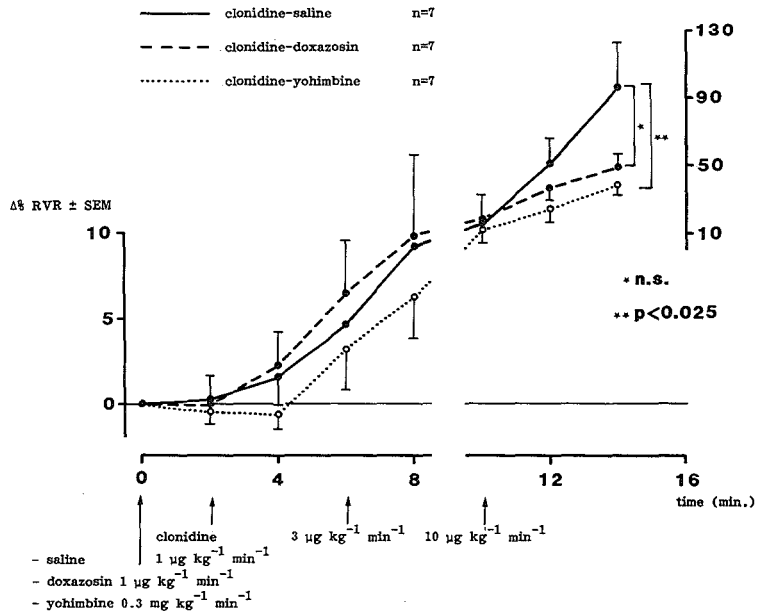


Fig. 7.7.2: The effects of infusion of incremental doses of clonidine on percentual changes in RVR. Prior to infusion of clonidine $\alpha_{1,2}$-antagonist- or saline infusion was started.

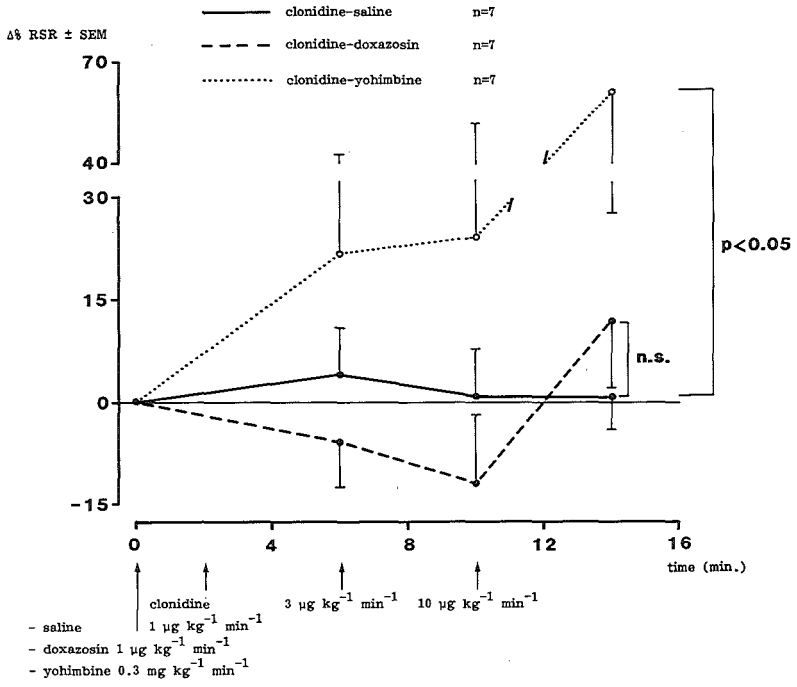


Fig. 7.7.3: The effects of infusion of incremental doses of clonidine on percentual changes in RSR. Prior to infusion of clonidine $\alpha_{1,2}$-antagonist- or saline infusion was started.

CHAPTER 8

Discussion.

8.1 Discussion with respect to dose response curves with alpha$_{1,2}$-agonists and alpha$_{1,2}$-antagonists.

In Chapter 7.2 experiments on cirazoline were described. In these series at a dose of 30 ugr kg^{-1} min^{-1} cirazoline no differences were established in comparison with saline infusion, although in comparison with former experiments with saline infusion the habitual pattern was not achieved. No apparent influence on RSR during cirazoline and saline infusion was observed. It should be noted that "spontaneous" decrements in RSR during saline experiments were more pronounced than in former experiments. In conclusion, cirazoline at this dosage does not elicit vasoactive or renin secretory responses in the isolated rat kidney.

Vandongen and Peart (1974) described that intrarenally infused methoxamine did not stimulate renin secretion, even when perfusion pressure was raised markedly. In other experiments (Vandongen et al, 1979), phenylephrine caused a significant elevation of perfusion pressure with concomitant decrease of flow, renin secretion tended to be reduced, these changes were not significant.

Osborn et al (1982[b]) infused methoxamine intrarenally in anaesthetized dogs. Only after provoking a fair vasoconstrictory response renin secretion was stimulated. This was confirmed by Hesse and Johns (1985); in their experiments, phenylephrine, at a low dose infused into the renal artery, without influence on renal haemodynamics, caused no alterations in renin release. At higher dosages, with a concomitant decrease in RBF and GFR, renin secretion increased significantly.

Our data, at a dose of cirazoline which does not affect flow, are consistent with Osborn et al and Hesse and Johns. Comparison with the data of Vandongen et al is difficult because of the changes in perfusion pressure in their experiments.

In Chapter 7.3 results of experiments with clonidine were described. Clonidine in our hands caused at a dose of 1.0 ugr kg^{-1} min^{-1} no significant pressor response; to the contrary, in comparison with control experiments a slight vasodilatation was observed. Renin secretion was not affected compared with control observations.

A subsequent series of experiments with clonidine (10 ugr kg^{-1} min^{-1}) revealed that, in this higher dose, clonidine elicits a substantial vasoconstrictory response; simultaneously RSR increased.

Vandongen and Greenwood (1975) also studied clonidine in the isolated rat kidney. Clonidine reduced isoprenaline-stimulated renin secretion without changes in renal perfusion pressures compared with experiments in which isoprenaline was infused separately (in both experiments renal perfusion pressure fell to approximately 80 mmHg). Non-stimulated RSR decreased during clonidine infusion.

Although we, too, observed lower values of RSR in absence of vasoactive effects, we assume that because of the variability of the model only correct interpretations may be made by comparison of changes in RSR. In that respect, our data are inconsistent with the data of Vandongen and Greenwood. Furthermore, they studied alterations in stimulated renin secretion, while we performed our experiments under "basal" conditions.

Wolff et al (1985) found in the rat kidney that α_2-agonists were neither potent nor efficient. In previous experiments in dogs Wolff et al (1984) demonstrated that clonidine, at dosages which varied from 0.1 to 10 microgram kg^{-1} min^{-1}, caused a vasoconstrictory response which was attenuated by yohimbine as well as by prazosine. This indicates that clonidine at this dosage possesses α_1- and α_1-agonistic properties, at least in the canine kidney. This was supported by Horn et al (1982) in vessel preparations.

Onesti et al (1971), Reid et al (1975), Pettinger et al (1976), Chevillard et al (1978) and Lowenstein (1980) described decrements in plasma renin activity after intravenous administration of clonidine. In 1978 Nolan and Reid proposed a centrally mediated decrease in sypathetic activity as underlying mechanism of renin suppression. However, since intraventricularly or intracisternally administered clonidine raised PRA, these data indicated that inhibition of renin secretion is mediated via activation of α-adrenoceptors in the kidney.

Sullivan and Drew (1980) found that clonidine (at comparable dosages) decreased noradrenaline outflow in canine saphenous vein strips. This was confirmed by Doxey et al (1977), Yamaguchi et al (1977) and Shepperzon et al (1981) in other preparations. Robie (1980) infused clonidine into the intact renal vascular bed at doses in approximating ours. At a higher dose (10 microgram kg^{-1} min^{-1}), a pressor response was observed with concomitant increased renin release.
Taking our data together, we conclude that clonidine (1.0 microgram kg^{-1} min^{-1}) does influence renal vascular resistance in comparison with saline infusion. This is perhaps due to a presynaptic effect (reduction of noradrenaline release), renin secretion is not affected. At a higher dosage (10 ugr kg^{-1} min^{-1}) clonidine induced both vasoconstriction and increased RSR.

Doxazosin experiments were described in Chapter 7.4. Doxazosin is a relatively selective α_1-adrenoceptor antagonist (de Leeuw et al, 1982; Vincent et al, 1983; Van Brummelen et al, 1983[a]; Thom et al, 1985; Meredith et al, 1985).
In our hands doxazosin at a dose of 1.0 ugr kg^{-1} min^{-1} did not alter renal flow, renal vascular resistance or renin secretion rate. In the literature conflicting data with regard to influence of α_1-antagonists on renin release are mentioned. Morganti et al (1982) described a rise in baseline plasma renin activity after prazosin without changes in arterial pressure, others (Graham and Pettinger, 1979; Pedrinelli et al, 1981; Colluci, 1982; Nicholson et al, 1985) observed increments in PRA with blood pressure reduction, whereas unchanged PRA values were noticed by Stokes and Weber (1974), Massingham and Hayden (1979), Wester (1979), de Leeuw et al (1980) and Hamilton and Reid (1982).
In the isolated perfused rat kidney prazosin (dose 0.01 mg kg^{-1} min^{-1} and 0.1 mg kg^{-1} min^{-1}) caused a slight vasodilatatory response during normothermic perfusion, while renin secretion was unchanged (Batenburg, 1983). De Leeuw et al (1986[a,b]) infused doxazosin into the renal artery of man. They observed slight vasodilatation concomitantly with some increase in renin secretion without a systemic effect on blood pressure.
Osborn et al (1982[b], 1983), Di Bona (1985) and Blair et al (1985)

demonstrated during normal perfusion pressure that renal nerve stimulated (up to 1.0 Hz) renin secretion was not attenuated by α_1- and α_2-antagonists (with exception of phenoxybenzamine). Our data support the opinion that α_1-adrenoceptors do not appear to be involved in renin release in absence of renal haemodynamic alterations.

In Chapter 7.5 experiments during non-vasoactive dosages of yohimbine infusions were described. We carried out our experiments at a dose of 0.3 mg kg^{-1} min^{-1}, which is a rather high dose in comparison with doses infused in man by others (Jie et al, 1984: 1 ugr kg^{-1} min^{-1}; De Leeuw et al, 1986[a,b]: 1.0, 3.0 and 10 ugr kg^{-1} min^{-1}), but comparable doses were used by Timmermans et al (1980[b]), Van Meel et al (1981) and Ruffolo et al (1984) in pithed rats and by Hamilton et al (1982) in conscious rabbits.
At a dose of 0.3 mg kg^{-1} min^{-1} yohimbine caused a slight and non-significant vasodilatation, renin secretion rate was not different in comparison with control experiments.
Fildes et al (1985) infused into the renal artery of anaesthetized dogs yohimbine at doses which ranged from 10 to 100 ugr kg^{-1} min^{-1}, mean arterial pressure, GFR and RBF were unaffected. De Leeuw et al (1986[a,b]) demonstrated marked vasodilatation in the human kidney and concomitant renin release during intrarenal infusion without systemic blood pressure alterations. This might be ascribed both to a postsynaptic α_2-adrenoceptor mediated effect or a combined presynaptic and postsynaptic effect of yohimbine. In the latter case presynaptically released noradrenaline would stimulate β_1-adrenoceptors, which mediate renin release. Jie et al (1986) observed at comparable doses that noradrenaline overflow was increased. Our data show that at a non-vasoactive dose of yohimbine renin secretion is unaffected.

8.2 Discussion with respect to combination experiments with alpha$_{1,2}$-agonists and alpha$_{1,2}$-antagonists.

8.2.1 Influence of renal flow.

In Chapter 7.6 and 7.7 results were given with regard to combined

experiments with α-agonistic and α-antagonistic drugs. Cirazoline is a very effective α_1-adrenoceptor agonist (Van Meel et al, 1980, 1981; Ruffolo and Yaden, 1984; Wolff et al, 1985). In pithed rats cirazoline increased diastolic blood pressure at dosages which ranged from 0.1-30 ugr kg^{-1} min^{-1}, this response was selectively antagonized by prazosin (0.1 mg kg^{-1} i.v.) and relatively unaffected by yohimbine (1.0 mg kg^{-1} i.v.).

Wolff et al (1985) observed after intrarenal cirazoline administration at a low dose very prominent local vasoconstrictory responses with minimal systemic effects; vasopressor effects were competitively antagonized by prazosin, indicating major α_1-adrenoceptor involvement.

These date could not be reproduced in our experiments. Higher dosages of cirazoline had to be infused before an obvious vasoconstrictory response could be established. Moreover, neither doxazosin nor yohimbine antagonized the responses significantly. Although doxazosin altered RVR more than yohimbine, no pharmacological proof for α_1-adrenoceptor mediated effects could be obtained. This may be due to inappropriate dosing of doxazosin (1.0 ugr kg^{-1} min^{-1}), but after intravenous administration similar dosages produced blood pressure decrements. Although different responses may be obtained during intrarenal or intravenous infusion of drugs (Fahri et al, 1982; Smits et al, 1986), we saw fit to maintain this dosage. As an additional variation, we infused antagonists two minutes prior to cirazoline, whilst in other studies an interval of 15 minutes was chosen (Van Meel et al, 1981; Horn et al, 1982; Wolff et al, 1984). As has been indicated before, we could not afford the "luxury" of spacing out the steps of our protocol too far, in view of the limited viability of our preparation.

Administration of clonidine provoked a measurable degree of vasoconstriction. This vasoconstriction was partly antagonized by infusion of yohimbine. Obviously clonidine induced postsynaptic α_2-adrenoceptor mediated effects. Doxazosin produced a slight displacement of the flow curve to the right, but this change did not attain statistical difference.

It appears from these data that vascular α_2-adrenoceptors are the prominent ones in the rat kidney, although we cannot state this with

utter certainty. Clonidine is not a selecetive α_2-agonist and exerts α_1-agonism. In addition, yohimbine in the dosage we used (0.3 mg kg^{-1} min^{-1}) is not completely α_2-selective (Di Bona, 1985). Hamilton and Reid (1982) mentioned that yohimbine in their experiments at dosages of 1 mg kg^{-1} min^{-1} or greater does exert α_1-antagonistic effects. This was confirmed by Timmermans et al (1985). Langer et al (1985) concluded that yohimbine is not sufficiently selective for postsynaptic α_2-adrenoceptors compared with α_1-adrenoceptors. Moreover, yohimbine proved not to be very effective in antagonizing vasoconstrictory responses to α_1- and α_2-agonist in dogs and cats (Shepperzon et al, 1981; Langer et al, 1985), in these experiments comparable or even higher dosages of yohimbine were used in comparison with our experiments.
Di Bona (1985) stated that the effects of yohimbine are both dose-dependent and related to animal species.

Schmitz et al (1981) perfused the isolated rat kidney both with phenylephrine and noradrenaline as α_1-selective agonists; these agonists were more potent than α-methyl-noradrenaline and clonidine; the antagonists used were prazosin and yohimbine. With the same drugs radioligand binding studies were carried out on rat renal membranes. Although both the α_1- and α_2-adrenoceptors appear to be present, the α_2-adrenoceptor predominate in a ratio of 3:1. Despite this preponderance of α_2-adrenoceptors the alpha-receptor-mediated renal vasoconstriction appeared to be mainly dependant on α_1-adrenoceptors.
Horn et al (1982) performed experiments on the renal vasculature of anaesthetized dogs pretreated with propranolol and hexamethonium. Clonidine was nearly as potent as phenylephrine. Whilst vasoconstriction induced by clonidine was in part antagonized by prazosin or yohimbine, phenylephrine- and methoxamine-induced vasoconstriction was effectively antagonized by prazosin and unaffected by yohimbine. Combined administration of prazosin and yohimbine reduced clonidine-induced vasoconstriction more effectively than did each of the components. Comparison of potency data of the agonists suggested that α_1-adrenoceptors predominate in the canine renal vascular bed. Similar data were described by Duval et al (1985).

Wolff et al (1984) infused phenylephrine, clonidine and guanabenz into the renal arteries of anaesthetized dogs. Guanabenz was 10 times weaker in eliciting vasoconstriction than clonidine. After α_1-adrenoceptor blockade with prazosin guanabenz still produced vasoconstriction. Clonidine constricted the vasculature both through α_1- and α_2-adrenoceptors since both prazosine and yohimbine (150 ugr kg^{-1} min_{-1}) attenuated renal blood flow decrements (dosage clonidine: 0.1-3.0 ugr kg^{-1} min^{-1}). Phenylephrine proved to be 10-fold more potent than clonidine. Denervation did not shift the curves. In 1985 Wolff et al presented data of experiments performed in anaesthetized rats. Several α_1- and α_2-selective agonists were infused into the renal vascular bed. Cirazoline and phenylephrine proved to be very potent compared with guanabenz, B-HT 920 and UK 14304. $Alpha_1$-agonists were competitively antagonized by prazosine. They concluded that the α_1-adrenoceptors are the only effective subtypes in mediating sympathetic vasoconstriction, α_2-adrenoceptors were assumed to be either sparse or inactive. As outlined in Chapter 3, however, in studies using α_2-selective radioligands and/or autoradiography and/or immunihistochemical techniques α_2-adrenoceptors predominate in the kidney over α_1-adrenoceptors (Young and Kuhar, 1980; McPherson and Summers, 1981; Schmitz et al, 1981; Snavely and Insel, 1981; Summers, 1984; Summers et al, 1985; Muntz et al, 1985; Insel et al, 1985; Umemura et al, 1986; Struyker Boudier et al, 1986).

In other studies (Vandongen and Peart, 1974; Vandongen et al, 1979; Gerber et al, 1981; Osborn et al, 1982^b), methoxamine and phenylephrine could induce vasoconstriction, but in these studies no competition curves were produced. Osborn et al (1983) described in dogs that prazosin antagonized intrarenal infusion of 2.0 microgram noradrenaline in contrast to yohimbine.

Hesse and Johns (1984) suggested that both α_1- and α_2-adrenoceptors in the renal vasculature of rabbits mediate vasoconstriction. This might also be true in man since De Leeuw et al (1986^b) demonstrated that both yohimbine and doxazosin produced vasodilatation. Yohimbine even elicited a more pronounced response.

8.2.2 Conclusion.

Most authors appear to be in favour of a predominant through α_1-adrenoceptor-mediated type of renal vasoconstriction in the rat, the dog and the cat. An α_2-adrenoceptor component in renal vasoconstriction is likely to be prominent in the rabbit and in man. Our data appear to support a dualistic view with regard to the relative contributions of the α_1- and α_2-adrenoceptor populations to α-adrenoceptor mediated renal vasoconstriction.
On the one hand, the α_1-agonist cirazoline, when titrated beyond the point of vasoconstrictory response, produced a huge decline in flow resulting in a several thousand-fold increase in calculated renal vascular resistance. This effect leveled off beyond a dose of 100 ugr kg^{-1} min^{-1}. Curiously enough, the dose response curve was shifted to the right more obviously by the α_2-antagonist (yohimbine) than by the α_1-antagonist (doxazosin). On the other hand, clonidine elicited a vasoconstrictory response, which steadily increased from a dose of 1.0 ugr kg^{-1} min^{-1} through 10 mgr kg^{-1} min^{-1}, but appeared to level off in the range of approximately 100% increase in RSR. This implies that the ceiling of the vasoconstrictory response to α_2-stimulation is likely to be attained earlier, and the deduction from this is that the potential of α_2-adrenoceptors (and/or its population density) is definitely less than that of α_1-adrenoceptors. More "logically" than in the former experiments, the dose response curve is moved to the right significantly by the α_2-antagonist yohimbine than by the α_1-antagonist doxazosin. Thus, there seems to be a relative preponderance of α_1- over α_2-mediated vasoconstriction in the rat kidney.

8.2.3 Influence on renin secretion rate.

In experiments with cirazoline as an agonist different patterns with regard to renin values and renin secretion rate were obtained without obvious differences in flow behaviour between the three groups. Comparison of cirazoline-saline with cirazoline-doxazosin infusions revealed that doxazosin reduced absolute renin values and RSR. Percentual alterations were not significantly different from each other (fig. 7.6.3). Cirazoline-yohimbine infusion induced non-significant

differences in the percentual changes in RSR in comparison with cirazoline-saline administration, but significant differences were observed in comparison with experiments in which cirazoline and doxazosin were infused.

In experiments with clonidine absolute renin values increased at the highest dosage of clonidine infused in all three groups concomitantly with vasoconstriction. This increase was not inhibited by $\alpha_{1,2}$-antagonists. Percentual changes in RSR revealed that yohimbine combined with clonidine elevated renin secretion significantly.

In previous experiments with a vasoconstrictory dosage of clonidine (10 ugr kg^{-1} min^{-1}) similar results were obtained. Cirazoline, at a dose eliciting renal vasoconstriction to a degree comparable with that during the highest dosage of clonidine, did not produce a similar pattern with regard to renin secretion rate . Whilst during cirazoline infusion RSR was reduced by doxazosin (but percentual changes in RSR did not change significantly), this was not observed when clonidine was combined with doxazosin. Unfortunately RSR could not be determined in cirazoline experiments at a dose of 300 ugr kg^{-1} min^{-1}. We are left, therefore, with a controversial result in this respect.

Osborn et al (1982[b]) observed in dogs that methoxamine induced renin secretion only at dosages which reduced RBF, GFR and sodium excretion.

Hesse and Johns (1985) observed in rabbits that renin secretion increased at doses of phenylephrine and UK 14304 which reduced renal blood flow and glomerular filtration rates. This is regarded to be consistent with the view that α-adrenoceptors do not directly stimulate renin secretion. Indirectly renin secretion may be stimulated through α-adrenoceptor mediated renal vasoconstriction and decreased sodium excretion (Kopp et al, 1981[b]; Osborn et al, 1982[b], 1983; Blair, 1983; Di Bona, 1985).

Gerber et al (1981) infused phenylephrine at vasoactive dosages into the renal artery of dogs and found that RSR increased. Pretreatment with indomethacin attenuated the rise in RSR. After infusion of phenylephrine in the non-filtering kidney renin secretion was not stimulated. This suggests that α-adrenergically stimulated renin release resulted from activation of the macula densa. Induction of

prostaglandin synthesis might thus be involved. Kopp et al (1981[b]) indeed proposed that prostaglandines modulate renin secretion induced by stimulation of α-adrenoceptors. By contrast, Seymour et al (1981) provided no evidence that adrenergically stimulated renin release was influenced by prostaglandines.
Summers (1984) demonstrated that stimulation of α_1-adrenoceptors leads to production of diacylglycerol and phosphatidic acid; production of diacylglycerol stimulates prostanoid synthesis. Alpha$_2$-adrenoceptor activation with concomitant decrement in cyclic AMP might stimulate phophatidyl-inositol turnover thereby facilitating diaglycerol production and subsequent prostanoid synthesis. Via such a mechanism α_2-adrenoceptors may facilitate effects of α_1-adrenoceptor activation, assuming that prostaglandines are essential for these effects.
Our results may indicate that in experiments with clonidine, when we assume mixed α_1- and α_2-adrenoceptor mediated effects at the highest dose, this mechanism might indeed be involved, whereas in experiments with cirazoline only α_1-mediated effects were observed. With comparable vasoactive effects cirazoline stimulates renin release less than clonidine does.

Yohimbine does not inhibit renin release in experiments with clonidine or cirazoline. On the contrary, in the clonidine-yohimbine experiments RSR was stimulated significantly, with a concomitant attenuation of vasoconstriction elicited by clonidine. This may be explained in two ways: on the one hand, we may presume that exclusive postsynaptic α_2-adrenoceptor stimulation inhibits renin release. When this receptor is blocked by yohimbine, renin release would be enhanced. On the other hand presynaptic α_2-adrenoceptor mediated noradrenaline release might be stimulated by yohimbine.

In the clonidine-yohimbine series of experiments yohimbine attenuated clonidine-induced vasoconstriction. It seems likely, therefore, that postsynaptic effects exist, although (as indicated before) concomitant α_1-adrenoceptor antagonism cannot be ruled out. At the same time a presynaptic effect remains to be considered because yohimbine may stimulate (preferentially) presynaptic α_2- as compared to postsynaptic

α_2-adrenoceptors (Starke et al, 1975; Drew, 1976; Doxey et al, 1977; Sullivan and Drew, 1980; Graham et al, 1980; Shepperzon et al, 1981; Rand et al, 1982; Hamilton et al, 1982; Hedler et al, 1983; Jie et al, 1986; Anonymus, Lancet, 1986). Yohimbine might occupy presynaptic α_2-adrenoceptors and via such a mechanism presynaptically released noradrenaline may stimulate β_1-adrenoceptors at the juxtaglomerular apparatus as described by Graham and Pettinger (1979) and Pedrinelli et al (1981).

Such a mechanism may be involved additionally in the study by De Leeuw et al (1986[a,b], 1987) in man. They infused intrarenally yohimbine and attained a vasodilatatory response with simultaneous renin secretion which was much more pronounced than the response to doxazosin. Keeton et al (1985) observed in conscious rats that yohimbine induced an increase in PRA. This effect was more closely related to the ability to increase plasma noradrenaline levels than to blood pressure reduction. On the other hand, Robie (1980) was unable to demonstrate presynaptic effects of yohimbine in the kidney.

In our experiments we did not observe increments in renin release during yohimbine infusion at non-vasoactive doses. It appears likely that only with concomitant vasoactive effects and/or effects at the macula densa renin secretion is stimulated. When we assume a presynaptic α_2-adrenoceptor effect elicited by yohimbine, this may provide an explanation for the contrasting data on renin secretion rate elicited by yohimbine or doxazosin. Whether or not an additional inhibitory effect of postsynaptic α_2-adrenoceptors on renin release exists cannot be ruled out with certainty, but this effect would be apparent only with concomitant α_1-adrenoceptor stimulation and influence upon the macula densa.

Contradictory data were obtained as to whether or not cirazoline influences presynaptic noradrenaline release. Van Meel et al (1981) provided no evidence for a presynaptic effect of this drug. Dubocowich et al (1984), however, estimated a 10 fold more selective effect of cirazoline on presynaptic than postsynaptic sites in experiments in which they measured (noradrenaline)overflow. Docherty et al (1983) and Story et al (1985) likewise proposed a presynaptic inhibitory effect on noradrenaline release mediated through α_1-adrenoceptors.

In addition, influence on re-uptake may interfere since the addition of uptake inhibitors reveals more distinct presynaptic effets. Alpha-adrenoceptor antagonists inhibit preferentially uptake I processes (Iversen, 1973; Graham and Pettinger, 1979; Ziegler et al, 1986).
It is unlikely that such mechanisms might have influenced our data but it cannot be ruled out with certainty.

8.2.4 Conclusion.

Several mechanisms might be involved in our experiments. The interpretation of some results is limited by the experimental set-up. A major problem was that the role of the macula densa could not be appraised. Clonidine at high doses induced increments in renin release, presumably by a direct vascular mechanism and/or activation of the macula densa. Renin release appears not to be entirely dependent on α_1-adrenoceptors, since doxazosin (at a dose of 1.0 ugr kg^{-1} min^{-1}) did not inhibit significantly the percentual changes in renin secretion rates. An inhibitory α_2-adrenoceptor mediated effect on renin release might be involved since yohimbine stimulated RSR, but this could not be dissociated from haemodynamic effects. Likewise, an additional presynaptic α_2-adrenoceptor mediated effect on noradrenaline may be involved, since yohimbine stimulated RSR both in combination with cirazoline or clonidine, compared with doxazosin. However, this effect appears only to be detectable when simultaneous vasoconstriction (and/or influence on the macula densa) exists.

CHAPTER 9

Epilogue.

In this chapter some reflections will be presented on the mechanisms which may be related to our data.
In Chapter 5 en 6 it was described that salbutamol caused a delayed response with regard to renin secretion. Majewski et al (1985) described that a facilitory effect of adrenaline on noradrenaline release was only obtained after 58 minutes (this study was carried out in pithed rabbits). This may be a reflection of uptake of adrenaline in the sympathetic nerve endings, and this period seems to be necessary for the accumulation of sufficient adrenaline levels before noradrenaline release is stimulated.
Our data may indicate that indeed some accumulation is necessary previous to presynaptically-mediated noradrenaline release.

With regard to alterations in flow induced by the α_1- and α_2-agonist. Our results with cirazoline and clonidine are consistent with a larger receptor reserve of α_1-adrenoceptors and fewer α_2-adrenoceptors, attained to the vasculature, in the rat kidney (Ruffolo et al, 1984; Hamilton et al, 1985 and Strandhoy et al, 1985). Since clonidine is a partial α_1-agonist, full saturation is required to obtain a maximal response. This might provide additional insight in the lesser vasoconstrictory response induced by clonidine than elicited by cirazoline. We did not estimate the receptor reserve in the kidney as proposed by Catt (1979), Pollet and Lewey (1981) and Ariens (1984).
In addition, α_2-adrenoceptor mediated contractions are associated with release of endothelium derived relaxant factor (EDRF) (Cocks and Angus, 1982; Furchgott, 1983; Miller et al, 1983; Strandhoy, 1985; McGrath and Reid, 1985; Summers et al, 1985), this may provide an additional explanation for the lesser vasoconstrictory response after infusion of clonidine.

With regard to the presumed involvement of prostaglandines on renin release after stimulation of the renal baroreceptor and macula densa (Gerber et al, 1981; Henrick, 1981; Osborn et al, 1984) by α_1-

agonists: complex interactions appear to exist between the prostaglandines, kallikrein-kinin and angiotensin systems (McGiff, 1984). Prostaglandines of the E-series inhibit and prostaglandine $F_{2\alpha}$ facilitate noradrenaline release (Malik and McGiff, 1975), both a dose dependency and species differences probably play a role here (Ching Shan Jin et al, 1981).

Effects on dopamine receptors in the kidney may be important (Lee, 1982; Mizoguchi et al, 1983). Dopamine agonists induce renin release, whereas they inhibit noradrenaline release.

Whether prostaglandines or dopamine receptors are involved in our experiments can, of course, not be excluded.

SUMMARY

In the present thesis we present data from experiments performed in the model of the isolated perfused rat kidney. This model has been developed in order to enable us to study the effects of infusion of various doses of pharmaca on vasoactivity and renin secretion without interference of complicating factors such as alterations in sympathetic tone, perfusion pressure or electrolyte composition. The single major disadvantage of the model was that it did not allow us to settle the influence of the macula densa.

In Chapter 1 the purpose of our investigation has been introduced and described.
Chapter 2 encompasses a general discussion on the renin-angiotensin system with special reference to the multiple factors which control secretion of renin.
In Chapter 3 the mechanisms of activation of adrenoceptors have been described, in addition, a general discussion on alpha- and beta-adrenoceptors has been presented with special reference to the function and distribution of these adrenoceptors in the kidney.
Chapter 4 deals with the methods used. In this chapter the perfusion technique has been described in more detail.
Chapter 5 describes the observations we obtained during infusion of $\beta_{1,2}$-agonists and $\beta_{1,2}$-antagonists. Initially dose-response experiments have been carried out in order to establish the non-vasoactive and vasoactive dose of the respective agonists. Next the presumed non-vasoactive dose has been infused at a constant rate and a separate series of experiments with $\beta_{1,2}$-antagonists was carried out. In subsequent experiments the β_1- or β_2-agonist was infused at incremental doses either with preceding and contemporary administration of the $\beta_{1,2}$-antagonist or with saline.
In Chapter 6 the data described in Chapter 5 have been discussed. It was obvious that infusion of prenalterol (β_1-agonist) augmented the renin secretion rate without a concomitant vasoconstrictory response. When atenolol (β_1-antagonist) was infused beforehand, the increment of renin secretion rate was prevented. It was argued that the β_1-adrenoceptor is localized near the juxtaglomerular cell. After infusing

the β_2-agonist salbutamol at incremental doses a slight vasodilatation ensued with elevated renin secretion rates. When the β_1-antagonist atenolol or the β_2-antagonist ICI 118.551 was infused beforehand, tapered vasodilatation occurred but contrasting data with regard to renin secretion rate were observed in that atenolol but not ICI 118.551 prevented the increment of renin secretion rate. It is proposed that vasodilatation may stimulate renin secretion rate, although this does not explain the phenomenon completely since atenolol antagonized vasodilatation more efficiently than ICI 118.551. A suitable explanation may be that salbutamol facilitates noradrenaline release via the presynaptic β_2-adrenoceptors, thereafter activating the postsynaptic β_1-adrenoceptor. When we accept this assumption it is justified to implicate that ICI 118.551 (at the dose infused) does not antagonize the presynaptic effects elicited by infusing salbutamol.

In Chapter 7 data on experiments with α_1- and α_2-agonists and antagonist have been shown. The protocol was similar to experiments carried out with the β-agonistic and antagonistic pharmaca. Cirazoline has been studied as an α_1-agonist, whilst clonidine has been infused in an attempt to clarify the α_2-adrenoceptor-mediated component of vasoconstriction.

In Chapter 8 the discussion on the data we obtained in experiments as described in Chapter 7 has been regarded. Cirazoline infused at incremental doses, elicited a fairly obvious vasoconstrictory response compared with clonidine. Neither doxazosin nor yohimbine antagonized significantly the vasoconstrictory response after infusing cirazoline to a significant degree. Therefore, the pharmacological proof that we were dealing with an alpha$_1$-adrenoceptor mediated effect has not been delivered. In a subsequent series of experiments clonidine, infused at incremental doses, elicited vasoconstriction, though less obvious than with cirazoline. This vasoconstriction was only attenuated by preceding infusion of yohimbine, thus indicating that it was α_2-adrenoceptor mediated. In agreement with the literature it appears likely, therefore, that vascular α_1-adrenoceptors functionally are more prominent than α_2-adrenoceptors. Some precautions, however, have to be taken into account because both clonidine and yohimbine, at the doses infused, may exhibit properties congruent to the α_1-adreno-

ceptor. It is obvious that experiments of the kind we reported may be fraught with uncertainties related to receptor-subtype specifity.

With regard to the renin secretion patterns; we observed an increase in RSR during the infusion of incremental doses of cirazoline as compared with saline. A preceding infusion of doxazosin did not attenuate the percentual changes in renin secretion rates. Clonidine at incremental doses elevated renin secretion; this rise was pronounced during the infusion of clonidine together with yohimbine, during infusion of clonidine-doxazosin renin secretion rate did not alter. These data seem to indicate that the postsynaptic α_2-adrenoceptor exerts an inhibitory action on renin release which apparently would only be recognizable in the presence of α_1-adrenoceptor mediated vasoconstriction. Alternatively, by infusing yohimbine facilitation of noradrenaline release via presynaptic α_2-adrenoceptors may be involved in the way of stimulating β_1-adrenoceptors. Unfortunately in our model the potential influence of the macula densa could not be appraised.

In Chapter 9 our observations have been interpreted in a general sense.

SAMENVATTING

In dit proefschrift worden de resultaten beschreven van een dierexperimenteel onderzoek waarbij het model van de geisoleerde geperfundeerde rattenier werd gekozen. Dit model is destijds ontwikkeld omdat een aantal variabelen beheerst kunnen worden die het vaatgedrag en de renine secretie beïnvloeden. Een nadeel is dat de invloed van de macula densa in de door ons gekozen opzet niet bestudeerd kon worden.

Hoofdstuk 1 omvat de inleiding waarin tevens de vraagstellingen geformuleerd worden.
In Hoofdstuk 2 worden de diverse aspecten van het renine-angiotensine systeem belicht.
In Hoofdstuk 3 wordt uitvoerig ingegaan op de adrenerge receptoren, met inbegrip van de activeringsmechanismen, functie en distributie in organen, met speciale aandacht voor de adrenerge receptoren in de nier.
In Hoofdstuk 4 worden de methoden beschreven, die gebruikt zijn in ons onderzoek.
In Hoofdstuk 5 worden de eigen waarnemingen beschreven zoals die verkregen zijn na infusie van beta-agonisten en beta-antagonisten. In de eerste plaats werden dosis-response curven geregistreerd teneinde een inzicht te verkrijgen in doseringen van de afzonderlijke farmaca welke een niet-vasoactieve of een vasoactieve respons veroorzaken. Tevens hoopten wij aldus inzicht te verkrijgen in de afzonderlijke invloed van deze farmaca op de reninesecretie. Vervolgens werden gecombineerde infusies toegepast teneinde het farmacologische bewijs van betrokkenheid van het te onderzoeken receptortype vast te stellen.
In Hoofdstuk 6 volgt de discussie over de verkregen gegevens. Hieruit blijkt dat activering van de β_1-adrenerge receptor aanleiding geeft tot toegenomen reninesecretie zonder begeleidende vaatreactie. Deze β_1-receptor is gesitueerd nabij de juxtaglomerulaire cel. Na infusie van salbutamol in opklimmende doseringen treedt vasodilatatie op, voorafgaande en gelijktijdige infusie van atenolol en ICI 118.551 doet de vasodilatatie afnemen, doch de reninesecretie wordt alleen

door atenolol geremd. Enerzijds lijkt er sprake te zijn van een vasculaire β_2-adrenerge receptor in de geisoleerde rattenier, maar anderzijds is de toegenomen reninesecretie hier niet geheel aan toe te schrijven.

Verondersteld wordt dat activering van presynaptische β_2-adrenerge receptoren hierbij een rol speelt waarbij de toegenomen noradrenaline-afgifte de postsynaptische β_1-adrenerge receptoren activeert. Dit veronderstelde presynaptische mechanisme wordt niet door ICI 118.551 geblokkeerd. Het feit dat atenolol ook vasodilatatie ten gevolge van salbutamol te niet doet pleit mogelijk voor een activering van postsynaptische α-adrenerge receptoren door een presynaptische β_2-afhankelijke noradrenaline release.

In Hoofdstuk 7 worden de resultaten weergegeven van eigen onderzoek betreffende alpha-agonisten en alpha-antagonisten. Het onderzoek werd uitgevoerd volgens hetzelfde protocol zoals beschreven wordt in Hoofstuk 5.

In Hoofdstuk 8 wordt nader ingegaan op de verkregen resultaten. Hieruit blijkt dat cirazoline een uitgesproken vasoconstrictie kan veroorzaken die slechts gedeeltelijk (niet significant) door beide alpha-antagonisten wordt tegengegaan. Deze vasoconstrictie is veel meer uitgesproken dan de vasoconstrictie ten gevolge van clonidine-infusie. Dit is in overeenstemming met de literatuurgegevens die wijzen op een met name door α_1-adrenerge receptoren bemiddelde vasoconstrictie in het niervaatstelsel. Dat er sprake is van vasculaire α_2-adrenerge receptoren lijkt aannemelijk omdat yohimbine (α_2-antagonist) de door clonidine geinduceerde vasoconstrictie significant doet verminderen. Echter, clonidine en yohimbine kunnen in de gebruikte doseringen niet alleen de α_2- maar ook de α_1-receptor beinvloeden, hetgeen de precieze vaststelling van welk type α-receptor betrokken is bemoeilijkt. Het plasma renine wordt door clonidine gestimuleerd in vasoactieve doseringen, cirazoline veroorzaakt geen duidelijke toename bij een vergelijkbare mate van vasoconstrictie. De procentuele veranderingen in de reninesecretie laten evenmin een eenduidig beeld zien. Duidelijk is wel dat yohimbine ten tijde van clonidine-infusie de reninesecretie bevordert, tijdens simultane infusie met cirazoline wordt dit niet waargenomen. Gedurende de simultane infusie met doxazosine en de respectievelijke

alpha-agonisten wordt een significant lagere procentuele reninesecretie berekend in vergelijking met de yohimbine-infusies. Mogelijk wijst dit op een remmende invloed van de postsynaptische α_2-adrenerge receptor; door en in combinatie met vasoconstrictie door prikkeling van de α_1-adrenerge receptor zou de reninesecretie aanmerkelijk kunnen stijgen. Anderzijds zou ook een remmende invloed op de presynaptische α_2-adrenerge receptor met als gevolg noradrenaline-release tot de mogelijkheden kunnen behoren. Een invloed op de macula densa konden wij helaas niet uitsluiten, wellicht speelt deze een belangrijke rol.

In Hoofdstuk 9 worden enkele kanttekeningen geplaatst bij de interpretatie van de resultaten.

REFERENCES

- Abrams WB. Satellite symposium on central α-adrenergic blood pressure regulating mechanisms. Hypertension 1984; 6 (suppl. II): II 87-II 93.
- Adler-Graschinsky E, Langer SZ. Possible role of a β-adrenoceptor in the regulation of noradrenaline release by nerve stimulation through a positive feed back mechanism. Br J Pharmac. 1975; 53: 43-50.
- Alquist RP. A study of the adrenotropic receptors. Am J Physiol. 1948; 153: 586-600.
- Altiere RJ, Douglas JS, Gillis CN. Temperature related effects on the binding characteristics of beta-adrenergic receptor agonists and antagonists by rabbit lung. Naunyn Schmiedeberg's Arch Pharmacol. 1981; 316: 278-287.
- Amery A, Billiet L, Fagard R et al. Atenolol and plasma renin concentration in hypertensive patients. Postgrad Med. 1974; 53: 116-119.
- Ammons WS, Kogama S, Manning JW. Neural and vascular interaction in renin response to graded renal nerve stimulation. Am J Physiol. 1982; 242: R 552-562.
- Anden NE. Uptake and release of dextro- and laevo-adrenaline in noradrenergic stores. Acta Pharmacol et Toxicol. 1964; 21: 59-75.
- Angus JA, Korner PI. Evidence against presynaptic α-adrenoreceptor modulation of cardiac sympathetic transmission. Nature. 1980; 286: 288-291.
- Angus JA, Bobik A, Jockman GP et al. Role of auto-inhibitory feed-back in cardiac sympathetic transmission assessed by simultaneous measurements of changes in ^{3}H-efflux and atrial rate in guinea pig atrium. Br J Pharmacol. 1984; 81: 201-214.
- Anonymous. Yohimbine: time for resurrection? Lancet. 1986; ii: 1194-1195.
- Aoi W, Wade MB, Rosner DR et al. Renin release by rat kidney slices in vitro: Effects of cations and catecholamines. Am J Physiol. 1974; 227: 630-634.

- Ariens EJ, Simonis AM. Physiological and pharmacological aspects of adrenergic receptor classification. Bioch Pharmacol. 1983; 32: 1539-1545.
- Ariens EJ. The dual action of partial agonists and partial antagonists. In: van Zwieten PA, ed. Physiological receptor antagonism, Uden: Actua Sandoz. 1984: 24-39.
- Ayers CR, Katholi RE, Carey RM et al. Acute and chronic intrarenal alpha- and beta-adrenergic receptor stimulation of renin release in the conscious dog. Hypertension. 1981; 3: 615-621.
- Barajas L, Latta H. A three-dimensional study of the juxtaglomerular apparatus in the rat. Lab Invest. 1963; 12: 257-269.
- Barajas L. The innervation of the juxtaglomerular apparatus. Lab Invest. 1964; 13: 916-929.
- Barajas L. Renin secretion: An anatomical basis for tubular control. Science. 1971; 172: 485-487.
- Barajas L. Anatomy of the juxtaglomerular apparatus. Am J Physiol. 1979; 237 (5): F 333-343.
- Barajas L. The juxtaglomerular apparatus: Anatomical considerations in feedback control of glomerular filtration rate. Fed Proc. 1981; 40: 78-86.
- Barajas L, Powers K, Wang P. Innervation of the renal cortical tubulus: A quantitative study. Am J Physiol. 1984; 247: F50-F60.
- Batenburg PL. Vaattonus en reninesecretie in de geisoleerde rattenier. Rotterdam: Thesis, 1983.
- Beckeringh JJ, Thoolen MJMC, de Jonge A et al. Differential effects of the calcium entry blocker D 600 on contractions of rat and guinea pig aortas, elicited by various alpha-1 adrenoceptor agonists. J Pharmacol Exp Ther. 1984; 229: 515-521.
- Berthelsen S, Pettinger WA, A functional basis for classification of α-adrenergic receptors. Life Sci. 1977; 21: 596-600.
- Bevan JA, Bevan RD, Laber I. Role of α-adrenoceptors in vascular control. Clin Sci. 1985; 68 (suppl. 10): 83S-88S.
- Birkenhäger WH, Krauss XH, Schalekamp MADH. Antihypertensive effect of propranolol: Observations on predictability. Folia Med Neerl. 1971; 14: 67-71.
- Bilski A, Dorries S, Fitzgerald JD et al. ICI 118.551, a potent β_2-adrenoceptor antagonist. Br J Pharm. 1980; 292 P.

- Blaine EH, Davis JO, Russel PL. Evidence of renal vascular receptor in control of renin secretion. Am J Physiol. 1971; 220(6): 1593-1597.
- Blaine EH, Davis JO. Evidence for a renal vascular mechanism in renin release: New observations with graded stimulation by aortic constriction. Circ Res 1971; 28: 29 (suppl. II): 118.
- Blaine EH, Zimmerman MB. Renin secretion during dynamic changes in renal perfusion pressure. Am J Physiol. 1979; 236(6): F546-51.
- Blair ML, Reid JA, Keil LC. Role of peripheral adrenoceptors and vasopressin in the suppression of plasma renin activity by L-dopa in carbidopa treated dogs. J Pharmacol Exp Ther. 1979; 210: 368-372.
- Blair ML. Inhibition of renin secretion by intrarenal α-adrenoceptor blockade. Am J Physiol. 1981; E682-688.
- Blair ML. Stimulation of renin secretion by α-adrenoceptor agonists. Am J Physiol. 1983; E37-44.
- Blair ML, Chen YH. Izzo JL. Influence of renal perfusion pressure on α- and β-adrenergic stimulation of renin release. Am J Physiol. 1985; 248: E317-E326.
- Boissier JR, Advenier C, Guidicelli JF et al. Studies on the nature of the bronchial β-adrenoceptors. Eur J Pharmacol. 1971; 15: 101-109.
- Bolli P, Kiowski W, Erne P et al. Enhanced alpha-adrenoceptor mediated vasoconstriction contributes to increased vascular resistance in essential hypertension. Neth J Med. 1984; 27: 140-145.
- Bolli P, Erne P, Kiowski W et al. The adrenaline-α_2-adrenoceptor mediated vasoconstrictor axis. Clin Sci. 1985; 68 (suppl. 10): 141S-146S.
- Brown MJ, Brown DC, Murphy MB. Hypokalaemia from beta$_2$-receptor stimulation by circulating epinephrine. N Engl J Med. 1983; 309: 1414-1419.
- Brown MJ, Struthers AD, Di Silvio L et al. Metabolic and haemodynamic effects of α_2-adrenoceptor stimulation and antagonism in man. Clin Sci. 1985; 68 (suppl. 10): 137S-139S.

- Van Brummelen P, Vermey P, Timmermans PBMWM et al. Preliminary evidence for a postsynaptic α_2-adrenoceptor in the vasculature of the human forearm. Br J Clin Pharmacol. 1983; 15: 134P-135P.
- Van Brummelen P, Jie K, Vermey P et al. Demonstration of a postsynaptic α_2-adrenoceptor contributing to basal vascular tone in man. J Hypertension 1983[a]; 1 suppl. 2: 254-256.
- Van Brummelen P, Jie K, Vermey P et al. Vascular α-adrenoceptors in man: interactions with adrenaline and noradrenaline. Clin Sci. 1985; 68 (suppl. 19): 151S-153S.
- Bryan LJ, Cole JJ, O'Donnel SR et al. A study designed to explore the hypothesis that beta-1 adrenoceptors are innervated receptors and beta-2 andrenoceptors are hormonal receptors. J Pharmacol Exp Ther. 1981; 216: 395-400.
- Bühler FR, Marbet G, Patel U et al. Renin suppressive potency of various beta-adrenergic blocking agents at supine rest and during upright exercise. Clin Sci Mol Med. 1975; 48: 61s-64s.
- Capponi AM, Valloton MB. Renin release by rat kidney slices incubated in vitro. Circ. Res, 1976; 39: 200-203.
- Carlsson E, Ablad B, Brändström B et al. Differentiated blockade of the chronotropic effects of various adrenergic stimuli in the cat heart. Life Sci. 1972; 11 part 1: 953-958.
- Catt KJ, Harwood JP, Aquilera G et al. Hormonal regulation of peptide receptors and target cell responses. Nature. 1979; 280: 109-116.
- Chang PC, van der Krogt JA, van Brummelen P. Demonstration of neuronal and extraneuronal uptake of circulating norepinephrine in the forearm. Eur J Clin Invest. 1986; 16 part 2; A58.
- Chevillard C, Pasquier R, Duchene N et al. Mechanism of inhibition of renin release by clonidine rats. Eur J Pharmacol. 1978; 48: 451-454.
- Ching Chan Lin, Iwao H, Puttkammer S et al. Prostaglandines and renin release in vitro. Am J Physiol. 1981; 240: E609-614.
- Clutter WE, Bier DM, Shah SD et al. Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and haemodynamic actions in man. J Clin Invest. 1980; 66: 94-101.

- Constantine JW, Lebel W. Complete blockade by phenoxybenzamine of α_1- but not of α_2-vascular receptors in dogs and the effects of propranolol. Naunyn Schmiedeberg's Arch. Pharmacol. 1980; 314: 149.
- Constantine JW, Lebel W, Archer R. Functional postsynaptic α_2- but not α_1-adrenoceptors in dog saphenous vein exposed to phenoxybenzamine. Eur J Pharmacol. 1982; 85: 325-329.
- Cocks TM, Angus JA. Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature. 1983; 305: 627-630.
- Cooke Cr, Brown TC, Zacherle BJ. The effect of altered sodium concentration in the distal nephron segments on renin release. J Clin Invest. 1970; 49: 1630-1638.
- Cryer PE, Rizza RA, Haymond MW. Epinephrine and norepinephrine are cleared through beta-adrenergic, but not alpha-adrenergic mechanisms in man. Metabolism. 1980; 11 suppl 1: 1114-1118.
- Cunningham J, van der Burg MJ, Holly JMP et al. Impaired response of plasma renin activity to tilting after renal transplantation. Clin Sci. 1981: 61: 69-73.
- Davies R, Geddes DM, Slater JDH et al. The specifity of β-adrenoceptors for the release of renin in man. Clin Sci Mol Med. 1975; 49: 14P.
- Davies JO, Freeman RH. Mechanisms regulating renin release. Physiol Rev. 1976; 56: 1-48.
- Davies B, Sudera D, Sever PS. Regulation of adrenoceptors in man during different adrenergic states. In: Kobinger W, Ahlquist RP, eds. Alpha and beta adrenoceptors and the cardiovascular system. Excerpta Medica, Princeton, 1984: 215-244.
- Derkx FHM, van Gool JMG, Wenting GJ et al. Inactive renin in human plasma. Lancet. 1976; ii: 496.
- Derkx FHM, Tan-Tjiong HL, Man in 't Veld AJ et al. Activation of inactive plasma renin by plasma and tissue kallikreins. Clin Sci. 1979[a]; 157: 351.
- Derkx FHM, Bouma BN, Tan Tjiong HL et al. Activation of inactive renin (prorenin) in human plasma: their connection with kinin formation, coagulation and fibrinolysis. Clin Sci. 1979[b]; 57: 89S.

- Derkx FHM, Brommer EJP, Boomsma FP et al. Activation of plasma prorenin by plasminogen activators in vitro and increase in plasma renin after stimulation of fibrinolytic activity in vivo. Neth J Med. 1984; 27: 124-128.
- Di Bona GF. Effects of mannitol diuresis and ureteral occlusion on distal tubular reabsorption. Am J Physiol. 1971; 221: 511.
- Di Bona GF. Neural control of renal function: Role of renal alpha adrenoceptors. J Cardiovasc Pharmacol. 1985; 7 (suppl. 8): S18-S23.
- Distler A, Keim HJ, Cordes U et al. Sympathetic responsiveness and antihypertensive effect of beta receptor blockade in essential hypertension. Am J Med. 1978; 64: 446-451.
- Docherty JR, McGrath JC. An examination of factors influencing adrenergic transmission in the pithed rat, with special reference to noradrenaline uptake mechanisms and postjunctional α-adrenoceptors. Naunyn Schmiedeberg's Arch Pharmacol. 1980; 313: 101-111.
- Docherty JR, Hyland L, Warnock P. A further examination of inhibitory α_1-adrenoceptors of rat vas deferens. Br J Pharmacol. 1984: 326P.
- Docherty JR, Hyland L. Vascular α_2-adrenoceptors can mediate nerve stimulation - evoked contractions. Clin Sci. 1985; 68 suppl. 10: 117S-120S.
- O'Donnell SR, Wanstall JC. The contribution of extraneuronal uptake to the trachea blood vessel selectivity of β-adrenoceptor stimulants in vitro in guinea pigs. Br J Pharmac. 1976; 57: 369-373.
- O'Donnel SR, Saar N, Wood LJ. The density of adrenergic nerves at various levels in the guinea pig lung. Clin Exp Pharmacol Physiol. 1978; 5: 325-332.
- Doxey JC, Smith LFC, Walker JM. Selectivity of blocking agents for pre- and postsynaptic α-adrenoceptors. Br J Pharmacol. 1977; 60: 91-96.
- Doxey JC, Roach AG, Samuel J. Effects of desipramine on stimulation induced contractions of the vas deferens of rats pretreated either chronically with desipramine or acutely with idazoxan. Clin Sci. 1985; 68 (suppl. 10): 155S-159S.

- Drew GM. Effects of α-adrenoceptor agonists and antagonists on pre- and postsynaptically located α-adrenoceptors. Eur J Pharmacol. 1976; 36: 313-320.
- Drew GM, Whiting SB. Evidence for two distinct types of post-synaptic α-adrenoceptors in vascular smooth muscle in vivo. Br J Pharmacol. 1979; 67: 207-215.
- Drew GM. What do antagonists tell us about α-adrenoceptors? Clin Sci. 1985; 68 suppl. 10: 15S-19S.
- Dubocovich ML, Langer SZ, Massingham R. Lack of correlation between presynaptic inhibition of noradrenaline release and organ responses during nerve stimulation. Br J Pharmacol. 1980; 69: 81-90.
- Duval N, Hicks PE, Langer SZ. Dopamine preferentially stimulates postsynaptic α_2-adrenoceptors in the femoral vascular bed, but α_1-adrenoceptors in the renal vascular bed of the anaesthetized dog. Eur J Pharmacol. 1985; 108: 265-272.
- Eikenburg DC, Schwartz DD. Enhanced stimulus induced neurotransmitter overflow in epinephrine-induced hypertensive rats is not mediated by prejunctional beta-adrenoceptor activation. Proceedings of the 11th Scientific Meeting of the International Society of Hypertension, 1986: p. 342.
- Elsner D, Saeed M, Sommer O et al. Sympathetic vasoconstriction sensitive to α_2-adrenergic receptor blockade. Hypertension. 1984; 6: 915-925.
- Enero MA, Langer SZ, Rothlin RP et al. Role of the α-adrenoceptor in regulating noradrenaline overflow by nerve stimulation. Br J Pharmacol. 1972; 44: 672-688.
- Fagard R, Eijskens E, De Looz H et al. The clearance of endogenous renin in the anhepatic anephric dog. Clin Sci. 1973; 45: 225S.
- Farhi ER, Cant JR, Barger AC. Interactions between intrarenal epinephrine receptors and the renal baroreceptor in the control of PRA in conscious dogs. Circ Res. 1982; 50: 477-485.
- Fildes RD, Eisner GM, Calcagno PL et al. Renal α-adrenoceptors and sodium excretion in the dog. Am J Physiol. 1985; 248: F128-133.

- Flavahan NA, McGrath JC. α_1-adrenoceptors can mediate chronotropic responses in the rat heart. Br J Pharmacol. 1981; 73: 586-588.
- Flavahan NA, Rimek TJ, Cooke JP et al. Characterization of postjunctional alpha$_1$- and alpha$_2$-adrenoceptors activated by exogenous or nerve-released norepinephrine in the canine saphenous vein. J Pharmacol Exp Ther. 1984; 230: 699-705.
- Fray JCS. Stretch receptor model for renin release with evidence from perfused rat kidney. Amer J Physiol. 1976; 231: 936.
- Fray JCS, Park CS. Influence of potassium, sodium, perfusion pressure, and isoprenaline on renin release induced by acute calcium deprivation. J Physiol 1979; 292: 363-372.
- Fray JCS. Stimulus-secretion coupling of renin. Circ Res. 1980; 47: 485-491.
- Freeman RH, Davis JO, Gotshall RW et al. The signal perceived by the macula densa during changes in renin release. Circ Res. 1974; 35: 307-316.
- Furchgott RF. Role of endothelium in responses of vascular smooth muscle. Circ Res. 1983; 53: 557-573.
- Gavendo S, Kapuler S, Servan T et al. β_1-Adrenergic receptors in kidney tubular cell membrane of the rat. Kidney Int. 1980; 17: 764-770
- Gerber JG, Olson RD, Nies AL. Interrelationship between prostaglandines and renin release. Kidney Int. 1981; 19: 816-821.
- Godfraind T, Egleme C, Al Osachie I. Role of endothelium in the contractile response of rat aorta to α-adrenoceptor agonists. Clin Sci. 1985; 68, suppl 10: 65S-71S.
- Goldberg MR, Robertson D. Evidence for the existence of vascular alpha$_2$-adrenergic receptors in humans. Hypertension 1984; 6: 551-556.
- Goldstein DS, Zimlichman R, Stall R et al. Plasma catecholamine and haemodynamic responses during isoproterenol infusions in humans. Pharmac Ther. 1986; 40: 233-238.
- Gottschalk CW, Leyssac PP. Proximal tubular function in rats with low insuline clearance. Acta Physiol Scand. 1968; 74: 453.

- Gotshall RW, Davis JO, Schade RE et al. Effects of renal denervation on renin release in sodium depleted dogs. Am J Physiol. 1973; 225: 344-349.
- Gotshall RW, Davis JO, Blaine EH et al. Increased renin release during renal arteriolar dilatation in dogs. Amer J Physiol. 1974; 227: 251.
- Graham RM, Pettinger WA. Effects of prazosin and phentolamine on arterial pressure, heart rate, and renin activity: Evidence in the conscious rat for the functional significance of the presynaptic α-receptor. J Cardiovasc Pharm. 1979; 1: 497-502.
- Graham RM, Stephenson WH, Pettinger WA. Pharmacological evidence for a functional role of the prejunctional alpha-adrenoceptor in noradrenergic neurotransmission in the conscious rat. Naunyn Schmiedeberg's Arch Pharmacol. 1980; 311: 129-138.
- Grant TL, Flavahan NA, Greig J. Attempts to uncover subtypes of α-adrenoceptors and associated mechanisms by using sequential administration of blocking drugs. Clin Sci. 1985; 68, suppl 10: 25S-30S.
- Gutkind JS, Enero MA. Effects of desimipramine on the cardiovascular responses to clonidine and guanabenz. Hypertension. 1986; 8 suppl.: I 184-I 186.
- Gutman FD, Tagawa H, Haber E et al. Renal arterial pressure, renin secretion and bloodpressure control in trained dogs. Amer J Physiol. 1973; 224: 66.
- Gutman Y. The mechanisms of PGE, alpha-adrenergic and beta-adrenergic regulation of catecholamine release: Evidence for different modes of action. In: Usdin E, Kopin Y, Barchas J, eds. Catecholamines: basic and clinical frontiers. New York; 1979: 277-279.
- Hamilton CA, Reid JL. Changes in α-adrenoceptors during long term treatment of rabbits with prazosin. J Cardiovasc Pharmacol. 1981; 3: 977-985.
- Hamilton CA, Reid JL. A postsynaptic location of $alpha_2$-adrenoceptors in vascular smooth muscle: In vivo studies in the conscious rabbit. Cardiovasc Res. 1982; 16: 11-15.

- Hamilton CA, Jones CR, Reid JL. α-Adrenoceptor regulation in vivo and in vitro in the rabbit. Clin Sci. 1985; 68 (suppl. 10): 125S-128S.
- Hamilton CA, Vincent J, Reid JL. Alpha-adrenoceptors and neural control of the circulation: Studies in experimental animals. In: Tarazi RC, Zanchetti A, eds. Physiology and pathophysiology of Hypertension, Chapter 7, Handbook of Hypertension, 1986.
- Hamilton CA, Reid JL, Zamboulis C. The role of presynaptic α-adrenoceptors in the regulation of blood pressure in the conscious rabbit. Br J Pharmac. 1982; 75: 417-424.
- Hartroft WS, Hartroft PM. New approaches in the study of cardiovascular disease: Aldosterone, renin, hypertension and juxtaglomerular cells. Fed Proc. 1961; 20: 845.
- Hartroft PM. Electron microscopy of nerve endings associated with juxtaglomerular (JG) cells and macula densa. Lab Invest. 1966; 15: 1127-1128.
- Hawthorn MH, Broadley KJ. Evidence from use of neuronal uptake inhibition that β_1-adrenoceptors, but not β_2-adrenoceptors, are innervated. J Pharm Pharmacol. 1982; 34: 664-666.
- Hedler L, Majewski H, Rump LC et al. Modulation of noradrenaline release in the rabbit through α-adrenoceptors. Br J Pharm. 1983; 43P.
- Heinsimer JA, Lefkowitz RJ. Adrenergic receptors: Biochemistry, regulation, molecular mechanism, and clinical implications. J Lab Clin Med. 1982; 100: 641-660.
- Henrich WL. Role of prostaglandines in renin secretion. Kidney Int. 1981; 19: 822-830.
- Hesse IFA, Johns EJ. The subtype of α-adrenoceptor involved in the neural control of renal tubular sodium reabsorption in the rabbit. J Physiol. 1984; 352: 527-538.
- Hesse IFA, Johns EJ. An in vivo study of the α-adrenoceptor subtypes on the renal vasculature of the anaesthetized rabbit. J Auton Pharm. 1984[a]; 4: 145-152.
- Hesse IFA, Johns EJ. The role of α-adrenoceptors in the regulation of renal tubular sodium absorption and renin secretion in the rabbit. Br J Pharm. 1985; 84: 715-724.

- Hoffman BB, Lefkowitz RJ. Radioligand binding studies of adrenergic receptors: New insights into molecular and physiological regulation. Ann Rev Pharmacol Toxicol. 1980; 20: 581-608.
- Hoffman BB, Lefkowitz RJ. Alpha-adrenergic receptor subtypes. N Engl J Med. 1980[a]; 302: 1390-1395.
- Hoffman BB, Lefkowitz RJ. Agonist interactions with α-adrenergic receptors. J Cardiovasc Pharmacol. 1982; 4: S14-S18.
- Hoffman BB, Michel TT, Brenneman TB et al. Interactions of agonists with platelet α_2-adrenergic receptors. Endocrinology. 1982; 110: 926-932.
- Holdaas H, Di Bona GF, Kil F. Effect of low-level renal nerve stimulation on renin release from non-filtering kidneys. Am J Physiol. 1981; 241: F156-F161.
- Horn PT. Kohli JD, Listinsky JJ et al. Regional variation in the alpha-adrenergic receptors in the canine resistance vessels. Naunyn Schmiedeberg's Arch Pharmacol. 1982; 318: 166-172.
- Insel PA, Snavely MD, Healy DP et al. Radioligand binding and functional assays demonstrate postsynaptic alpha$_2$-receptors on proximal tubulus of rat- and rabbit kidney. J Cardiovasc Pharm. 1985; 7, suppl. 8: S9-S17.
- Iversen LL. Catecholamine uptake processes. Br Med Bull. 1973; 29: 130-135.
- Jie K, van Brummelen P, Vermey P et al. Identification of vascular postsynaptic α_1-and α_2-adrenoceptors in man. Circ Res. 1984; 54: 447-452.
- Jie K, van Brummelen P, van Zwieten PA. Evidence for regulation of noradrenaline release by presynaptic α_2-adrenoceptors in humans. Eur J Clin Invest. 1986; 16 part II: A58.
- de Jonge A, Santing PN, Timmermans PBMWM et al. Physiological role of prejunctional α_2-adrenoceptors in the heart of spontaneously hypertensive rats. Br J Pharmac. 1983: 27P
- Johns EJ, Richards HK, Singer B. Effect of adrenaline, noradrenaline, isoprenaline and salbutamol on the production and release of renin by isolated renal cortical cells of the cat. Br J Pharmac. 1975; 53: 67-73.

- Johns EJ. An investigation into the type of β-adrenoceptor mediating sympathetically activated renin release in the cat. Br J Pharmac. 1981; 73: 749-754.
- Johnson BF, Smith IK, Labrooy J et al. The nature of the β-adrenoceptor controlling plasma renin activity in man. Clin Sci Mol Med. 1976; 51: 113S-118S.
- Johnson JA, Davis JO, Witty RT. Effects of catecholamines and renal nerve stimulation on renin release in the non-filtering kidney. Circ Res. 1971; 29: 646-653.
- Johnson JA, Davis JO, Gotshall RW et al. Evidence for an intrarenal beta receptor in control of renin release. Am J Physiol. 1976; 230: 410-418.
- Kalsner S, Chi Chung Chan. Adrenergic antagonists and the presynaptic receptor hypothesis in vascular tissue. J Pharmacol Exp Ther. 1979; 211: 257-264.
- Kalsner S. The presynaptic receptor controversy. Tips. 1982; 11-14.
- Kauker ML. Inhibition of water reabsorption in the collecting tubule by guanabenz. Kidney Int. 1982; 21: 279.
- Keeton TK, Pettinger WA. The dominance of adrenergic mechanisms in mediating hypotensive drug induced renin release in the conscious rat. J Pharmacol Exp Ther. 1979; 208: 303-309.
- Keeton TK, Biediger AM, Pfister SL. A comparison of the haemodynamic and neuroendocrine effects of α_2-adrenoceptor antagonists. Fed Proc. 1985; 44: 1571.
- Khayat A. Gonda S, Sen S et al. Responses of juxtaglomerular cells suspensions to various stimuli. Hypertension. 1981; 3: 157-167.
- Kho TL, Zaal GA, Wester A et al. Haemodynamic and endocrine changes during antihypertensive treatment with clonidine and its derivate st-600. Clin Sci Mol Med. 1976; 51: 641S-643S
- Kirchheim H, Ehmke H, Fischer S et al. Sympathetic modulation of the pressure dependent renin release in conscious dogs. In: Renal nerves and hypertension. Proceedings of a satellite symposium of the 11th Scientific Meeting of the ISH, Maastricht. 1986.

- Kobinger W, Pichler L. α_1- and α_2-adrenoceptor subtypes: Selectivity of various agonists and relative distribution of receptors as determined in rats. Eur J Pharmacol. 1981; 73: 313-321.
- Kopp U, Aurell M, Svensson L et al. Effect of prenalterol, a β_1-adrenoceptor agonist, on renin secretion rate in the anaesthetized dog. Acta Pharmacol Toxicol. 1981[a]; 49: 230-235.
- Kopp U, Aurell M, Sjolander M et al. The role of prostaglandines in the alpha- and beta-receptor mediated renin release response to graded renal nerve stimulation. Pflügers Arch. 1981[b]; 391: 1-8.
- Kopp U, Di Bona GF. Interaction of renal β_1-adrenoceptors and prostaglandines in reflex renin release. Am J Physiol. 1983; 244: F 418-424.
- Kopp U, Di Bona GF. Interaction between neural and non-neural mechanisms controlling renin secretion rate. Am J Physiol. 1984; 246: F620-624.
- Korner PI. Role of central monoaminergic systems and of beta-adrenoceptors in cardiovascular regulation. In: Alpha and beta adrenoceptors and the cardiovascular system. Excerpta Medica, Princeton, 1984: 72-105.
- Krausz MM, Feuerstein G, Feuerstein N et al. The role of the renin angiotensin system in mediation of adrenal catecholamine secretion in the cat induced by intrarenal β-adrenergic stimulation. Circ Res. 1980; 47: 835-839.
- Lafontan M, Berlan M. Characterization of physiological agonist selectivity of human fat cell α_2-adrenoceptors: adrenaline in the major stimulant of the α_2-adrenoceptors. Eur J Pharmacol. 1982; 82: 107-111.
- Lands AM, Arnold A, McAuliff JF et al. Differentiation of receptor systems activated by sympathomimetic amines. Nature. 1967; 214: 597-598.
- Langer SZ. Presynaptic regulation of catecholamine release. Biochem Pharmacol. 1974; 23: 1793-1800.
- Langer SZ, Adler-Graschinsky E, Giorgi O. Physiological significance of α-adrenoceptor mediated negative feedback mechanism regulating noradrenaline release during nerve stimulation. Nature. 1977; 265: 648-650.

- Langer SZ, Massingham R, Shepperzon NB. Differential sensitivity to prazosin blockade of endogenously released and exogenously administered noradrenaline: Possible relationship to the synaptic location of α_1- and the extra-synaptic location of α_2-adrenoceptors in dog vascular smooth muscle. Br J Pharm. 1980[a]; 123P.
- Langer SZ, Massingham R, Shepperzon NB. Presence of post-synaptic α_2-adrenoceptors of predominantly extrasynaptic location in the vascular smooth muscle of the dog hind limb. Clin Sci. 1980[b]; 59: 225S-228S.
- Langer SZ, Shepperzon NB. Postjunctional α_1-adrenoceptors and the role of neuronal uptake. J Cardiovasc Pharmacol. 1982; 4: S8-S13.
- Langer SZ, Hicks PE. Alpha-adrenoceptor subtypes in blood vessels: physiology and pharmacology. J Cardiovasc Pharmacol. 1984; 6: S547-558.
- Langer SZ, Duval N, Massingham R. Pharmacology and therapeutic significance of α-adrenoceptor subtypes. J Cardiovasc Pharmacol. 1985; 7 (suppl. 8): S1-S8.
- Lee MR. Dopamine and the kidney. Clin Sci. 1982; 62: 439-448.
- Leenen FHH, Redmond DP, McDonald RH. Alpha and beta adrenergic induced renin release in man. Clin Pharmacol Ther. 1975; 31-38.
- Lees GM. A hitch-hiker's guide to the galaxy of adrenoceptors. Br Med J. 1981; 283: 173-178.
- de Leeuw PW. Vasoregulation and renal function in essential hypertension. Rotterdam, Thesis, 1978.
- de Leeuw PW, Wester A, Willemse PJ et al. Effects of prazosin on plasma noradrenaline and plasma renin concentrations in hypertensive subjects. J Cardiovasc Pharmacol. 1980; 2 (suppl. 3): S361-S372.
- de Leeuw PW, Ligthart JJ, Smout AJMP et al. Within patients comparison of prazosin and UK-33274, a new alpha-adrenoreceptor antagonist. Eur J Clin Pharm. 1982; 23: 397-401.
- de Leeuw PW, van Es PN, Vermey P et al. Contribution of alpha-adrenoceptors to renal vascular tone in hypertensive humans. Eur J Clin Invest. 1986[a]; 16 part II: 343.

- de Leeuw PW, van Es PN, Tchang PT et al. Efferent renal nerve activity in man. Renal nerves and hypertension. Proceedings of a satellite symposium of the 11th Scientific Meeting of the ISH. 1986[b], Maastricht.
- de Leeuw PW, van Es P, de Bos R et al. Role of α_1- and α_2-adrenergic receptors in the human hypertensive kidney. Hypertension. 1987; 9 (suppl. III): III 210-III 212.
- Lefkowitz RJ, Caron MG, Stiles GL. Mechanisms of membrane-receptor regulation. N Eng J Med. 1984; 310: 1570-1579.
- Lowenstein J. Clonidine. Ann Int Med. 1980; 92: 74-77.
- Lynen PJ, Amery AK, Fagard RH. The effects of β-adrenoceptor blockade on renin, angiotensin, aldosterone and catecholamines at rest and during exercise. Br J Clin Pharmac. 1979; 7: 175-181.
- Majewski H, McCullock MW, Rand MJ et al. Adrenaline activation of prejunctional β-adrenoceptors in guinea pig atria. Br J Pharmac. 1980; 71: 435-444.
- Majewski H, Hedler L, Schurr C et al. Modulation of noradrenaline release in the pithed rabbit: A role for angiotensin II. J Cardiovasc Pharmacol. 1984; 6: 888-896.
- Majewski H, Hedler L, Schurr C et al. Dual effect of adrenaline on noradrenaline release in the pithed rabbit. J Cardiovasc Pharmacol. 1985; 7: 251-257.
- Majewski H, Alade PI, Rand MJ. A possible involvement of adrenaline in stress induced increases in blood pressure in rats. Proceedings of the 11th Scientific Meeting of the International Society of Hypertension. 1986: 60.
- Malik U, McGiff JC. Modulation by prostaglandines of adrenergic transmission in the isolated perfused rabbit and rat kidney. Circ Res. 1975; 36: 599-109.
- Man in 't Veld AJ, Boomsma F, Schalekamp MADH. Regulation of α and β-adrenoceptor responsiveness studies in patients with chronic autonomic failure. Br J Clin Pharmac. 1983; 15: 507S-519S.
- Man in 't Veld AJ, Schalekamp MADH. Mechanism of action of β-blockers in hypertension: vasodilatation via presynaptic β-receptors. In: Van Zwieten ed. Physiological receptor antagonism. Sandoz, Uden, 1984: 50-68.

- Man in 't Veld AJ, van den Meiracker A, Vincent HH et al. Beta adrenoceptor blocking drugs. Eur J Clin Invest. 1986; 16 part II: A60.
- Markwardt F, Glusa A, Hoffman A. Studies on α-adrenoceptors of blood platelets. Naunyn Schmiedeberg's Arch Pharmac. 1980; 313 suppl: R22.
- McGiff JC. Interactions of prostaglandines with the kallikrein-kinin and renin-angiotensin systems. Clin Sci. 1980; 59: 105S-116S.
- McGrath JC, Reid JL. Commentary on workshop on α-adrenoceptors. Clin Sci. 1985; 68 suppl. 10: 1S-7S.
- McPherson GA, Summers RJ. [^{3}H] Prazosin and [^{3}H] clonidin binding to α-adrenoceptors in membranes prepared from regions of rat kidney. J Pharm Pharmacol. 1981; 33: 189-191.
- van Meel JCA, Timmermans PBMWM, van Zwieten PA. Selectivity of some agonists for postsynaptic α_1- and α_2-adrenoceptors in the vascular system of the pithed rat. Naunyn Schmiedeberg's Arch Pharm. 1980; 313 suppl: R22.
- van Meel JCA, de Jonge A, Kalkman HO et al. Vascular smooth muscle contraction initiated by postsynaptic α_2-adrenoceptor activation is induced by an influx of extracellular calcium. Eur J Pharmacol. 1981; 69: 205-208.
- van Meel JCA, de Jonge A, Timmermans PBMWM et al. Selectivity of some alpha adrenoceptor agonists for peripheral α_1- and α_2-adrenoceptors in the normotensive rat. J Pharmacol Exp Ther. 1981[a]; 219:760-767.
- Meredith PA, Vincent J, Elliot HL et al. The clinical pharmacology of doxazosin. In: Proceedings of the 2nd European Meeting on Hypertension. 1985: 356.
- Meurer KA, Maxrath L, Helber A et al. Uber die beteiligung β_1- und β_2-adrenerger receptoren an der renin freisetzung. Verh Deutsch Gesellschapft Inn Med. 1976; 82: 1296-1299.
- Meurer KA, Lang R, Hombach V et al. Effects of a β_1- selective adrenergic agonist in normal human volunteers. Klin Wochenschr. 1980; 58: 425-427.
- de Mey J, Vanhoutte PM. Uneven distribution of postjunctional alpha$_1$- and alpha$_2$-like adrenoceptors in canine arterial and venous smooth muscle. Circ Res. 1980; 48: 875-884.

- Michelakis AM, Caudle J, Liddle GW. In vitro stimulation of renin production by epinephrine, norepinephrine, and cyclic AMP. Proc Soc Exp Biol Med. 1969; 130: 748.
- Michelakis AM, McAllister RG. The effect of chronic adrenergic receptor blocade on plasma renin activity in man. J Clin Endocrin. 1972; 34: 386-394.
- Miller RC, Mony M, Schini V et al. Endothelial mediated inhibition of contraction and increase in cyclic GMP levels evoked by the α-adrenoceptor agonist B-HT 920 in rat isolated aorta. Br J Pharmac. 1984; 83: 903-908.
- Minneman KP, Molinoff PB. The pharmacological specificity of beta 1 and beta 2 adrenergic receptors in rat heart and lung in vitro. Mol Pharmacol. 1979[a]; 16: 21-33.
- Minneman KP, Hegstrand LR, Molinoff PB. Simultaneous determination of beta-1 and beta-2-adrenergic receptors in tissues containing both receptor subtypes. Mol Pharmacol. 1979[b]; 16: 34-46.
- Minneman KP, Hedberg A, Molinoff PB. Comparison of beta adrenergic receptor subtypes in mammalian tissues. J Pharmacol Exp Ther. 1979[c]; 211: 502-508.
- Minneman KP, Dibner MD, Wolfe BB et al. β_1- and β_2-adrenergic receptors in rat cerebral cortex are independently regulated. Science. 1979[d]; 204: 866-868.
- Minneman KP, Molinoff PB. Classification and quantitation of β-adrenergic receptor subtypes. Biochem Pharmacol. 1980; 29: 1317-1323.
- Mitchell HC, Pettinger WA. The hypernoradrenergic state in vasodilatator drug-treated hypertensive patients: Effect of clonidine. J Cardiovasc Pharmacol. 1980; 2: 1-7.
- Mizoguchi H, Dzau VJ, Siwek LG et al. Effect of intrarenal administration of dopamine on renin release in conscious dogs. Am J Physiol. 1983; 244: H39-H45.
- Mogil RA, Itskovitz HD, Russel JH et al. Renal innervation and renin activity in salt metabolism and hypertension. Am J. Physiol. 1969; 216: 693-697.
- Morel F. Sites of hormone actions in the mammalian nephron. Am J Physiol. 1981; 1: F159-F164.

- Morganti A, Sola C, Palermo A et al. α_1-Adrenoceptor blockade dissociation of its effects on renin release and arterial blood pressure in man. Clin Sci. 1981; 61: 307S-309S.
- Morris BJ, Nixon RL, Johnston CI. Release of renin from glomeruli isolated from rat kidney. Clin Exp Pharmacol Physiol. 1976; 3: 37-47.
- Motulsky HJ, Insel PA. Adrenergic receptors in man: Direct identification, physiologic regulation and clinical alterations. N Engl J Med. 1982; 307: 18-29.
- Motulsky HJ, Snavely MD, Hughes RJ et al. Interaction of verapamil and other calcium channel blockers with α_1- and α_2-adrenergic receptors. Circ Res. 1983; 52: 226-231.
- Muntz KM, Garcia C, Hagler HK. α_1-Receptor localization in rat heart and kidney using autoradiography. Am J Physiol. 1985; 249: H512-H-519.
- Münzel PA, Healy DP, Insel PA. Autoradiographic localization of β-adrenergic receptors in rat kidney slices using [^{125}I] iodocyenopindolol. Am J Physiol. 1984; 246: F240-F245.
- Naftilan AJ, Oparil S. The role of calcium in the control of renin release. Hypertension. 1982; 4: 670-675.
- Nakane H, Nakane Y, Roux A et al. Effects of selective and non-selective beta adrenergic agents on renin secretion in isolated perfused rat kidney. J Pharmacol Exp Ther. 1980; 212: 34-38.
- Nicholson JP, Resnick LM, Pickering FG et al. Relationship of blood pressure response and the renin angiotensin system. Am J Med. 1985; 78: 241-244.
- Nolan PL, Reid IA. Mechanism of suppression of renin secretion by clonidine in the dog. Circ Res. 1978; 42: 206-211.
- Nolly HL, Reid IA, Ganong WF. Effect of adrenergic agents on renin release in vitro. Fed Proc 1974; 33: 253.
- Oates HF, Stoker LM, Monaghan JC et al. The beta-adrenoceptor controlling renin release. Arch Int Pharmacodyn. 1978; 234: 205-213.

- O'Brien JW, Flavahan NA, Grant TL et al. Influence of blood gases, Ca^{2+}-entry blockade and angiotensin converting enzyme inhibition on pressor responses to α-adrenoceptor agonists: Evidence in vivo for subtypes of response independent of receptor subtype. Clin Sci. 1985; 68 suppl. 10: 99S-104S.
- Onesti G, Schwartz AB, Kim KE et al. Antihypertensive effect of clonidine. Circ Res. 1971; 28-29 suppl. II: II53-II69.
- Osborn JL, Di Bona GF, Thames MD. Beta-1-receptor mediation of renin secretion elicited by low frequency renal nerve stimulation. J Pharmacol Exp Ther. 1981; 216: 265-269.
- Osborn JL, Thames MD, Di Bona GF. Role of macula densa in renal nerve modulation of renin secretion. Am J Physiol. 1982[a]; 242: R367-371.
- Osborn JL, Di Bona GF, Thames MD. Role of renal α-adrenoceptors mediating renin secretion. Am J Physiol. 1982[b]: F620-626.
- Osborn JL, Holdaas H, Thames MD et al. Renal adrenoceptor mediation of antinatriuretic and renin secretion responses to low frequency renal nerve stimulation in the dog. Circ Res. 1983; 53: 298-305.
- Osborn JL, Kopp U, Thames MD et al. Interactions among renal nerves, prostaglandines and renal arterial pressure in the regulation of renin release. Am J Physiol. 1984; 247: F706-713.
- Pedrinelli R, Sassano P, Arzilli F et al. Mediation of renin release in essential hypertension by α-adrenoceptors. J Cardiovasc Pharmacol. 1981; 3: 1153-1161.
- Pettinger WA, Keeton TK, Campbell WB et al. Evidence for a renal α-adrenergic receptor inhibiting renin release. Circ Res. 1976; 38: 338-346.
- Pettinger WA, Smyth DD, Umimura S. Renal α_2-adrenoceptors: Their locations and effects on sodium excretion. J Cardiovasc Pharmacol. 1985; 7 (suppl. 8): S24-27.
- Pollet RJ, Levey GS. Principles of membrane receptor physiology and their application to clinical medicine. Ann Int Med. 1980; 92: 663-680.
- Rand MJ, McCulloch MW, Story DF. Feedback modulation of noradrenergic transmission. Tips. 1982: 8-11.

- Rand MJ, Majewski H. Adrenaline mediates a positive feedback loop in noradrenergic transmission: Its possible role in development of hypertension. Clin Exper Hypert. 1984; A6 (1 and 2): 347-370.
- Reid IA, McDonald DM, Pachnis B et al. Studies concerning the mechanism of suppression of renin secretion by clonidine. J Pharmacol Exp Ther. 1975; 192: 713-721.
- Reid JL. Alpha-adrenoceptors in man. In: Kobinger W, Alquist RP eds. Alpha and beta adrenoceptors and the cardiovascular system. Princeton: Excerpta Medica, 1984: 161-177.
- Richards HK, Noble AR, Munday KA. Isoprenaline induced secretion of active and inactive renin in anaesthetized rabbits and by kidney cortex slices. Clin Sci. 1981; 61: 679-684.
- Roach AG, Berridge TL, Dexter DT et al. Effects of selective α_1- and α_2-adrenoceptor antagonists on plasma glucose and insulin levels in rats. Fed Proc. 1985; 44: 391.
- Robie NW. Evaluation of presynaptic α-receptor function in the canine renal vascular bed. Am J Physiol. 1980; 239: H 422-H 426.
- Ruffolo RR, Rosing EL, Waddell JE. Receptor interactions of imidazolines: Affinity and efficacy for alpha adrenergic receptors in rat aorta. J Pharmacol Exp Ther. 1979; 209: 429-436.
- Ruffolo RR, Waddell JE, Yaden EL. Postsynaptic alpha adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of alpha-2-adrenergic receptors in rat aorta. J Pharmacol Exp Ther. 1981; 217: 235-240.
- Ruffolo RR, Waddell JE, Yaden EL. Heterogenecity of postsynaptic alpha adrenergic receptors in mammalian aortas. J Pharmacol Exp Ther. 1982; 221: 309-314.
- Ruffolo RR, Yaden EL. The existence of spare α_1-adrenoceptors but not spare α_2-adrenoceptors in the vasculature of the pithed rat. J Cardiovasc Pharmacol. 1984; 6: 1011-1019.
- Ruffolo RR. Relative agonist potency as a means of differentiating α-adrenoceptors and α-adrenergic mechanisms. Clin Sci. 1985; 68 (suppl. 10): 9S-14S.
- Russell MP, Moran NC. Evidence for lack of innervation of β_2-adrenoceptors in the blood vessels of the gracilis muscle of the dog. Circ Res. 1980; 46: 344-352.

- Salvetti A, Polli L, Arzilli F et al. Effects of salbutamol and metoprolol on plasma renin activity and plasma potassium of normal subjects and hypertensive patients. J Endocrinol Invest. 1978; 1: 1-8.
- Schalekamp MADH, Vincent MH, Man in 't Veld AJ. Adrenaline, stress, and hypertension. Lancet 1983; i: 362.
- Schalekamp MADH, Man in 't Veld AJ. Mono-aminergic receptors and bloodpressure. Neth J Med. 1985; 28 (2): 83-90.
- Schmidt HHHW, Schurr C, Hedler L et al. Local modulation of noradrenaline release in vivo: presynaptic β_2-adrenoceptors and endogenous adrenaline. J Cardiovasc Pharmacol. 1984; 6: 641-649.
- Schmitt H, Schmitt-Jubeau H. Alpha-adrenoceptors and central cardiovascular control. In: Kobinger N, Ahlquist RP, eds. Alpha and beta adrenoceptors and the cardiovascular system. Excerpta Medica, Princeton, 1984: 45-71.
- Schmitz JM, Graham RM, Sagalowsky A et al. Renal alpha-1 and alpha-2 adrenergic receptors. Biochemical and Pharmacological Correlations. J Pharmacol Exp Ther. 1981; 219: 400-406.
- Schümann HJ. What role do α- and β-receptors play in the regulations of the heart? Eur Heart J. 1983; 4 suppl. A: 55-60.
- Schümann HJ, Lues I. Postjunctional α-adrenoceptors in the isolated saphenous vein of the rabbit. Naunyn Schmiedeberg's Arch Pharmacol. 1983; 323: 328-334.
- Sealy JE, Moon C, Laragh JH. Plasma prorenin: cryoactivation and relationship to renin substrate in normal subjects. Am J Med. 1976; 61: 731.
- Seymour AA, Davis JO, Echtenkamp SF. Adrenergically induced renin release in conscious indomethacin treated dogs and rats. Am J Physiol. 1981; 240: F515-F521.
- Shanks RG. The physiological role of alpha- and beta-adrenoceptors in regional circulation. In: Kobinger W, Ahlquist RP, eds. Alpha and Beta adrenoceptors and the cardiovascular system. Excerpta Medica, Princeton, 1984: 109-122.
- Shepherd JT, Vanhoutte PM. Local modulation of adrenergic transmission. Circulation. 1981; 64: 655-665.

- Shepperzon NB, Langer SZ. The effects of the 2-amino-tetrahydronaphtalene derivate M_7, a selective α_2-adrenoceptor agonist in vivo. Naunyn Schmiedeberg's Arch Pharmacol. 1981; 318: 10-13.
- Shepperzon NB, Duval N, Massingham R et al. Pre- and postsynaptic alpha adrenoceptor selectivity studies with yohimbine and its stereoisomers rauwolscine and coryanthine in the anaesthetized dog. J Pharmacol Exp Ther. 1981; 219: 540-546.
- Shoji T, Tsuru H, Shigei T. A regional difference in the distribution of postsynaptic alpha-adrenoceptor subtypes in canine veins. Naunyn Schmiedeberg's Arch Pharmacol. 1983; 324: 246-255.
- Shulkes AA, Gibson RR, Skinner SL. The nature of inactive renin in human plasma and amniotic fluid. Clin Sci. 1978; 55: 41.
- Skinner SL, McCubbin JN, Page IH. Renal baroreceptor control of renin secretion. Science. 1963; 141: 814-816.
- Skinner SL, McCubbin JN, Page IH. Control of renin secretion. Circ Res. 1964; 15: 64-75.
- Smits JFM, Struyker Boudier HAJ, Kleinjans ICS et al. Nervous control of the kidney and hypertension. Renal nerves and hypertension. Abstract of a satellite symposium of the 11th Meeting of the ISH. Maastricht 1986.
- Smyth DD, Umemura S, Pettinger WA. Renal α_2-adrenergic receptors multiply and mediate sodium retention after prazosin pretreatment. Hypertension. 1986; 8: 323-331.
- Snavely MD, Insel PA. Characterization of alpha adrenergic receptor subtypes in the rat renal cortex. Mol Pharmacol. 1982; 22: 532-546.
- Snavely MD, Motulsky HJ. Moustafa E et al. β-adrenergic receptor subtypes in the rat renal cortex. Circ Res. 1982; 51: 504-513.
- Snedecor GW, Cochran WG. Statistical methods. The Iowa State University Press, Ames, Iowa, USA, sixth edition 1967.
- Staessen J, Cottaert A, de Schaepdrijver A et al. Effects of β_1-adrenoceptor agonism on plasma renin activity in normal man. Br J Clin Pharmacol. 1983; 16: 553-556.
- Starke K, Borowski E, Endo T. Preferential blockade of presynaptic α-adrenoceptor by yohimbine. Eur J Pharmacol. 1975; 34: 385-388.

- Starke K. Regulation of noradrenaline release by presynaptic receptor systems. Rev Physiol Biochem Pharmacol. 1977; 77: 1-124.
- Starke K, Docherty JR. Types and functions of peripheral α-adrenoceptors. J Cardiovasc Pharmacol. 1982; 4: 53-57.
- Stevens MJ, Moulds RFW. Factors determining α-adrenoceptor mediated responses of human digital and metacarpal veins. Clin Sci. 1985; 68 (suppl. 10): 31S-33S.
- Stiles GL, Caron MG, Lefkowitz RJ. β-adrenergic receptors: biochemical mechanisms of physiological regulation. Physiol Rev. 1984; 64: 661-742.
- Stjärne L, Brundin J. Dual adrenoceptor mediated control of noradrenaline secretion from human vasoconstrictor nerves: Facilitation by β-receptors and inhibition by α-receptors. Acta Physiol Scand. 1975; 94: 139-141.
- Stjärne L, Brundin J. β_2-adrenoceptors facilitating noradrenaline secretion from human vasoconstrictor nerves. Acta Physiol Scand. 1976; 97: 88-93.
- Stokes GS, Weber MA. Prazosin: Preliminary report and comparative studies with other antihypertensive agents. Br Med J. 1974; II: 298.
- Story DF, Standford Starr CA, Rand MJ. Evidence for the involvement of α_1-adrenoceptor in negative feedback regulation of noradrenergic transmitter release in rat atria. Clin Sci. 1985; 68 (suppl. 10): 111S-115S.
- Strandhoy JW, Morris M, Buckalew M. Renal effects of the antihypertensive guanabenz in the dog. J Pharmacol Exp Ther. 1982; 221: 347-352.
- Strandhoy JW. The role of alpha-2-receptors in the regulation of renal function. J Cardiovasc Pharmacol. 1985; 7 (suppl. 8): S28-S33.
- Strang KD. De rol van renale alpha receptoren bij de regulatie van de reninesecretie door de geisoleerde rattenier. Thesis, Rotterdam, 1978.
- Struyker Boudier HAJ, Janssen BJA, Vervoort-Peters LHTM. Adrenoceptors in the kidney. In: Renal nerves and hypertension, abstracts of a satellite symposium of the 11th Meeting of the ISH. Maastricht, 1986.

- Sullivan AT, Drew GM. Pharmacological characterization of pre- and postsynaptic α-adrenoceptors in dog saphenous vein. Naunyn Schmiedeberg's Arch Pharmacol. 1980; 312: 249-258.
- Summers RJ, Kuhar MJ. Autoradiographic localization of β-adrenoceptors in the kidney. Eur J Pharmacol. 1983; 91: 305-310.
- Summers RJ. Renal α-adrenoceptors. Fed Proc. 1984; 43: 2917-2922.
- Summers RJ. Stephenson JA, Lipe S et al. α_2-Adrenoceptors in dog kidney: autoradiographic localization and putative functions. Clin Sci. 1985; 68 suppl. 10: 105S-109S.
- Taugner R, Hackenthal E, Rix E et al. Immunocytochemistry of the renin-angiotensin system: renin, angiotensinogen, angiotensin I, angiotensine II, and converting enzyme in the kidneys of mice, rats, and tree shrews. Kidney Int. 1982; 22 (suppl. 12): 33.
- Thames MD, DiBona GF. Renal nerves modulate the secretion of renin mediated by non-neural mechanisms. Circ Res. 1979; 44: 645-652.
- Tharp MD, Hoffman BB, Lefkowitz PJ. α-Adrenergic receptors in human adipocyte membranes: direct determination by ^{3}H-yohimbine binding. J Clin Endocrinol Metab. 1981; 52: 709-714.
- Thom S, Calvete J, Hayes R et al. Human vascular smooth muscle responses mediated by α_2-mechanisms in vivo and in vitro. Clin Sci. 1985; 68 (suppl. 10): 147S-150S.
- Thurau K, Schnerrman J, Nagel W et al. Composition of tubular fluid in the macula densa segment as a factor regulating the function of the juxtaglomerular apparatus. Circ Res. 1967; 20-21, suppl. 11: 79-89.
- Thurau K, Grüner A, Mason J et al. Tubular signal for the renin activity in the juxtaglomerular apparatus. Kidney Int. 1982; 22, suppl. 12: S55-S62.
- Tigerstedt R, Bergmann PG. Niere und Kreislauf. Skand Arch Physiol. 1898; 8: 223.
- Timmermans PBMWM, van Zwieten PA. Postsynaptic α_1- and α_2-adrenoceptors in the circulatory system of the pithed rat: selective stimulation of the α_2-type by B-HT 933. Eur J Pharmacol. 1980[a]; 63: 199-202.

- Timmermans PBMWM, van Meel JCA, van Zwieten PA. Evaluation of the selectivity of α-adrenoceptor blocking drugs for postsynaptic α_1- and α_2-adrenoceptors in a simple animal model. J Autonom Pharmacol. 1980[b]; 1: 53-60.
- Timmermans PBMWM, de Jonge A, van Zwieten PA. Discrepancy between peripheral and central α-adrenergic activities with 2,3- and 2,5-substituted derivates of clonidine. Naunyn Schmiedeberg's Arch Pharmacol. 1980[c]; 313 (suppl.): R22.
- Timmermans PBMWM, van Zwieten PA. Mini review: the post-synaptic α_2-adrenoceptor. J Autonom Pharmacol. 1981[a]; 1: 171-183.
- Timmermans PBMWM, Schoop AMC, Kwa HY et al. Characterization of α-adrenoceptors participating in the central hypotensive and sedative effects of clonidine using yohimbine, rauwolscine and corynanthine. Eur J Pharmacol. 1981[b]; 70: 7-15.
- Tobian L, Tomboulian A, Janecek L. The effect of high perfusion pressure on the granulation of juxtaglomerular cells in an isolated kidney. J Clin Invest. 1959; 38: 605.
- Tobian L, Broden M, Money J. The effect of unilateral renal denervation on the secretion of renin. J Lab Clin Med. 1964; 64: 1011.
- Umemura S, Yasuda G, Uchino K et al. Existence of renal alpha-1 and alpha-2-adrenoceptors in the human kidney. In: Proceedings of the 11th Scientific Meeting of the International Society of Hypertension. 1986: 254.
- U'Prichard DC, Snijder SH. Distinct α-noradrenergic receptors differentiated by binding and physiological relationships. Life Sci. 1979;24: 79-88.
- Vander AJ, Miller R. Control of renin secretion in the anaesthetized dog. Am J Physiol. 1964; 207(3): 537-546.
- Vander AJ. Effect of catecholamines and the renal nerves on renin secretion in anaesthetized dogs. Am J Physiol. 1965; 209(3): 659-662.
- Vander AJ. Control of renin release. Physiol Rev. 1967; 47: 359-382.
- Vandongen R. Peart WS, Boyd GW. Adrenergic stimulation of renin secretion in the isolated perfused rat kidney. Circ Res. 1973; 32: 290-296.

- Vandongen R. Direct intrarenal action of catecholamines on renin secretion. Clin Exp Pharmacol Physiol. 1975; suppl. 2: 103-107.
- Vandongen R, Peart WS. The inhibition of renin secretion by alpha adrenergic stimulation in the isolated rat kidney. Clin Sci Mol Med. 1974; 47: 471-479.
- Vandongen R, Peart WS. Calcium dependence of the inhibitory effect of angiotensin on renin secretion in the isolated perfused kidney of the rat. Br J Pharmac. 1974[a]; 50: 125-127.
- Vandongen R. Intrarenal stimulation of renin secretion by frusemide in the isolated kidney of the rat. Br J Pharm. 1977; 60: 73-76.
- Vandongen R, Strang KD, Poessé MH et al. Suppression of renin secretion in the rat kidney by a non-vascular α-adrenergic mechanism. Circ Res. 1979; 45: 435-439.
- Vanhees L, Fagard R. Hespel P et al. Renin release: β_1- or β_2-receptor mediated. N Engl J Med. 1985; 312: 123-124.
- Vanhoutte PM, Cooks JP, Lindblad LE et al. Modulation of post-junctional α-adrenergic responsiveness by local changes in temperature. Clin Sci. 1985; 68 suppl. 10: 121S-123S.
- Vincent HH, Man in 't Veld AJ, Boomsma F et al. Elevated plasma noradrenaline in response to β-adrenoceptor stimulation in man. Br J Clin Pharmacol. 1982; 13: 717-721.
- Vincent HH, Boomsma F, Man in 't Veld AJ et al. Beta receptor stimulation by adrenaline elevates plasma noradrenaline and enhances the pressor responses to cold exposure and isometric exercise. J of Hypertension. 1983; 1 suppl. 2: 74-76.
- Vincent HH, Man in 't Veld AJ, Schalekamp MADH. Effects of beta-2-adrenoceptor blockade on plasma noradrenaline, renin and bloodpressure. In: Proceedings of the second European Meeting on Hypertension. 1985: 554.
- Vincent J, Elliot HL, Meredith PA et al. Doxazosin, an α_1-adrenoceptor antagonist: pharmacokinetics and concentration effect relationship in man. Br J Clin Pharmacol. 1983; 15: 719-725.
- Wagermark J, Ungerstedt U, Ljungqvist A. Sympathetic innervation of the juxtaglomerular cells of the kidney. Circ Res. 1968; 22: 149-153.

- Wahrenberg H, Engfeldt P, Arner P et al. Adrenergic regulation of lipolysis in human adipocytes. J Clin Endocrin Metab. 1986; 63: 631-638.
- Waterfall JF, Rhodes KF, Lattimer N. Studies of α_2-adrenoceptor antagonist potency in vitro: comparisons in tissues from rats, rabbits, dogs and humans. Clin Sci. 1985; 68 (suppl. 10): 21S-24S.
- Weber MA, Stokes GS, Gain J. Comparison of the effects on renin release of beta adrenergic antagonists with differing properties. J Clin Invest. 1974; 54: 1413-1419.
- Weinberger MH, Aoi W, Henry DP. Direct effect of beta adrenergic stimulation on renin release by the rat kidney slice in vitro. Circ Res. 1975; 37: 318-324.
- Wester A. Vasodilatatie en beta-adrenerge blokkade bij de behandeling van hypertensie patienten. Thesis, Rotterdam, 1979.
- Westfall TC. Local regulation of adrenergic neurotransmission. Physiol Rev. 1977; 57: 660-707.
- Wikberg JES. The pharmacological classification of adrenergic α_1- and α_2-receptors and their mechanisms of actions. Acta Physiol Scand. 1979; suppl. 468: 1-99.
- Wilffert B, Timmermans PBMWM, van Zwieten PA. Extrasynaptic localtion of alpha-2 and noninnervated beta-2 adrenoceptors in the vascular system of the pithed normotensive rat. J Pharmacol Exp Ther. 1982[a]; 221: 762-768.
- Wilffert B, Gouw MAM, de Jonge A et al. Indications for vascular alpha- and beta-2-adrenoceptors in synapses of the muscarinic pathways in the pithed normotensive rat. J Pharmacol Exp Ther. 1982[b]; 223: 219-223.
- Wilffert B, Gouw MAM, Timmermans PBMWM et al. Interaction between β_2-adrenoceptor mediated vasodilatation and α_2-adrenoceptor mediated vasoconstriction in the pithed normotensive rat. J Cardiovasc Pharmacol. 1983; 5: 822-828.
- Wilffert B, de Jonge A, Thoolen MJMC et al. Adrenoceptors in the heart. In: Van Zwieten, ed. Physiological receptor antagonism, Actua Sandoz, Uden, 1984: 116-129.

- Williams RS. Selectivity of prenalterol for adrenergic receptor subtypes: a potential mechanism of inotropic selectivity. J Cardiovasc Pharmacol. 1983; 5: 266-271.
- Wilton A, Joshi PIS. Adrenergic receptors and renin release. Lancet. 1977; i: 698-699.
- Winer N, Deenbandhu SC, Mying Sun Soon et al. Adrenergic receptor mediation of renin secretion. J Clin Endocrin. 1969; 29: 1168-1175.
- Witty RT, Davis JO, Schade RE et al. Mechanisms regulating renin release in dogs with thoracic caval constriction. Circ Res. 1972; 31: 339-347.
- Wolff DW, Buckalew VM, Strandhoy JW. Renal α_1- and α_2-adrenoceptor mediated vasoconstriction in dogs: comparison of phenylephrine, clonidine and guanabenz. J Cardiovasc Pharmacol. 1984; 6: S793-S798.
- Wolff DW, Gesek FA, Strandhoy JW. In vivo assessment of rat renal vascular α-adrenoceptors. Fed Proc. 1985; 44: 1571.
- Yamaguchi N, de Champlan J, Nadeau RA. Regulation of norepinephrine release from cardiac sympathetic fibers in the dog by presynaptic α- and β-receptors. Circ Res. 1977; 41: 108-117.
- Yamaguchi I, Kopin IJ. Differential inhibition of alpha-1- and alpha-2-adrenoceptor mediated pressor responses in pithed rats. J Pharmacol Exp Ther. 1980; 214: 275-281.
- Young WS, Kuhar MJ. α_2-Adrenergic receptors are associated with the renal proximal tubulas. Eur J Pharmacol. 1980; 67: 493-495.
- Ziegler MG, Chernow B, Woodson LC et al. The effect of propranolol on catecholamine clearance. Clin Pharmacol Ther. 1986; 40: 116-119.
- Zukowska-Grojec Z, Bayorh MA, Kopin IJ. Effect of desipramine on the effects of α-adrenoceptor inhibitors on pressor responses and release of norepinephrine into plasma of pithed rats. J Cardiovasc Pharmacol. 1983; 5: 297-301.
- van Zwieten PA, Thoolen MJMC, Timmermans PBMWM. The hypotensive activity and side effects of methyldopa, clonidine and guanfacine. Hypertension. 1984: 6 suppl II: II28-II33.
- van Zwieten PA. Centrally acting antihypertensives. Eur J Clin Invest. 1986; 16 part II: A 60.

Nawoord

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CURRICULUM VITAE

Hessel van Houten werd in 1953 te Sint Nicolaasga (FR) geboren. Hij bezocht het Bogerman Lyceum te Sneek alwaar in 1970 het einddiploma HBS-B werd behaald. In hetzelfde jaar begon de medische studie aan de Rijksuniversiteit te Groningen. Alvorens deze studie in 1977 werd afgesloten was hij assistent op de beademingsafdeling, hoofd Prof. Dr. H. Sluiter, van het Academisch Ziekenhuis te Groningen.
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In 1979 werd aangevangen met de opleiding tot internist in het Zuiderziekenhuis te Rotterdam onder leiding van Prof. Dr. W.H. Birkenhäger, waarna in 1984 inschrijving in het specialistenregister plaatsvond. In 1985 en 1986 was hij werkzaam op de interne afdeling als chef de clinique. In januari 1987 werd de overstap naar het Academisch Ziekenhuis te Leiden gemaakt alwaar de promovendus werkzaam is op de afdeling Endocrinologie en Stofwisselingsziekten (Hoofd: Prof. Dr. H.M.J. Krans) en zich met name bezighoudt met pancreastransplantaties bij de mens.