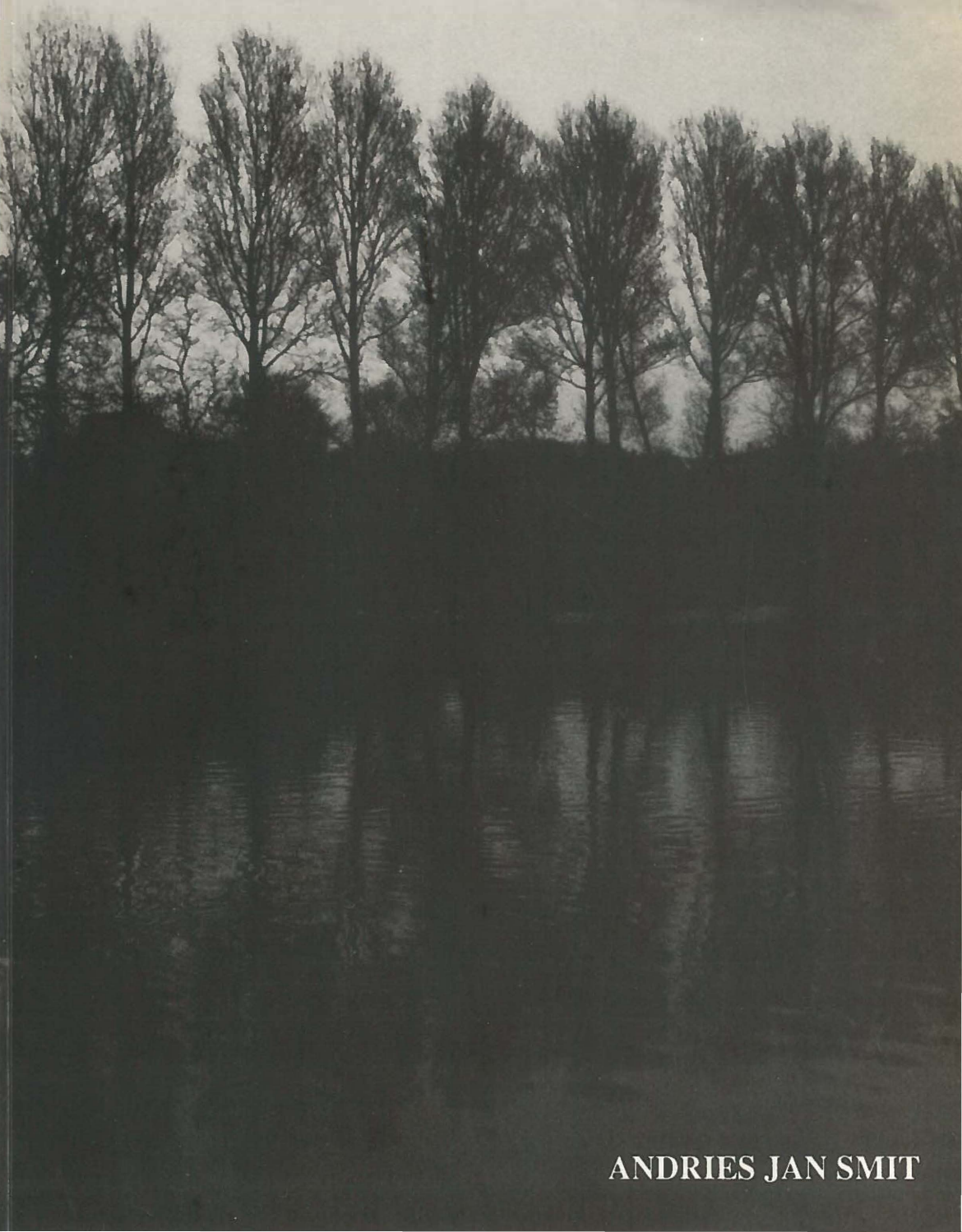
Renal effects of exogenous and endogenous dopamine

Smit, Andries Jan

RENAL EFFECTS OF EXOGENOUS AND ENDOGENOUS DOPAMINE

STUDIES ON THE RENAL HAEMODYNAMIC AND NATRIURETIC EFFECTS OF DOPAMINE IN MAN



ANDRIES JAN SMIT

RENAL EFFECTS OF EXOGENOUS AND ENDOGENOUS DOPAMINE

STUDIES ON THE RENAL HAEMODYNAMIC AND NATRIURETIC EFFECTS OF DOPAMINE IN MAN

Stellingen behorende bij het proefschrift van Andries Jan Smit.

"Renal effects of exogenous and endogenous dopamine"

Groningen, 20 juli 1988.

I Er bestaat geen renale dosis dopamine.

II Selectiviteit van dopamine-agonisten is niet op voorhand een gunstige eigenschap bij gebruik van deze middelen voor de behandeling van decompensatio cordis.

III De waarde van dopamine-agonisten voor de behandeling van chronische nierinsufficiëntie zal eerder gelegen zijn in hun diuretische dan in het renaal hemodynamische effect.

IV Plasma dopaminespiegels hebben geen betekenis voor de klinische diagnostiek.

V Bij onderzoek naar renale effecten van cytostatica moet rekening gehouden worden met de zoutretinerende werking van dopamine antagonisten die als antiemetica gelijktijdig toegediend worden.

VI Zoutbeperking dient de eerste stap te zijn bij de behandeling van cystinurie.

VII Bij intraveneuze digitalisatie is op farmacokinetische gronden gebruik van bolusinjecties van digoxine niet logisch en verdient infusie de voorkeur.

VIII Een goed opgezet vergelijkend onderzoek hoort niet voortijdig afgebroken te worden wanneer bij tussentijdse analyse een nieuwe behandeling een beter resultaat heeft dan de traditionele wijze van behandeling.

IX Geneesmiddelonderzoek bij hartfalen besteedt onvoldoende aandacht aan functieveranderingen in de nier.

X De snelle ontwikkeling in niet-chirurgische interventionele technieken ter behandeling van vanouds operatief benaderde aandoeningen kan een spanningsveld oproepen tussen de bedrijvers van deze technieken en de snijdende specialisten. Het valt te hopen dat dit spanningsveld zich even gericht en ten bate van de patiënt ontlaadt als de energie waarvan gebruik gemaakt wordt bij een aantal van deze technieken.

XI Behandeling van decompensatio cordis hoort in specialistische handen gezien de hoge mortaliteit van deze aandoening en de beschikbaarheid van effectieve maar niet altijd risicoloze behandelingsmogelijkheden.

XII Het vermogen tot oordelen lost op in alcohol maar het oordeel over het oplossend vermogen van alcohol is niet eensluidend.

XIII Op dissertaties bestaande uit een in boekvorm gebundelde verzameling nadrukken van reeds gepubliceerde artikelen is de benaming 'naschrift' meer van toepassing dan die van 'proefschrift'.

XIV De hoge impact factor van publikatie in de pers rechtvaardigt het invoeren van een citation index voor laatste stellingen van een proefschrift.

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RENAL EFFECTS OF EXOGENOUS AND ENDOGENOUS DOPAMINE

STUDIES ON THE RENAL HAEMODYNAMIC AND NATRIURETIC EFFECTS OF DOPAMINE IN MAN

Proefschrift

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. S.K. Kuipers
in het openbaar te verdedigen op woensdag 20 juli 1988
des namiddags te 4.00 uur

door

Andries Jan Smit

geboren te Leeuwarden

Promotores: Prof. Dr. W.D. Reitsma
Prof. Dr. H. Wesseling
Prof. Dr. A.J.M. Donker

Referent : Dr. S. Meijer

Aan mijn ouders

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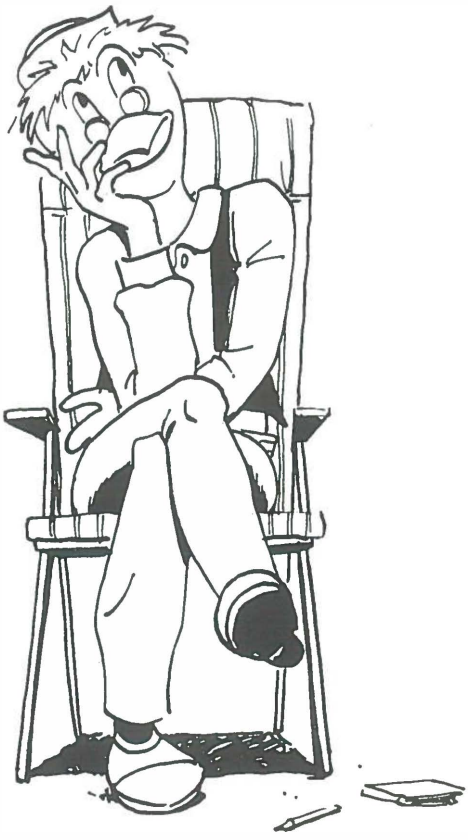

CHAPTER 1

DOPAMINE AND THE KIDNEY: A REVIEW

INTRODUCTION

It is now more than a quarter of a century ago that the effects of dopamine on renal function were first described. The renal and cardiovascular effects of dopamine made the agent attractive as a drug in various clinical situations in which cardiovascular or renal function is compromised. In the next decade dopamine indeed found widespread clinical use and has not yet lost its position since. Dopamine also became one of the most extensively studied substances in medical research. Although most early studies on the physiology of dopamine were devoted to its cardiovascular actions, its essential role in the central nervous system became soon established and shifted the balance to a preponderance of biochemical and behavioural studies. The existence of several types of dopamine receptors in the central nervous system has found recognition and has been complemented by a comparable classification of dopamine receptors in peripheral organs. A major contribution was formed by the development of selective antagonists for these receptors. Some of these antagonists became valuable drugs in psychiatric and gastrointestinal medicine. Their therapeutical value in cardiovascular or renal diseases, however, was limited. In this field of medicine they mainly served as a research tool for the pharmacological evaluation of the effects of dopamine. However, recent years have witnessed the advent of selective dopamine agonists which meanwhile have grown to clinical practice. This may have important implications in renal and cardiovascular medicine: besides facilitating the study of the renal effects of dopamine receptor stimulation in man, the development of these selective and often orally active dopamine agonists not only jeopardizes the till now undisputed place of dopamine in clinical practice, but also opens new therapeutical perspectives. Use of such drugs in for example hypertensive emergencies, chronic renal insufficiency and congestive heart failure is now under clinical investigation. Although several reviews have addressed the renal effects of dopamine in the past, these new developments justify renewed consideration of the subject [1-5]. This review deals with the relation between dopamine and the kidney. After a historical introduction, the effects of dopamine on various receptors will be reviewed. The renal actions of exogenous and endogenous dopamine on the renal vasculature and on natriuresis will be considered separately. Special attention will be devoted to the natriuretic action of dopamine. After an interlude on the relation between dopamine and some hormones, the role of dopamine in hypertensive and oedematous states will be discussed. Finally, the therapeutical perspectives of some newly developed selective dopamine agonists and antagonists will be mentioned.

HISTORY

The first description of the renal effects of dopamine in man dates from the early sixties. Goldberg described the natriuretic action of dopamine in four patients with congestive heart failure [6]. In the next year McDonald extended this observation by performing renal function studies in nine normal subjects and in six patients with congestive heart failure [7]. He administered the maximal dose which had been found not to increase the blood pressure in the same individual in a previous experiment, resulting in doses ranging from 2.6 to 7.1 μg/kg/min in normal subjects, and from 1.3 to 3.6 μg/kg/min in patients with congestive heart failure. Using these doses he found a mean increase of inulin clearance from 109 to 126 ml/min in healthy subjects and from 61 to 80 ml/min in patients. PAH-clearance rose from 507 to 798 ml/min in normal controls, and from 217 to 321 ml/min in patients. Sodium excretion rose from 171 to 575 μmol/min (246 to 828 mmol/day) in healthy volunteers and from 82 to 337 μmol/min (118 to 485 mmol/day) in patients.

This digression on his results is made for several reasons: first of all these results nicely demonstrate the major renal effects of dopamine with a marked increase in renal blood flow and natriuresis, and a more modest increase in glomerular filtration rate. Secondly, his results show that the propitious response to dopamine is conserved and possibly even enhanced in patients with congestive heart failure thereby stressing its potential therapeutic value. Thirdly, the study of McDonald in humans is remarkable because it gave the first description of the renal effects of dopamine, not even investigated before in animal studies. A marked contrast existed in this respect between dopamine and all other catecholamines known at that time. The naturally occurring catecholamines epinephrine and norepinephrine both induce a fall in ERPF and natriuresis [8]. Only isoproterenol had been found to induce a slight increase in ERPF [9]. The unusual effects of dopamine formed a major stimulus for the subsequent discovery of dopamine receptors. Finally, McDonald's results may serve to highlight the importance of dosage considerations in the use of dopamine. Administration of much larger doses, usually in the milligram range, in almost all previous studies probably postponed recognition of the unique effects of dopamine for decades.

Dopamine, first synthesized in 1910 independently by Barger and Dale from Wellcome Laboratories, and by Mannich and Jacobsohn in Berlin, was tested in the same year by Dale as part of a large series of catecholamines for its pressor potency [10 11]. A dose 35 to 50 times that of adrenaline was needed to result in a sub-maximal pressor response in the cat, which made dopamine not different from many other newly synthesized catecholamines. The pressor effect of dopamine was confirmed in later studies. Both Tainter and Raymond-Hamet found a depressor effect of dopamine in studies in the early thirties, but they considered this to be due to pretreatment with ergotoxine and yohimbine, respectively [12 13].

In 1942 Holtz and Credner from Rostock University, who had demonstrated the abundant presence of dopa-decarboxylase in the kidneys of several species in earlier studies, reported on the blood pressure effects of the intravenous administration of urine of animals and humans given the amino acid l-dioxyphenylalanine (L-dopa) [14 15].

They correctly assumed that dopa would be converted by dopa-decarboxylase to dopamine, which substance they expected to be excreted in the urine. Urine portions were collected before and after oral or intravenous administration of dopa, and assayed for the presence of dopamine. Considerable quantities of dopamine were found in the urine collected after dopa administration in several species. This urine was injected subsequently in cats, rabbits and guinea-pigs. In cats, after a small initial fall in blood pressure a marked pressor response occurred. However, in guinea-pigs and rabbits, injection of comparable amounts of urine caused no pressor response whatsoever. Subsequent experiments revealed that direct intravenous injection of dopamine consistently caused a depressor response in these animals. Holtz et al ascribed this depressor effect of smaller quantities of dopamine or of urine collected after dopa administration, not to dopamine itself but to a metabolite of dopamine formed after amine oxidation. Unfortunately, they did not consider in their comments the small initial depressor response to dopamine which preceded the rise in blood pressure in the cats and which their metabolite hypothesis failed to explain. It took another 16 years before Hornykiewicz was able to refute Holtz's assumption in a study in guinea-pigs, in which a monoamine-oxidase inhibitor prolonged rather than abolished the depressor response to dopamine [16]. Holtz and coworkers found dopamine to be present in the urine not only after administration of dopa but also before, thereby for the first time demonstrating its presence in normal urine. They also noted the frequently occurring pressor response to intravenous injection of this control urine, but did not comment on depressor responses observed in other instances. Dopamine was considered by them to possess pressor effects only and to function as a precursor of epinephrine. But despite their incorrect interpretation of the results, Holtz's article deserves to be considered as a landmark study in yet another respect. They also found that most of the dopamine in the urine was present there in a conjugated form. Up to 40 % of the dopa they administered could be recovered as dopamine in the urine, thereby illustrating the quantitative importance of dopamine formation and excretion in the kidney. We will return to some of these aspects of urine dopamine excretion in a later section.

After the depressor effect of dopamine had been confirmed in the study of 1958 of Hornykiewicz in guinea-pigs, Burn et al reported a fall in blood pressure in cats when low doses of dopamine were infused [17]. In early studies in man dopamine was reported to cause an increase in blood pressure, even when infused in doses considered at that time as relatively low, ranging from 5.3 to 11.6 µg/kg/min [18]. A hypotensive effect of dopamine in man was first reported in 1966 by McNay in patients with severe hypertension who were also treated with phenoxybenzamine: a dose of 1 to 1.5 µg/kg/min resulted in a marked fall (37 mmHg) in mean arterial pressure [19].

Extensive pharmacological studies during the sixties showed that the vasodepressor effect of dopamine could not be blocked by conventional blocking agents like alpha- or beta-blockers, antihistamines and atropine [20 21]. This led to the theory that the hypotensive effect of dopamine resulted from stimulation of a separate receptor for dopamine [1]. As mentioned earlier, the study of McDonald on the renal effects of dopamine in man offered decisive support to this theory: the renal haemodynamic effects of

dopamine were different from those reported for all other catecholamines. Subsequent studies in different species using specific antagonists or non-specific vasodilating agents eventually confirmed the existence of a separate, previously unknown dopamine receptor [22-25]. For subsequent research on dopamine and existing or newly developed dopamine agonists and antagonists, it was of major importance that the kidney offered a suitable model to study their effects on a relatively selective basis. Such studies revealed that dopamine, besides stimulating its own receptors, also directly or indirectly influenced other receptors. The various effects of its influence on these receptors served to explain the divergent action of dopamine on blood pressure described above. The action of dopamine on these various receptors will now be described.

DOPAMINE AND ITS RECEPTORS

In this section we will limit ourselves to a discussion of receptor studies of dopamine outside the central nervous system. Dosage considerations are included here. As discussed above, for many of the studies the original animal models using blood pressure effects were abandoned in favour of models using the vascular response in specific organs. The pioneer studies of Goldberg and his co-workers used the renal vasculature of dogs in vivo, as most other groups did later [26 27]. Other vascular beds, like the cerebral, hepatic or ileal vessels have been less extensively studied, but results were comparable to those obtained in the kidney [22 28-31]. In all these vascular beds dopamine administration results in a vasodilatory response which underlies the systemic depressor response to dopamine mentioned earlier [22]. The renal vascular model, as used by the group of Goldberg and in their wake by several others, was first described in 1965 by McNay et al and has been used in essentially the same way up till now [23]. It consists of a pentobarbitone-anaesthetized dog, usually pretreated with the aselective alpha-blocker phenoxybenzamine and a beta-blocker, in whom drugs are injected directly into the renal artery while renal blood flow is measured with electromagnetic flow probes and systemic blood pressure is recorded from a carotid or femoral artery. In the original study of McNay, dose-dependent renal vasodilation was the response to dopamine in doses ranging from 0.75 to 6 μg/kg/min. For doses above 12 μg/kg/min a progressively stronger and longer-lasting vasoconstrictory response was observed. This vasoconstrictory response could be reversed to vasodilation by pretreatment with phenoxybenzamine [32]. Other studies showed that the renal vasodilatory response was not inhibited by beta-adrenoceptor blocking agents or antagonists of other effector systems like atropine or antihistamines, or by preventing metabolism of dopamine. Administration of dopamine metabolites resulted in no or qualitatively different renal vascular responses [33]. The same renal vascular responses were found in other models of renal vasodilation, including those in humans [7 34 35].

For the early characterized dopamine antagonists like haloperidol and other butyrophenones, and phenothiazines specificity was limited to a rather narrow dose range [24]. Used in higher doses they also inhibited the effects of e.g. isoprenaline or

bradykinin. Later more selective and potent antagonists of dopamine-induced vasodilation were discovered, e.g. sulpiride and SCH-23990 [36 37].

The availability of such increasingly potent and selective antagonists allowed a distinction to be made between two types of dopamine receptors in peripheral tissues. Goldberg proposed a classification in DA_1 and DA_2 dopamine receptors [38] (Table 1).

Table 1: **Peripheral dopamine receptors: renal effects.**

	DA_1	DA_2
Prototype location and functional model for structure-activity studies of agonists and antagonists	blood vessels (renal arteries, afferent and efferent arterioles): smooth muscle relaxation results in vasodilation	postganglionic sympathetic nerves (renal nerves): presynaptic inhibition of norepinephrine release
Other locations and effects:	renal tubules: diuresis,natriuresis	unknown whether DA_2 receptors exert direct influence on renal tubules or juxtaglomerular cells
	juxtaglomerular cells: renin release(?)	
		adrenal cortex: inhibition of aldosterone release
Dopamine dosage necessary for receptor stimulation	millimolar	micromolar
Agonists:	SKF82526(fenoldopam) SKF38393 others: epinine, DPDA, A-6,7-DTN	LY171555(quinpirole) LY141865, N-0437 others: apomorphine, bromocriptine
Antagonists:	*selective DA_1* ◄———	———► *selective DA_2*
	SCH23990 SKF83566	R-sulpiride haloperidol cis-flupenthixol domperidone

DA_1 dopamine receptors

The DA_1 dopamine receptor subserves smooth muscle relaxation. It seems to be located postsynaptically in various organs [39]. Renal vasodilation in a model as described above has served to study structure-activity relations of agonists and antagonists. Millimolar doses of dopamine are required to stimulate DA_1 receptors [40]. The R-enantiomer of sulpiride was used in initial studies as a selective antagonist of this receptor, but was later replaced by SCH23990 as a prototype antagonist. Most DA_1 antagonists also inhibit DA_2 receptors with the exception of SCH23990. SKF83566, the 7-bromo analogue of SCH23990, also selectively inhibits DA_1 dopamine receptors [41]. For antagonists like sulpiride or flupenthixol, the relative antagonist activities on DA_1 and DA_2 receptors depend on the enantiomer used. For example R-sulpiride has considerable DA_1 antagonist activity, whereas S-sulpiride is more potent as a DA_2 antagonist [42].

As for the DA_1 agonists, initially only epinine (N-methyl-dopamine) was found to act as a full agonist, and apomorphine as a partial agonist, in the renal vascular bed and it took another 10 years before the group of the N-N-di-substituted catecholamines, of which DPDA (N-N-di-propyl-dopamine) became the first representative, was found to possess dopamine agonist activity [43]. They were complemented by A-6,7-DTN and later followed by some benzazepine derivatives like SKF38393 and fenoldopam (SKF-82526-J) [44] [45]. The latter two drugs have now become the most important representatives of selective DA_1 agonists, used not only for pharmacological studies but in the case of SKF82526-J also in clinical studies [46]. Considering the intrarenal distribution of DA_1 receptors, they have been demonstrated in renal arteries of dogs and humans, in afferent and effent arterioles, in renal tubules and in mesangial cells [47-51]. At the cellular level Felder recently described autoradiographic techniques which enabled him to study the quantitative distribution of DA_1 receptors along the renal tubule [52]. An even distribution of DA_1 dopamine receptors along the entire proximal tubule was found. On a more functional level the same author demonstrated that phospholipase-C activity in renal cortical membranes was selectively stimulated by DA_1 dopamine agonists, but not by DA_2 dopamine agonists [53]. Phospholipase-C is involved in sodium transport mechanisms in the proximal tubule by alterations in cytosolic calcium. This may form a link between the tubular localisation of DA_1 receptors and the presumed direct tubular natriuretic effects of dopamine. We will return to this subject later.

DA_2 dopamine receptors

Stimulation of DA_2 dopamine receptors results in the inhibition of norepinephrine release from postganglionic sympathetic nerves in response to nerve stimulation [54] [55]. Several methods have been employed to characterize this receptor, and its agonists and antagonists: in most studies the effects of agonists and antagonists on the physiological response to electrical stimulation of postganglionic sympathetic nerves were used [56]. In

such models a DA_2 agonist will decrease, and a DA_2 antagonist enhance the physiological effects of nerve stimulation. Bogaert et al used the femoral vascular bed with apomorphine as a standard agonist, while Goldberg et al utilized dipropyl-dopamine (DPDA) as an agonist and (S)-sulpiride as a reference antagonist in the same preparation [57] [58]. Lokhandwala et al obtained their results in isolated perfused rat kidneys [59]. In all these vascular beds, DA_2 agonists inhibited the vasoconstrictor response to sympathetic nerve stimulation. In initial studies, presynaptic dopamine receptors had been detected by an in vitro technique of Langer et al, who recorded a reduction in the release of radioactive norepinephrine from electrically stimulated postganglionic sympathetic nerves when dopamine agonists were added to the perfusion fluid [55]. Micromolar quantities of dopamine are sufficient to stimulate DA_2 receptors. Remarkably, the conformational requirements for DA_2 receptor activation are much less strict than those for DA_1 agonists and they are active at a dose range far below that of DA_1 agonists [60]. Apomorphine is equal or even greater in activity as a DA_2 agonist than dopamine itself, but also seems to possess partial agonist activity at the DA_1 receptor. Other DA_2 agonists include bromocriptine, many of the ergot derivatives, piribedil and dimethyl A-5,6-ADTN. More selective agonists like LY141865, LY17155 (quinpirole, the S-enantiomer of LY141865) and N-0437 have been developed in recent years [61-63]. Most DA_2 antagonists lack DA_1 antagonist activity as illustrated by domperidone which does not inhibit dopamine-induced renal vasodilation in phenoxybenzamine-pretreated dogs [64]. Within the kidney, Jose et al showed DA_2 receptors to be present in renal arteries of dogs [65]. These DA_2 receptors are not linked to renal nerves. Receptor stimulation results in vasodilation. Felder found evidence for DA_2 receptors in rat glomeruli [66].

Comparison of the DA_1/DA_2 and the D_1/D_2 dopamine receptor models

It should be stressed here that still unexplained discrepancies exist for several dopamine antagonists between the classification of peripheral dopamine receptors and the dopamine receptor scheme proposed by Kebabian and Calne for dopamine receptors in the central nervous system. In the central nervous system evidence for the existence of several dopamine receptors has been collected. These receptors have been characterized by biochemical techniques like receptor binding assays or stimulation of dopamine sensitive adenylate cyclase, while the effects of stimulation or inhibition of these receptors usually are studied by behavioural approach [67] [68]. A division is made in D_1 dopamine receptors, which stimulate adenylate cyclase, and D_2 dopamine receptors, which do not. Some authors favour a subdivision of the D_2 receptors based on affinity characteristics of various agonists and antagonists for these receptors. Studies of dopamine receptors in the kidney using comparable biochemical or receptor binding techniques are scarce: the binding characteristics of both dopamine and haloperidol differ markedly from those observed in the central nervous system [52] [69-71]. Whereas all DA_1 agonists share stimulation of adenylate cyclase with the D_1 agonists and a DA_2 agonist like apomorphine behaves as a D_2 agonist, this close resemblance is not found

for the antagonists. Sulpiride, especially the R(-)-enantiomer, is listed as a D_2 antagonist in the Kebabian-classification, but is one of the most potent antagonists of DA_1 receptor dependent renal vasodilation [72].

Dopamine and adrenoceptors

It is remarkable that the vascular dopamine receptor has strict conformational requirements while on the other hand dopamine itself as the only naturally occurring agonist for this receptor has an extremely flexible structure allowing it to stimulate other structurally unrelated receptors as well. A short examination of the other receptors stimulated by dopamine is useful, as these receptors are important for some of the renal effects of dopamine.

Alpha-adrenoceptors

Dopamine directly stimulates alpha-adrenoceptors, resulting in vasoconstriction in the kidney. Alpha-1 receptors seem to contribute more to this vasoconstrictory action of dopamine than alpha-2 receptors. Contrary to what is generally believed, the alpha-adrenoceptor action of dopamine is not limited to the higher doses.

Although dopamine is a precursor of norepinephrine, and the alpha-adrenoceptor stimulation during infusion of dopamine might seem to result from metabolism of dopamine to norepinephrine, a direct action of dopamine on alpha-adrenoceptors had been shown already in the studies of Raymond-Hamet and Gurd using cocaine or reserpine pretreatment which was confirmed later [13 73 74]. Stimulation of both alpha-1- and alpha-2-adrenoceptors leads to vasoconstriction. Duval showed that in anaesthetized dogs, the vasoconstrictory action of dopamine in the kidney is preferentially blocked by the alpha-1-blocker prazosin and hardly inhibited by the alpha-2-blocker idazoxan [75]. As mentioned earlier, reversal of a vasoconstrictory response to dopamine in doses above 12 µg/kg/min into a vasodilatory response after phenoxybenzamine pretreatment showed the role of the alpha-adrenoceptor in the renal effects of dopamine. It should be pointed out that alpha-adrenoceptor stimulation also occurs at much lower doses of dopamine. In the study of McNay in hypertensive patients, the depressor effect of dopamine only became evident after phenoxybenzamine pretreatment [19]. Probably a balance between vasodilation due to dopamine receptor stimulation, and a vasoconstrictory response to alpha-adrenoceptor stimulation, is reached during infusion of dopamine in doses below the just mentioned 12 µg/kg/min.

Beta-adrenoceptors

The effects of dopamine on the beta-adrenoceptor were initially studied by McNay in the femoral vascular bed, and further elucidated by studies of Black and McDonald measuring myocardial stimulation [76 77]. Myocardial stimulation by dopamine is due to a stimulation of beta-1-adrenergic receptors. Dopamine is a very weak agonist of, or has no effect at all, on beta-2-adrenoceptors [78]. Dopamine has also been shown to directly stimulate beta-adrenoceptors within the kidney [79]. Steinhausen et al studied the

influence of beta-blockers on dopamine-induced renal vasodilation [80]. They found in in vivo studies of the effects of dopamine on renal microvessels, that propranolol enhanced the dilator response of most preglomerular vessels and efferent arterioles to intermediate concentrations of dopamine. At higher concentrations of dopamine the tendency of preglomerular vessels to constrict was abolished, and often reversed to a dilatory response, by propranolol and also alpha-blockers; however, only propranolol abolished the constriction of efferent arterioles induced by high concentrations of dopamine.

Some authors have also suggested that dopamine acts on serotonine receptors [81].

RENAL EFFECTS OF DOPAMINE

In this part we will first describe and discuss the effects of exogenous dopamine. Data on endogenous dopamine will be summarized in the next section. For historical reasons we will first discuss the renal effects of exogenous administration of dopamine and dopamine agonists. A note of warning is perhaps justified. Several problems are encountered when studying the renal effects of exogenous dopamine in man. In the first place one should consider the fact that the kidney itself is one of the main producers of dopamine [82]. Infusions of dopamine, even in doses as low as 0.03 µg/kg/min, result in plasma levels of free dopamine far above base-line levels [83]. Plasma levels may increase more than a thousandfold during infusions of higher doses of dopamine. Dopamine doses in the range used for clinical purposes also result in urine dopamine levels exceeding base-line excretion rates. Thus, caution is warranted in drawing conclusions on the physiological renal effects of endogenous dopamine from the results of studies using pharmacological doses of dopamine. We will, therefore, consider separately studies assessing the renal effects of exogenous and endogenous dopamine. In vivo studies also have to take into account the variety of receptors stimulated by dopamine on the one hand, and the influence of the extrarenal effects of dopamine on the other hand. The latter include the effects on systemic haemodynamics resulting from myocardial stimulation and from the effects on other vascular beds, the effects mediated via the nervous system, and hormonal effects. The use of selective agonists and antagonists for the various receptors, and the development of several in-vitro models, have facilitated the separation of the renal effects of dopamine. Recently, studies employing selective agonists and antagonists in isolated renal vessels and tubules have shed more light on the contribution of various dopamine receptors and the various parts of the nephron to the renal actions of dopamine.

EXOGENOUS DOPAMINE

Systemic haemodynamic effects of exogenous dopamine

A short extension on the systemic haemodynamic effects of various doses of dopamine is justified because of their contribution to the renal effects. Especially the dose ranges separating the depressor and the pressor response, respectively, are important. Setler described depressor responses of dopamine in doses of 1 and 3 μg/kg given as a bolus injection in anaesthetized dogs, while she found a pressor response in the dose range of 9 to 81 μg/kg [84]. Robie, using doses from 1.25 to 20 μg/kg/min in anaesthetized dogs, found an increase in mean arterial pressure at doses above 5 μg/kg/min [85]. For all doses except the 20 μg/kg/min dose, a decrease in systemic vascular resistance was found. Hsieh found a rise in mean arterial pressure in the 5-20 μg/kg/min dose range in dogs; addition of phentolamine, however, resulted in a fall in mean arterial pressure below the control values [86]. As for other species, Chapman demonstrated an increase in mean arterial pressure in rats at doses of 3 μg/kg/min and higher, which was reversed to a depressor response on addition of phenoxybenzamine [87]. Horn found depressor responses of dopamine for a dose range of intravenous bolus injections of 1.8-116 μg/kg in guinea pigs [88].

In man Breckenridge, and later Hollenberg, found no effects of dopamine on blood pressure up to doses of 3 μg/kg/min [89 90] (Table 2). Using long-term infusions of dopamine in doses of 0.5 to 1.25 μg/kg/min, Orme also found no effects on blood pressure or heart rate [91]. In the study of McNay in hypertensive patients and mentioned before, dopamine resulted in a marked fall in mean arterial pressure at doses of 1 to 1.5 μg/kg/min when they had been pretreated with phenoxybenzamine. Horwitz observed an increase in blood pressure, mainly systolic, for doses varying from 5.3 to 11.6 μg/kg/min [18]. McDonald showed that changes in pulse pressure were evident for doses of 2.6 to 7.1 μg/kg/min, while mean arterial pressure remained unchanged due to opposite effects of dopamine on systolic (increased) and diastolic (decreased) pressure [7]. Systemic vascular resistance falls in the dose range of 2.5 to 12 μg/kg/min and returns to, or rises above, base-line values at doses of 20 μg/kg/min and higher [18 86 92].

Renal effects of exogenous dopamine

As has been described in the historical paragraph, the unique vascular properties of dopamine were not recognized for decades because dopamine was given in too large doses to recognize its hypotensive potency. Up to the sixties no adequate dose-response studies of exogenously administered dopamine had been performed. However, as soon as the renal vascular effects of dopamine had been described and the renal vasculature was used to characterize the vascular dopamine receptor, animal studies of the dose-response effects of infused dopamine profligated.

Table 2: **Dose dependency of renal effects of dopamine.**

Dose of dopamine (μg/kg/min)	Effect	Author
Systemic:		
1-1.5	fall in blood pressure during alpha-blockade	McNay
1-2	no effect	Breckenridge
3	no effect	Hollenberg
2.6-7.1	increase in pulse pressure, MAP unchanged	McDonald
5.3-11.6	rise in blood pressure, mainly systolic	Horwitz
2.5-12	fall in systemic vascular resistance (SVR)	Horwitz, Maskin
above 20	rise in SVR	Maskin
Renal:		
RBF		
1-2	77 % increase at 1, and 122 % increase at 2 μg/kg/min	Breckenridge
2	maximal increase, patients with congestive heart failure	Maskin
3	maximal increase	Hollenberg
2.6-7.1	57 % increase in PAH-clearance	McDonald
175-700 μg/min	48 % increase in PAH-clearance	Ramdohr
GFR		
2.6-7.1	16 % increase in inulin clearance	McDonald
Sodium excretion		
175-700 μg/min	more than 100 % increase at 175, slight further increase up to 700 μg/min	Ramdohr
2.6-7.1	236 % increase	McDonald
5-10	45 % increase for 5 and 146 % for 10 μg/kg/min, patients with congestive heart failure	Beregovich
3	35 % increase for 3, not for 0.03 or 0.3 μg/kg/min	Levinson
2-5	164 % increase at 2, no further increase at 5 μg/kg/min, patients with chronic renal failure	Baglin
Renin release		
1.3-3	suppression of PRA	Barnardo
3-30	increase in renin secretion at 3 and 30 μg/kg/min, not at lower doses	Ball

Renal haemodynamic effects of exogenous dopamine

Infusion of dopamine results in a dose-dependent increase in renal blood flow and a less marked rise of the glomerular filtration rate.

Intravenous infusion of dopamine in anaesthetized animals leads to an increase in renal blood flow in a dose range of 1 to 10 μg/kg/min, the upper limit depending on the animal species used. A maximum in renal blood flow was reached at 5 μg/kg/min in the study in dogs of Hsieh, and remained constant at higher doses when phenoxybenzamine was added. Robie in experiments with dogs observed a maximal response at 10 μg/kg/min [85 86]. However, the same group found that in dogs pretreated with an alpha-blocker like phenoxybenzamine or phentolamine, renal blood flow continues to rise up to doses of 40 μg given as a bolus injection into the renal artery [72]. In rats an increase in renal blood flow occurred up to doses of 12 μg/kg dopamine infused into the renal artery. Glomerular filtration rate starts to rise at doses of 0.5 μg/kg/min in dogs [93]. For doses up to 7 μg/kg/min, an increase in inulin clearance above control values has been shown [7]. Meyer found comparable dose ranges for anaesthetized and conscious dogs [94]. Meyer also compared the intravenous route of dopamine administration with an infusion into the renal artery and observed similar responses of renal blood flow for an intravenous dose of 6 μg/kg/min and an intra-arterial dose of 0.6 μg/kg/min.

Compared to the numerous dose-response studies in animals, data on the dose-response effects of dopamine on renal haemodynamics in man are relatively scarce (Table 2). McDonald found a mean increase in PAH-clearance from 507 to 798 ml/min using dopamine doses ranging from 2.6 to 7.1 μg/kg/min [7]. Inulin clearance rose from 109 to 126 ml/min. Ramdohr observed an increase in PAH-clearance from 782 ml/min to 1161 and 1121 ml/min for doses of 175 and 350 μg/min, respectively [95]. Inulin clearance, however, did not change significantly. Breckenridge gave doses of 1 and 2 μg/kg/min dopamine to hypertensive patients with unilateral renal disease, which resulted in an increase of renal blood flow, measured by an indicator dilution technique in the unaffected kidney, of 77.1 and 121.6 %, respectively [89].

Intrarenal distribution of blood flow

Studies on the intrarenal distribution of blood flow during administration of dopamine indicate an increase in cortical blood flow. Hardaker et al studied the effects of dopamine (in a mean dose of 4.6 μg/kg/min intravenously) on the distribution of intrarenal blood flow in anaesthetized dogs [96]. Using microsphere techniques, a redistribution of blood flow to the inner third of the cortical area was observed, with an overall increase in renal blood flow of 26 %. Neiberger, using ^{85}Kr-washout techniques in dogs, found a redistribution to the subcortical outer medullary area, where the flow increased 50 % using a dopamine dose of 6 μg/kg/min [97]. In the rat Chapman observed increases in both cortical and medullary blood flow using doses of 20 and 65 nmol/kg/min dopamine [87]. Hollenberg performed the only reported study in man on redistribution of renal blood flow during dopamine infusion. Employing a dose of 3 μg/kg/min dopamine, an increase in cortical renal blood flow occurred [90].

In vitro renal vascular effects of dopamine

Hughes found relaxant activity of the DA_1 agonist fenoldopam in preconstricted human renal artery segments, which was competitively blocked by sulpiride and SCH23990 [50]. Edwards investigated the effects of topical dopamine and dopamine agonists on isolated renal microvessels from rabbits, observed by light microscopy [49]. Dopamine caused a dose-dependent relaxation both of norepinephrine-induced and spontaneous tone in afferent and efferent arterioles of glomeruli, while relaxant activity was much less outspoken in interlobular arteries. In a later study, using fenoldopam and SKF87516 as selective DA_1 dopamine receptor agonists, he found that this relaxation was apparently due to stimulation of DA_1 receptors [98]. His results are partly in contrast to those of Steinhausen et al. They used a split hydronephrotic rat kidney model, enabling them to directly visualize renal vessels in vivo [80]. They observed the effects of locally applied dopamine on these vessels. Dopamine produced a dose-dependent dilation of arcuate and interlobular arteries, and of afferent glomerular arterioles. In efferent arterioles the magnitude of the response to the lower doses of dopamine was less marked than in the preglomerular vessels (an increase in diameter of 9 ± 2 % for the efferent arteriole versus 27 ± 10 % for arcuate arteries). However, for technical reasons, they could not observe the effects of dopamine on the very narrow region of the efferent arteriole near to the glomerulus. Probably, precisely this segment represents a high-resistance postglomerular region with a significant influence on glomerular haemodynamics, and might be more responsive to dopamine. It seems therefore from these in vitro studies that DA_1 receptor stimulation is essential for the renal vasodilatory action of dopamine.

Natriuretic effects of exogenous dopamine

In the initial description of the renal effects of dopamine by McDonald et al, the increase in natriuresis and diuresis was also stressed (Table 2). Natriuresis rose from 171 to 575 µmol/min in the dose range of 2.6-7.1 µg/kg/min [7]. Levinson saw no effect of dopamine in doses of 0.03 and 0.3 µg/kg/min on diuresis, natriuresis and kaliuresis; for a 3 µg/kg/min dose only natriuresis increased [83]. Orme later used long-term infusions of dopamine in doses ranging from 0.5 to 1.25 µg/kg/min in hypertensive patients with renal function impairment and found an increase of diuresis and natriuresis on the first day. However, on the second day diuresis and natriuresis had returned to control values [91]. These results agree with those of Baglin in patients with chronic renal failure using a dopamine dose of 2 µg/kg/min [99]. In patients with congestive heart failure Beregovich found significant increases in diuresis and natriuresis for dopamine doses of 5 or 10 µg/kg/min, but not for a 1 µg/kg/min dose [100]. In vitro studies of the natriuretic effects of dopamine agonists are discussed in a later paragraph. Data concerning the effects of dopamine infusion on the excretion of other electrolytes or on other aspects of tubular function are scant. In the study of Baglin just mentioned, an increase of potassium and calcium excretion was found for a 2 µg/kg/min dopamine dose.

Repeated or prolonged administration of dopamine

No tachyphylaxis for dopamine and DA_1 or DA_2 agonist activity has been observed after repeated administration, at least in acute studies. However, prolonged infusions of dopamine result in a loss of effect. Orme evaluated the effects of dopamine infusion continued for 36 to 105 hours in hypertensive patients with renal function impairment [91]. He used a dose which was just below that initially found to cause an increase in blood pressure and which varied between 0.5 and 1.25 µg/kg/min. After an increase in GFR and PAH-clearance during the first hours of dopamine infusion, both returned to base-line values after 48 hours. The increase in diuresis and natriuresis was also transient. Tachyphylaxis for the systemic haemodynamic effects of dopamine has also been observed during long-term infusions in patients with congestive heart failure. Steady state levels of plasma dopamine are reached within 15 minutes after starting a dopamine infusion [83 101].

ENDOGENOUS DOPAMINE

Origin of dopamine in plasma and urine

Dopamine is formed from L-dopa by dopa-decarboxylase. Dopamine normally circulates in plasma. However, in man 98-99 % of plasma dopamine is conjugated, i.e. considerably more than norepinephrine or epinephrine [102 103]. Conjugated dopamine is biologically inactive. Species differences exist in the degree and form of conjugation; in man sulphoconjugates predominate [104]. Free dopamine levels are very low and were up to a few years ago often considered undetectable. Most authors now use HPLC with electrochemical detection; gas chromatography-mass fragmentography and radio-enzymatic methods are also used. Hjemdahl reviewed methods for plasma catechol-amine assays a few years ago and reported values for free dopamine of 0.1-0.2 nmol/l (= 15-30 pg/ml) [105]. Plasma dopamine has been reported to rise in reaction to exercise and acute surgical stress [106]. Plasma dopamine falls after sodium restriction [107]. In studies of Cuche and Wang, free dopamine levels, assayed with a radio-enzymatic method, amounted in normal volunteers to 36 ± 8 and 50 ±10 pg/ml, respectively, while plasma sulphoconjugated dopamine levels varied around 5568 and 2490 pg/ml [104 108]. Radioenzymatic assays tend to show higher values and considerable variability, possibly due to a higher degree of deconjugation with enzymatic methods [105]. Why dopamine is sulphoconjugated to such an extent is unknown. Kuchel assumed that a sulphoconjugation barrier in the intestine may protect the body against the catecholamines present in food [109]. However, Unger, studying efflux of conjugated catecholamines from different organs in the dog, found that conjugated dopamine was released together with free catecholamines from the adrenals after surgical stress. He proposed that under these circumstances dopamine sulphate, released from the adrenals, might serve as a transport form of dopamine or as a direct precursor of

norepinephrine [110]. Deconjugation enzymes, shown to be present in the kidney, liberate dopamine which would subsequently be excreted in the urine. In some discrepancy to his hypothesis is the fact that adrenalectomized rats still are capable of markedly increasing levels of dopamine in the urine upon salt loading. Besides the adrenals the sympathetic nervous system and the kidneys have been proposed as sources of plasma dopamine. As for the latter, Ball proposed the kidney as a source of plasma dopamine on the basis of clearance studies. He determined plasma and urine free dopamine simultaneously in normals and calculated a urinary clearance for dopamine of 1996 ml/min (range 402-3844 ml/min) [111]. His conclusion, however, was disputed by Kopp who found negative venoarterial dopamine concentration differences in dogs [112].

Concerning the urinary excretion of dopamine the hypothesis of tubular production of dopamine from dopa is supported by a large body of evidence. As mentioned above, the kidney is rich in dopa-decarboxylase. This enzyme appears to be preferentially located in renal tubules. Administration of carbidopa, a peripheral dopa-decarboxylase inhibitor, results in a fall in urine dopamine [113 114]. When a large quantity of L-dopa is given to human volunteers, this leads to a marked increase in plasma L-dopa and in urine dopamine while plasma dopamine rises much less [115]. Administration to normal volunteers of γ-L-glutamyl-L-dopa, which is converted by γ-glutamyltransferase and subsequently by dopa-decarboxylase into dopamine resulted in a 500-fold increase in urine dopamine and a natriuresis without any change in plasma dopamine, while γ-L-glutamyl-L-tyrosine failed to increase urine dopamine or sodium excretion [116]. Baines and Chan showed that tritiated L-dopa, injected in proximal tubules of both innervated and denervated rat kidneys, is converted to urine dopamine, while no conversion to dopamine occurred with injected L-tyrosine [117]. These two studies seem to implicate a minor role of dopaminergic nerves, which like other peripheral autonomic nerves contain tyrosine hydroxylase and therefore should be able to convert both forms of tyrosine, in the formation of urine dopamine. In dogs given L-dopa, Ball demonstrated, measuring arteriovenous differences across the kidney, a fall in L-dopa concentrations accompanied by a rise in plasma and urine dopamine [82]. Suzuki also concluded that urinary free dopamine is mainly derived from plasma dopa in studies using isolated perfused rat kidneys [118]. Finally, Hagege and Richet observed dopamine histofluorescence in proximal tubules of rat kidneys incubated with L-dopa, which could be abolished by pretreatment with a dopa-decarboxylase inhibitor [119].

Apart from this tubular conversion of L-dopa to dopamine, renal dopaminergic nerves have also been proposed to contribute to urine dopamine excretion. Bell first demonstrated dopamine-containing neuronal elements in dog kidneys and he and his coworkers later provided persuasive arguments for the presence of specific dopaminergic nerves within the kidney [26 120-122]. Dinerstein and co-workers showed that dopaminergic neurons were mainly located at the glomerular vascular pole in contrast to norepinephrine-containing neurons which were preferentially present in the periadventitial layer of arcuate arteries [123]. In the interlobular arteries a mixture of dopamine- and norepinephrine-containing fibers was found. It is of interest that

dopaminergic fibres ended in close proximity to the juxtaglomerular apparatus, suggesting their involvement in the control of renin release. Kopp evaluated the effects of renal nerve stimulation on venous and urinary outflow of dopamine, and other catecholamines, in anaesthetized dogs [111]. While in a denervated and unstimulated kidney a negative venoarterial concentration difference was found for all catecholamines and low-level renal nerve stimulation only increased venous norepinephrine outflow, high-level renal nerve stimulation increased renal venous outflow of dopamine and norepinephrine. Urinary excretion of norepinephrine but not of dopamine increased both during low-level and high-level renal nerve stimulation. Phenoxybenzamine pretreatment counteracted the haemodynamic response to high-level renal nerve stimulation and stimulated venous and urinary outflow of dopamine and norepinephrine. In another study by Bradley et al on renal venous outflow of free and conjugated dopamine and norepinephrine during renal nerve stimulation, it was found that renal nerve stimulation reduced catecholamine extraction by the kidney [124]. They concluded from the high dopamine/norepinephrine ratio in kidney tissue, and from renal venous outflow of dopamine during renal nerve stimulation, that specific dopaminergic nerves probably exist in the dog kidney.

Functional significance of endogenous dopamine

Studies on the functional significance of endogenous renal dopamine production and secretion, even if limited to the kidney, are hampered by the lack of direct measures of dopamine secretion. Plasma dopamine has no relation to the supposed physiological renal effects of dopamine. Plasma dopamine levels correlate inversely with sodium excretion whereas urine dopamine excretion is directly correlated with natriuresis [109 110 125 126]. Levinson, performing graded infusions of dopamine in normal man only observed a natriuretic effect of dopamine at supraphysiological concentrations both of plasma and urine dopamine [83]. Plasma levels of dopamine exceeding 200 times base-line levels and, therefore, far exceeding the physiological range, were needed to stimulate renin release during dopamine infusions in dogs [127].

Urine dopamine mainly reflects tubular secretion of dopamine and may not reflect at all the activity of dopamine on the renal vasculature and hormonal secretion, or the excitation of dopaminergic nerves. Indirect techniques, using dopamine antagonists for example, have been used to circumvent this problem. An exception to such indirect techniques have been the studies on the relation between natriuresis and dopamine excretion in the urine. Most of this work stems from the group of Lee, Ball and Oates. They explored the influence of several manoeuvers on urine dopamine excretion. Administration of oral or intravenous sodium chloride, but also of oral potassium- or ammonium chloride, resulted in an increase in urine dopamine while oral sodium bicarbonate has no such effect [111]. It seems therefore that the chloride ion is important in this dopamine response. While sodium chloride infusion results in an increase in urine dopamine, expansion of the effective circulating volume with albumin infusions does not [128]. An increase in dietary sodium from 10 mmol to 200 mmol is accompanied

by an approximately 50 % increase in urine dopamine [126]. Ingestion of a protein-meal leads to a comparable increase in urine dopamine excretion [129]. Administration of fludrocortisone to normals leads to an initial fall in urine dopamine but parallel with the mineralocorticoid escape, urine dopamine rises again [130]. Frusemide and progesterone both increase urine dopamine while oestrogens reduce urine dopamine excretion [131 132]. Pregnancy is also associated with an increase in urine dopamine [133].

Several authors have chosen the indirect pathway of using dopamine antagonists to study the role of endogenous dopamine (Table 3). These studies seem to indicate that only during volume expansion renal blood flow and GFR depend to some extent on endogenous dopamine: in man, in oncological patients who had been prehydrated before receiving chemotherapy, metoclopramide given in high doses decreased renal plasma flow [134]. Pelayo et al found in hydropenic anaesthetized rats that cis-flupenthixol inhibited the increase in GFR (but not SNGFR) and $FENa^+$% during Ringer loading [135]. Manoogian failed to detect an effect of metoclopramide given in a low dose of 10 mg on renal blood flow in normal man, while the increase in renal blood flow during dopamine 1 µg/kg/min was blocked [136]. In a study of Hahn and Wardell metoclopramide did not affect base-line renal blood flow or renal vascular resistance in anaesthetized dogs while the effects of exogenously administered dopamine were blocked [137]. Goldberg found no influence of bulbocapnine or sulpiride on base-line renal blood flow in dogs while both dopamine antagonists again specifically inhibited dopamine-induced increases in renal blood flow [72]. In a study of Schmidt, employing isolated perfused kidneys of rats pretreated with phenoxy-benzamine and sotalol, flupenthixol- or sulpiride-isomers had no influence on base-line renal vascular resistance while dopamine-induced renal vasodilation was inhibited [138]. Chapman showed that intravenous infusions of sulpiride reduced cortical renal blood flow in rats without influencing overall renal blood flow [87]. Frederickson found no influence of SCH23990 on base-line renal blood flow in anaesthetized dogs pretreated with phenoxybenzamine and propranolol [139].

Inhibition of dopamine generation gives results comparable to those of dopamine antagonists. McClanahan gave carbidopa to conscious dogs during acute volume expansion with saline while measuring renal haemodynamics, natriuresis and hormonal status [140]. Carbidopa reduced both the increase in sodium and dopamine excretion but did not influence ERPF or GFR.

Studies using dopamine antagonists may implicate a more important role of endogenous renal dopamine in maintaining natriuresis and diuresis. Krishna found a marked inhibition of natriuresis by metoclopramide after volume expansion in normal volunteers in sodium balance [141]. Bradley performed a comparable study, infusing 60 ml/kg 0.9 % NaCl in 2 hours in conscious dogs, but failed to observe any influence of the DA_1 antagonist SCH23990 or the DA_2 antagonist domperidone on subsequent natriuresis [142]. However, no sodium balance had been attained before the studies were performed. Although Schnermann found no influence of haloperidol, SCH23990 or sulpiride on GFR in saline- or water-diuretic rats, these antagonists resulted in a clear decrease in diuresis and sodium excretion [143]. Imondi also showed that sulpiride 120 mg intraperitoneally inhibited saline-induced diuresis in rats [144]. Brown and

Table 3: **Effects of dopamine antagonists on renal function.**

Species, pretreatment	Antagonist, dose	Hydration	RBF	GFR	sodium excretion	Author
1) Human	metoclopramide, 2.5 mg/kg	75-200 ml/h	-13 %	n.m.	n.m.	Israel, 1986
2) Human	metoclopramide, 10 mg i.v.	no	no change	n.m.	n.m.	Manoogian, 1986
3) Human	metoclopramide, 10 mg/h	2 l saline in 4 hours	n.m.	n.m.	abolition of increase in UNa.V	Krishna, 1985
4) Human	domperidone, 40 mg i.v.	water immersion	nm	no change	inhibited increase in UNa.V	Coruzzi, 1986
5) Human	domperidone, 40 mg i.v.	no	n.m.	n.m.	no change	Bennett, 1982
		lower-body positive pressure	n.m.	n.m.	inhibited increase in UNa.V, but not diuresis	idem
6) Dog anaesth	metoclopramide, 1 and 10 mg i.v.	no	no change	n.m.	n.m.	Hahn, 1980
7) Dog anaesth, POB	bulbocapnine, up to 9.2 x 10^{-7} M in renal artery	not mentioned	no change	n.m.	n.m.	Goldberg, 1979
	sulpiride, up to 5.8 x 10^{-6} M in renal artery	not mentioned	no change	n.m.	n.m.	
8) Dog anaesth, POB, propranolol	SCH23990, 0.5 µg/kg/min	no	no change	no change	no change	Frederickson, 1985
9) Dog conscious	carbidopa, each 8 h 1 mg/kg	30 ml/kg/h saline	no change	no change	inhibited	McClanahan, 1985
10) Dog conscious	SCH23990, 0.5 µg/kg/min	60 ml/kg saline for 2 h	n.m.	n.m.	no change	Bradley, 1986
	domperidone, 1 and 10 µg/kg/min	idem	n.m.	n.m.	no change	
11) Rat anaesth	flupenthixol, 5-10 µg/kg/min	hydropenic	n.m	no change	no change	Pelayo, 1983
		Ringer-loading	n.m.	inhibited increase	inhibited increase	
12) Rat anaesth	sulpiride, 0.7 x 10^{-6} M/kg/min	no	no change	n.m.	n.m.	Chapman, 1980

continued:

Species, pretreatment	Antagonist, dose	Hydration	RBF	GFR	sodium excretion	Author
13) Rat	haloperidol, 100 µg	22.5 ml/h saline	n.m.	no change	25 % fall	Schnermann, 1986
	SCH23990, 100 µg			no change	30 % fall	
	sulpiride			no change	no change inhibited	
14) Rat	sulpiride, 120 mg i.p.	saline	n.m.	n.m.	increase in diuresis	Imondi, 1979
	haloperidol				no change	
15) Rat	sulpiride, 0.5 mg/kg/day i.p.	saline+ DOCA 25 mg	n.m.	n.m.	65 % fall	Brown, 1979
	domperidone, 1 mg/kg/day i.p.				60 % fall	
16) Isolated perfused rat kidneys, POB, sotalol	flupenthixol (cis-isomer), 10^{-9}-10^{-7} M	no	no change in RVR		n.m.	Schmidt, 1983
	sulpiride-isomers, up to 10^{-4} M		no change in RVR		n.m.	
17) Isolated perfused rat kidneys, POB, propranolol	haloperidol, 10^{-4} M in perfusate	no	increase in RVR 4 %	fall 20 %	no change	Baines, 1986
	SCH23990, 10^{-5} M in perfusate		increase in RVR 4%	fall 20 %	fall ±35 %	
	(+)-butaclamol, 10^{-4} M in perfusate		increase in RVR 2 %	fall 15 %	no change fall 57 %	
	carbidopa, 5 mg/kg intra-peritoneally		n.m.	fall 7 %		

n.m. = not measured. POB = phenoxybenzamine. RVR = renal vascular resistance. anaesth = anesthized. i.p. = intra peritoneally. For references see text.

Dollery observed that domperidone as well as sulpiride inhibited the increase in sodium excretion in DOCA-saline treated rats, while the excretion of dopamine in the urine was unaffected [145]. Coruzzi found that domperidone blunted the natriuresis following water immersion in eight normal subjects, while no changes were seen in the creatinine clearance and the water immersion-induced changes in diuresis, or in the suppression of the renin-aldosterone system [146]. Domperidone was also found to abolish the natriuretic, but not the diuretic response to lower-body positive pressure [147]. These results with domperidone also illustrate that DA_2 dopamine antagonists have a clear antinatriuretic effect and that the effects of dopamine antagonists on natriuresis do not necessarily depend on changes in renal haemodynamics, a subject to which we will return later.

Baines and Drangova surmise from observations in isolated perfused rat kidneys after acute or chronic denervation, that dopamine released from quiescent renal nerves participates in the regulation of glomerular filtration, while dopamine from tubules may increase sodium excretion [148]. Chronic denervation of the kidney, which removes neural dopamine production, and carbidopa, which totally inhibits dopamine production, lead to a similar fall in GFR and natriuresis. Acute renal denervation on the other hand is followed by a natriuresis which can be attenuated by the DA_1 antagonist SCH23990. Acute renal denervation is associated with a short-lived release of dopamine from the severed nerves. Chapman presented evidence that renal nerve stimulation during alpha-blockade results in renal vasodilation, which can be blocked by sulpiride [87]. In the earlier mentioned study of Kopp using high-level renal nerve stimulation, comparable results emerged with an increase in renal venous outflow of dopamine and norepinephrine, and a reduction of renal blood flow, which was reversed by phenoxybenzamine pretreatment [112].

DOPAMINE AND THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

Most studies concerning the relation between dopamine and renin secretion used infusions of dopamine or administration of dopamine agonists and, therefore, hardly address physiological circumstances. It is questionable whether the physiological plasma dopamine levels bear any relation to renin release. It may well be possible that endogenous dopamine released from dopaminergic nerves terminating in juxtaglomerular cells or generated in tubules and reaching the juxtaglomerular apparatus in higher than systemic concentrations influence renin secretion. Theoretically, the expected actions of dopamine on renin secretion are rather complex. In reviewing studies which investigated the influence of dopamine agonists or antagonists on renin secretion, the question arises whether they were specifically stimulating or blocking, respectively, DA_1 or DA_2 dopamine receptors, or whether their action was due to stimulation of other types of receptors or to indirect effects. Dopamine has beta-adrenergic activity which is known to increase renin activity by a direct action on the cells of the juxtaglomerular apparatus. Alpha-adrenergic activity of dopamine, resulting

in vasoconstriction, would also tend to increase renin activity which might again be modulated because dopamine-dependent stimulation of alpha-2-adrenoceptors inhibits sympathetic activity. Finally, in vivo the situation is further complicated by the effects of dopamine on blood pressure, cardiac output and regional blood flow distribution, all of which indirectly influence renin activity.

Bell and Lang reported that the increase in renin secretion caused by hypotensive haemorrhage and acute suprarenal aortic constriction, was inhibited by ergometrine, considered by them as an aspecific dopamine vascular antagonist [149]. In contrast the increase in plasma renin activity (PRA) produced by frusemide diuresis is not attenuated by ergometrine. Ball, investigating the effects of dopamine infusions at various rates on the renin-angiotensin-aldosterone system in dogs, found no influence of dopamine on renin release in the physiological dose range [127]. Plasma levels exceeding 200 times base-line were needed to stimulate renin release. Other studies using dopamine infusions had rather conflicting results. Wilcox infusing dopamine in doses of 200 to 600 µg/min, found an elevation of PRA in normals, whereas norepinephrine in a dose causing a similar pressor response resulted in a fall in PRA [150]. On the other hand Barnardo demonstrated suppression of PRA during infusion of lower doses of dopamine (1.3 to 3 µg/kg/ min) [151]. In animal studies Imbs reported that intrarenal infusions of 6 µg/kg/min dopamine induced an increase in renin secretion, which could be blocked by haloperidol, but not by phentolamine or propranolol [152]. In earlier studies higher doses of dopamine had been used, resulting in a marked alpha- and beta-adrenoceptor stimulation. Both alpha- and beta-adrenergic receptor stimulation result in a rise of PRA [153] [154]. The latter studies can, therefore, not be considered to represent the influence of dopamine receptor stimulation on renin release. In vitro studies of Quesada and Henry showed an increase in renin release upon addition of dopamine which could be blocked by propranolol but not by haloperidol, thus implicating beta-adrenoceptor stimulation of renin release [155]. Lopez recently reported that dopamine increased in vitro renin release and tissue renin content [156]. As for dopamine agonists, again variable results were observed: Carey found no changes in PRA in six normal subjects in sodium balance after addition of the DA_2 agonist bromocriptine 2.5 mg [157]. However, Edwards found higher PRA levels in normal individuals using 7.5 mg of bromocriptine [158]. In dogs, Imbs reported an increase in renin secretion during apomorphine, which could be abolished by haloperidol [159]. In conclusion, the results of the studies on the relation between dopamine and renin are rather equivocal. Perhaps use of selective agonists and antagonists will clarify this relation.

The effects of dopamine on aldosterone secretion fall outside the direct context to this review; however, some remarks deserve to be made because of the importance of aldosterone secretion for sodium balance. Aldosterone seems to be under tonic dopaminergic suppression [160] [161]. Dopamine antagonists result in a marked increase in aldosterone levels without a rise in PRA [162] [163]. Several authors imply the DA_2 dopamine receptor to be responsible for the effects of dopamine on aldosterone secretion [164-166]. The interrelationships of dopamine with other hormonal systems present in the kidney or influencing kidney function are discussed elsewhere.

DOPAMINE AND NATRIURESIS

The relation between the renal haemodynamic and the natriuretic effects of dopamine has long been the subject of considerable debate (Table 4). Schmidt suggested that the natriuretic effect of dopamine is secondary to its vasodilatory effects [167]. She measured

Table 4: **Mechanisms of dopamine-induced natriuresis.**

Effect	Receptors involved	Mechanism leading to natriuresis
Systemic haemodynamic:		
Positive inotropy	beta	increase in ECV
Systemic vasodilation	DA_1, DA_2, beta	increase in ECV
Renal:		
Renal vasodilation	DA_1, DA_2	fall in RVR favouring fall in tubular sodium reabsorption
Direct tubular	DA_1	direct inhibition of NaK-ATP-ase
Other:		
Inhibition of aldosterone release	DA_2	inhibition of NaK-ATP-ase in distal collecting tubule
Inhibition of sympathetic transmission, both ganglionic and postganglionic	DA_2	vasodilation and fall in renin release

ECV = Effective Circulating Volume RVR = Renal Vascular Resistance

renal haemodynamics and natriuresis in hydrated or dehydrated anaesthetized dogs during dopamine infusions. During dehydration a parallel increase in renal blood flow and GFR was found, without an increase in natriuresis. In the hydrated dog on the contrary, dopamine increased renal blood flow while GFR remained constant, reflected by a fall in filtration fraction. An increase in sodium excretion was found, probably due to diminished proximal sodium reabsorption. Other groups performing in vivo studies also found no natriuresis in the absence of a dopamine-induced fall in filtration fraction [168 169].

On the other hand McGiff described a natriuretic effect of dopamine even when GFR and renal blood flow had been reduced to below control levels by acute aortic constriction [170]. In a more direct way substantial evidence has now been collected to support the theory that dopamine may exert a direct tubular natriuretic effect, independent of renal vasodilation. Pelayo reported that in anaesthetized rats cis-

flupenthixol attenuated the natriuresis of saline loading without changes in renal perfusion pressure or GFR [135]. His results were confirmed in a study of Jose in denervated kidneys of saline-loaded rats, in which cis-flupenthixol as well as SCH23990 impaired the natriuresis of acute renal denervation without influencing GFR [171]. In the isolated perfused rat kidney Bell found a dopamine-induced natriuresis independent of changes in perfusion flow rate or GFR [149]. Baines also concluded from studies in isolated perfused kidneys of chronically denervated rats that dopamine, produced in renal tubules, did not influence GFR but directly stimulated sodium excretion by a mechanism which was SCH23990 and, therefore, DA_1 receptor dependent [148]. Finally, in vitro studies of Aperia showed direct tubular effects of dopamine on tubular Na/K-ATP-ase and sodium reabsorption [172] [173]. As mentioned before, Felder demonstrated that in renal cortical tubular membranes phospholipase-C activity is selectively stimulated by DA_1 agonists. Phospholipase-C is involved in proximal tubular sodium transport by altering cytosolic calcium concentrations [174]. A third mechanism by which dopamine may exert natriuretic effects is its inhibitory action on aldosterone release. Antinatriuretic effects of dopamine antagonists have been linked to their potent stimulation of aldosterone release. Dopamine seems to have a tonic inhibitory influence on aldosterone secretion under physiological circumstances. It is, therefore, difficult to imagine how administration of pharmacological doses of dopamine, associated with only a slight fall in plasma aldosterone concentration, can exert an important natriuretic effect. In a recent study by Jose et al in saline-loaded adrenalectomized rats evidence was provided that the antinatriuretic action of the dopamine antagonist cis-flupenthixol does not depend on the presence of the adrenals [175]. Finally, the earlier described redistribution of renal blood flow to cortical areas during dopamine administration may mirror a preferential perfusion of cortical nephrons. Redistribution of blood flow to these nephrons is accompanied by a fall in renal vascular resistance. Cortical nephrons with relatively short loops of Henle are presumed to have an impaired ability for sodium reabsorption [176].

As the renal vasodilatory action of dopamine seems to depend mainly on DA_1 receptor stimulation and the inhibition of aldosterone secretion by dopamine has been ascribed to DA_2 receptor stimulation, use of selective agonists and antagonists for DA_1 and DA_2 dopamine receptors may again offer new possibilities to delineate the contribution of the renal vasodilatory, the direct tubular and the aldosterone-release inhibiting actions to the dopamine-induced natriuresis. Jose infused fenoldopam into renal arteries of dogs, resulting in a dose-dependent increase of renal blood flow and absolute and fractional sodium excretion. Reduction of RBF to control levels by renal artery constriction no more than partly reversed the increase in natriuresis, thereby implicating that the natriuretic effect of selective DA_1 dopamine receptor stimulation cannot be accounted for completely by haemodynamic mechanisms [177]. In another study of this group the effects of selective dopamine antagonists on GFR and sodium excretion were assessed in dogs during the natriuresis following acute unilateral renal denervation. Aselective dopamine receptor blockade with cis-flupenthixol resulted in a more marked reduction of sodium excretion than selective DA_1 dopamine receptor blockade with SCH23990 [178].

The interrelations between the effects of dopamine on renal haemodynamics and natriuresis also depend on the influence of dopamine on renin secretion, which has been discussed above.

INTERACTION OF DOPAMINE WITH OTHER RENAL HORMONAL SYSTEMS

The involvement of dopamine in the secretion of other hormones on the one hand, and its role as a mediator or modulator of the effects of a hormone on the other, is too broad a subject to be adequately covered in this review. For example the secretion of most pituitary hormones is affected by dopamine while sex hormones on their turn influence urine free dopamine excretion. We will restrict ourselves here to a discussion of the interaction of dopamine with hormones that have a major influence on renal function.

Vasopressin

Lightman and Forsling claimed that vasopressin release from the pituitary is inhibited by dopamine in man, but Rowe et al could not confirm this [179] [180]. Ball and co-workers found an increase in plasma vasopressin; however, the doses of dopamine were so high that emesis, also a powerful stimulus of vasopressin, occurred [127]. Metoclopramide stimulates vasopressin secretion while domperidone, which does not cross the blood-brain barrier, had no such effect [181]. Muto showed that dopamine inhibited the hydro-osmotic effect of vasopressin in cortical collecting tubules of rabbit kidneys while dopamine alone failed to affect hydro-osmosis at this level [182].

Prostaglandins

Prostaglandins do not seem to influence urine dopamine production. Indomethacin fails to influence urine free dopamine excretion, and the stimulation of free dopamine excretion by frusemide is not inhibited by indomethacin [183] [184]. Results on the influence of dopamine on urinary prostaglandin production or on the role of prostaglandins as mediators of the effects of dopamine, are conflicting. Nadjer found that indomethacin and ibuprofen prevented the increase in renal blood flow induced by intravenous infusion of 1 μg/kg/min dopamine in normal man [185]. He did not measure urine dopamine release. His results have been confirmed by Yeyati et al who found a reversal of the effects of dopamine 6 μg/kg/min intravenously on renal vasodilation and natriuresis in healthy volunteers by indomethacin 2 mg/kg [186]. In the study of Nadjer, dopamine caused an augmentation of the urinary excretion of 6-keto-$PG_{F1\alpha}$, the stable metabolite of prostacycline which could be prevented by metoclopramide. Vikse failed to observe significant changes in PG_{E_2} excretion in dogs during infusion of 1 μg/kg/min dopamine [187]. In studies of Barnett using isolated rat glomeruli and cultured rat mesangial cells, indomethacin did not influence the ability of dopamine to attenuate the angiotensin II-associated reductions in glomerular and mesangial planar surface

area [188]. Directly determining mesangial cell PG_{E2} production, dopamine was also shown not to change either basal or angiotensin II-stimulated synthesis of PG_{E2}.

Atrial natriuretic factor

Recently, much attention has been given to the role of dopamine in the natriuretic actions of atrial natriuretic factor (ANP). Katoh found that the diuresis and natriuresis during ANP-infusion into the rat renal artery was markedly attenuated by haloperidol and also by carbidopa [189]. SCH23990 and R-sulpiride also inhibit the diuresis and natriuresis induced by intravenous infusions of ANP [190]. Wilkins confirmed Katoh's results with carbidopa in a study in normal man using infusions of high doses of ANP [191]. Petterson et al were also able to antagonize the diuretic response to ANP with haloperidol; however, another antagonist, selective for the DA_2 dopamine receptor, domperidone, fails to inhibit the natriuretic and diuretic effects of ANP [192 193]. Dopamine infusions did not influence ANP levels in studies of Shenker and Tulassay [194 195]. On the other hand evidence for a stimulatory effect of dopamine on ANP release has been found by Hollingsworth-Dajani et al, while metoclopramide blunts the ANP response to saline-induced volume expansion [196]. In conclusion, it seems that dopamine may have a role as a mediator of the natriuretic effect of ANP, whereas its effect on ANP-release is still a matter of debate.

ROLE OF RENAL DOPAMINE IN SOME HYPERTENSIVE AND OEDEMATOUS DISORDERS

It has become clear that dopamine is a renal vasodilatory and natriuretic substance which is generated intrarenally under physiological circumstances. An absolute or relative failure in the intrarenal formation of this catecholamine might contribute to the genesis or maintenance of hypertensive and oedematous disorders.

Essential hypertension

Disturbances in both central and peripheral dopaminergic mechanisms have been implicated in the pathogenesis of hypertension in animal models and in humans [5 197 198]. Plasma levels have been found to be lower in both established and borderline hypertension, while free and total dopamine and HVA (a metabolite of dopamine) excretion in the urine is decreased in essential hypertension [199-201]. On the other hand Kuchel et al reported higher levels of plasma conjugated dopamine and also observed surges of plasma dopamine in patients with essential hypertension [202 203]. Harvey et al identified patients with essential hypertension in whom urinary free dopamine paradoxically fell after addition of dietary salt [204]. Especially patients with essential hypertension whose blood pressure rises after an increase in salt intake (salt-sensitive) have decreased dopaminergic activity [205]. Sowers suggested that dopaminergic activity is decreased in essential hypertension, leading to an altered control of

norepinephrine secretion [206]. On the other hand patients with essential hypertension show an exaggerated natriuretic response to intravenous dopamine (given in a dose of 3 μg/kg/min) or to the dopamine pro-drug γ-glutamyl-dopa [207-209]. Dopamine agonists lower blood pressure in patients with essential hypertension, but not or hardly in normotensive control subjects [210 211]. For fenoldopam this hypotensive response in essential hypertension was accompanied by a marked increase in renal blood flow (from 379 to 640 ml/min, more than in healthy controls), while GFR showed minor or no changes [212 213].

Chronic renal failure

In patients with chronic glomerulonephritis oral sodium loading does not result in an increase in urinary dopamine output [214]. Casson concluded from these data that abnormal retention of sodium and water in glomerulonephritis may partly be due to a failure to mobilise dopamine in the kidney. With the dopamine agonist ibopamine chronic administration led to a significant increase in creatinine clearance (23 % after 3 months, 31 % after 6 months) in a group of 21 patients with impaired renal function (mean creatinine clearance 29 ml/min) [215]. Effects on natriuresis were not measured.

Results from other studies on the other hand support the assumption that an increase in endogenous renal dopamine generation may exist in chronic renal failure. Metoclopramide injections caused a fall in sodium excretion in normokalaemic patients with stable chronic renal failure (GFR 31 ± 7 ml/min), but not in patients with the same degree of renal function impairment and hyperkalaemia, or in normal individuals [216]. The same authors also found an exaggerated rise in plasma aldosterone concentration after metoclopramide compared to the normal controls, perhaps implying that hyperaldosteronism was responsible for the antinatriuresis rather than inhibition of direct natriuretic effects of dopamine by metoclopramide. We recently reported an impaired response of ERPF and FF to infusion of dopamine 2 μg/kg/min both in a group of patients with IgA-nephropathy and in a larger group of 131 patients with various renal diseases [217 218]. The increase in ERPF and GFR during dopamine infusion was related to base-line GFR. Below a GFR of 50 ml/min/1.73 m^2 dopamine failed to affect GFR and ERPF. An increase in endogenous renal dopamine generation might explain both the impaired effect of exogenous dopamine on renal haemodynamics in our studies and the fall in natriuresis during metoclopramide in the just-mentioned normokalaemic patients with chronic renal failure. Fenoldopam in a dose of 50-200 mg/day causes a fall in sodium excretion, GFR and renal blood flow in acute experiments in hydrated patients with moderate renal function impairment [219]. Tulassay observed a small increase of 14 % in ERPF and no rise in GFR during infusion of 2 μg/kg/min dopamine in pediatric patients with advanced renal failure (mean GFR 17,8 ml/min/1.73 m^2) [220]. These results also argue in favour of either an increase in endogenous dopamine generation or an impaired responsiveness to dopamine. Further studies are needed to clarify the importance of renal dopamine in patients with chronic renal failure.

Congestive heart failure

An increase in sodium intake from 10 to 100 mmol/day resulted in suppression of sympathetic nervous system activity with minimal changes in systemic haemodynamics in patients with congestive heart failure [221]. Two subgroups could be identified in the response to the 100 mmol diet: the first group rapidly achieved sodium balance, the other avidly retained sodium and water. Although initial renin-angiotensin system activity was higher in the second group, both showed suppression of PRA and urinary aldosterone secretion during the increase in dietary sodium. The patients with high PRA had high plasma dopamine levels [222 223]. In contrast to normals and to the group of patients with congestive heart failure and low PRA, a fall in plasma dopamine levels occurred during sodium repletion. Unfortunately, urine dopamine levels were not measured. Interestingly, the degree of renal blood flow reduction in patients with congestive heart failure is known to be correlated with PRA levels [224 225]. Dopamine infusion in patients with congestive heart failure results in striking increases in ERPF and GFR, and in a pronounced natriuresis. Ramdohr observed a mean increase of 70 % in PAH-clearance, and of 36 % in inulin-clearance using a 175 µg/min dopamine dose, while the same dose of dopamine given to normals resulted in a 48% rise in PAH-clearance and no significant change in inulin clearance [226]. Rosenblum had comparable results with increases of ERPF, GFR and sodium excretion of 79, 38 and 486 %, respectively, during infusion of a dopamine dose of 2.6 µg/kg/min in patients with congestive heart failure [227]. Maskin even observed an increase in renal blood flow of 99 % at 2 µg/kg/ min dopamine in congestive heart failure, while enalapril, inducing a comparable increase in cardiac index, improved renal blood flow by 35 % [228]. Hilberman compared dopamine with dobutamine in doses of 5 and 3.5 µg/kg/min, respectively, causing identical increases in cardiac index in patients with a modest impairment of left ventricular function and renal function (ERPF 375 ml/min) [229]. Comparable responses of ERPF (604 ml/min after dopamine) and GFR occurred for dopamine and dobutamine; however, the natriuretic and diuretic response to dopamine was more prominent. Thus, the renal haemodynamic and the natriuretic response to dopamine infusions seem to be markedly enhanced in patients with congestive heart failure. It is not clear whether this is due solely to differences in systemic haemodynamic effects of dopamine between normals and patients with congestive heart failure or also to altered responsiveness of the kidney to exogenous dopamine. It is also conceivable that defective renal dopamine generation is a contributory factor in the sodium retention and in the exaggerated response to exogenous dopamine in the patients with congestive heart failure.

As for dopamine precursors and dopamine agonists, oral levodopa is known to increase renal plasma flow in patients with severe congestive heart failure [230]. Ibopamine induced a sustained increase in natriuresis and diuresis in congestive heart failure, associated with a moderate increase in creatinine clearance [231 232]. Dopexamine, a compound with marked beta-2-adrenergic and DA_1-dopaminergic activity, resulted in a pronounced increase in creatinine clearance and a moderate diuresis and natriuresis in

part of the patients with congestive heart failure [233]. The effects of other dopamine agonists and of dopamine antagonists on renal haemodynamics or natriuresis have not been evaluated in these patients.

Cirrhosis

Both dopamine and one of its pro-drugs, ibopamine, increased renal blood flow, GFR and natriuresis in cirrhotics [234-236]. Domperidone did not have an effect on spironolactone-induced natriuresis in such patients. However, in cirrhotics with ascites metoclopramide results in a fall in sodium excretion and also inhibits the natriuretic effects of spironolactone [237 238]. Both a stimulatory effect of metoclopramide on aldosterone secretion, as described earlier, and an effect on renal haemodynamics have been implicated as factors contributing to its antinatriuretic action. In conclusion, while the renal response in cirrhosis does not seem to be qualitatively different from normals, insufficient data are available to draw conclusions on possible quantitative differences.

THERAPEUTICAL IMPLICATIONS FOR THE FUTURE

While progressively selective antagonists of the two types of peripheral dopamine receptors have been available for some years, their usefulness for clinical purposes is limited as far as renal effects are concerned. Selective dopamine agonists offer better therapeutical perspectives in this regard. However, the development of selective dopamine agonists has lagged some years behind: the structural requirements for such agonists appeared to be quite strict. Another important prerequisite for dopamine agonists to make them clinically useful is that they are orally effective. SKF38393 was found to have selective but partial agonist activity at DA_1 dopamine receptors without significant alpha- or beta-adrenergic activity [239]. It has been used for scientific purposes for some years; however, important effects on the central nervous system prohibited clinical use [240]. SKF82526-J, also called fenoldopam, another benzazepine derivative and first reported in 1980, better fulfilled the characteristics for a clinically useful selective DA_1 agonist. It does not have significant alpha-, beta-adrenergic or DA_2 agonist activity and does not cross the blood-brain barrier [241]. Moreover it is orally active. Extensive clinical studies have now been performed. Projected indications for clinical use include congestive heart failure, chronic renal insufficiency and hypertensive emergencies. The rationale for its use in congestive heart failure is based on its potential to reduce afterload via systemic arteriolar vasodilation and to induce increases in renal blood flow and natriuresis [242]. The very pronounced increases in renal blood flow, glomerular filtration rate and natriuresis observed in patients with congestive heart failure during administration of dopamine or dopamine agonists, more pronounced than during for example converting enzyme inhibition, make a drug like fenoldopam especially attractive. So far no results have been published on the renal haemodynamic and natriuretic effects of fenoldopam in patients with congestive heart

failure. The propitious systemic haemodynamic effects have been confirmed in acute studies [242]. The renal haemodynamic properties of fenoldopam with a marked rise in renal blood flow accompanied by a fall in filtration fraction, probably reflect a predominantly postglomerular vasodilation and therefore, a fall in intraglomerular pressure. As intraglomerular hypertension has been linked to the development of glomerular sclerosis and progressive renal function loss in patients with renal disease, fenoldopam may be attractive in such patients to retard renal function loss. However, the disappointing effects of dopamine infusions in acute studies in patients with chronic renal failure justify caution in these expectations [217 218]. This is strenghthened by the preliminary results of a study of Stom et al in patients with mild chronic renal failure: fenoldopam paradoxically resulted in a fall in renal blood flow, GFR and natriuresis [219]. Initial studies have fulfilled the expectation that the systemic vasodilatory actions of fenoldopam make the drug effective in the treatment of hypertension [212 243]. Preservation or even augmentation of renal blood flow forms an attractive property of fenoldopam among drugs which may be of use in achieving controlled hypotension [244].

A second drug which has become available for clinical studies is SB-7505 or ibopamine, the di-isobutyryl ester of epinine. It is orally active but lacks the selectivity of fenoldopam. Most effects after oral administration are not due to ibopamine itself but to the metabolite epinine to which it is de-esterified. Epinine is one of the already longer known drugs active at dopamine receptors but also at adrenergic receptors. Renal vasodilation due to DA_1 dopamine receptor stimulation was observed in experimental studies in dogs after intravenous ibopamine and epinine [245]. Results of clinical studies on its effect on renal function have been conflicting: in a study of Harvey ibopamine in doses of 150 and 600 mg did not affect creatinine excretion or natriuresis in normal volunteers, while Incerti et al observed an increase of both GFR and sodium excretion with a dose of 50 mg in an acute study [246 247]. In a study of Melloni, who gave 100 mg/day, an increase in creatinine clearance, diuresis and natriuresis was observed after 6 days [248]. Improvement of systemic haemodynamics but also of renal function was observed in patients with congestive heart failure in acute studies [232 249]. In patients with moderate renal insufficiency, a sustained improvement of creatinine clearance up to 6 months after starting ibopamine was established [250]. Best results were obtained in patients with nonglomerular diseases.

Clinical data on another dopamine agonist, the ergoline derivative cianolergine are scant [251]. It is orally active and has no significant central nervous system side effects, but a supposed specificity for DA_1 receptors has not been validated. It failed to influence renal function (assessed by creatinine clearance and natriuresis only), PRA and plasma aldosterone concentration in patients with essential hypertension.

Dopexamine is a drug which preferentially stimulates both DA_1 and beta2-adrenergic receptors [252-254]. Beta-2-adrenergic stimulation, resulting in systemic vasodilation, may offer additional advantages in patients with congestive heart failure. It is undergoing clinical trials in patients with congestive heart failure. Initial reports show a

marked improvement in renal function in patients with low-output congestive heart failure [255].

Recently developed selective DA_2 agonists, like N-0437, LY141865 and LY171555, also called quinpirole, have sofar not been shown to influence renal haemodynamics in experimental studies. The effects on natriuresis and the renin-angiotensin-aldosterone system have not been reported yet [256]. A vasodilatory response may be expected with these drugs due to their inhibitory effect as DA_2 agonists on sympathetic nervous system activity by inhibition of norepinephrine release at postganglionic sympathetic nerve terminals, by inhibitory influences on sympathetic nerve transmission at sympathetic ganglia, and by their effects on the central nervous system. Indeed, bromocriptine, a less selective DA_2 agonist, resulted in a fall in blood pressure and systemic vascular resistance in an acute study in patients with congestive heart failure [257]. Quinpirole is being evaluated clinically for its potential usefulness as an antihypertensive agent [258].

Preliminary reports have appeared on experimental studies with a selective dopamine-beta-hydroxylase (DBH)-inhibitor, SKF102698 [259]. This long-acting drug leads to a marked rise in endogenous dopamine levels, concomitant with a fall in norepinephrine, resulting in a 10-fold increase in the dopamine/NE-ratio. A fall in blood pressure in spontaneously hypertensive rats was accompanied by a marked increase in diuresis. Initial clinical studies confirm the antihypertensive effects, while orthostatic hypotension, a major problem in a recently reported patient with congenital dopamine-beta-hydroxylase deficiency, was not observed (Berkowitz B A, unpublished observations) [260]. Results on renal function have sofar not been reported.

In conclusion, the attractive characteristics of some of the dopamine analogues mentioned above and the propitious preliminary clinical results warrant furher studies.

SUMMARY

The history of dopamine research has more than an anecdotal role in this review: it illustrates the importance of dosage considerations for dopamine and apparent paradoxes in the effects of this substance due to its stimulation of multiple receptors with partly opposite effects. The lack of adequate "dose-finding studies" resulted in a delay of the discovery of the unusual properties of dopamine as a catecholamine for decades. Dopamine was long considered as merely one of several epinephrine analogues with weak pressor potency. After its depressor activity in low doses had been found it took another 15 years before the unique renal effects of dopamine were discovered and led to the recognition that dopamine not only stimulates adrenergic receptors but also has its own receptor. Further research eventually revealed the presence of two peripheral dopamine receptors.

The effects of dopamine on the two dopamine receptors, called DA_1 and DA_2 dopamine receptors and on other receptors are subsequently described in general terms. Stimulation of DA_1 dopamine receptors results in smooth muscle relaxation; renal

vasodilation is often used as a model to study structure-activity relations for DA_1 agonists, of which fenoldopam is now the prototype representative, and for DA_1 antagonists like SCH23990. DA_2 dopamine receptor stimulation results in inhibition of norepinephrine release from postganglionic sympathetic nerve terminals. Selective DA_2 agonists like quinpirole and DA_2 antagonists like domperidone are available. As mentioned before dopamine also exhibits alpha-adrenergic and some beta-1-adrenergic activity.

The renal effects of exogenous and endogenous dopamine are examined separately. Plasma and urine dopamine levels during administration of dopamine in usual doses far exceed physiological levels. It is therefore possible that the effects of exogenous dopamine are not representative of those of endogenous dopamine. Exogenous dopamine results in an increase of renal blood flow and glomerular filtration rate accompanied by a redistribution of flow to cortical areas. In vitro these effects of dopamine are reflected by a relaxation of several intrarenal blood vessels. Dopamine infusion also results in natriuresis.

Studies on the role of endogenous dopamine required assays for plasma and urine free dopamine levels, as almost all dopamine circulates in a conjugated and biologically inactive from. When these assays eventually became available, it was apparent that plasma levels show a poor correlation with the supposed renal effects of endogenous dopamine like natriuresis. Urine dopamine excretion, studied during several physiological manoeuvers, does correlate directly with sodium excretion. Studies using inhibition of dopamine generation or dopamine antagonists also implicate a role for endogenous dopamine in maintaining natriuresis and diuresis, while renal blood flow and GFR only depend on endogenous dopamine during volume expansion. Aldosterone seems to be suppressed by dopamine in physiological circumstances, the influence of endogenous dopamine on renin secretion is not established beyond doubt.

Dopamine-induced natriuresis may be explained by several mechanisms: besides a direct effect of dopamine on tubular NaKATPase, for which in vitro evidence has recently been found, its renal haemodynamic effects, causing renal vasodilation, and its suppression of aldosterone secretion have been held responsible for an increase in sodium excretion. As different dopamine receptors seem to be implicated in these effects, studies using selective agonists and antagonists may further elucidate the contribution of these respective mechanisms.

Several studies indicate a role for dopamine as a mediator of the natriuretic effects of atrial natriuretic factor (ANP). Studies on the relation of dopamine to renal prostaglandins and to vasopressin are inconclusive.

Some oedematous and hypertensive disorders may be associated with a failure in the intrarenal formation of dopamine. Although no firm conclusions are possible, data relevant for this hypothesis are discussed for essential hypertension, chronic renal failure, congestive heart failure and cirrhosis. In essential hypertension disturbances in both central and peripheral dopaminergic mechanisms have been observed. Essential hypertension is associated with decreased plasma and urine free dopamine levels; in part of the patients with essential hypertension urine free dopamine paradoxically falls

after addition of dietary salt. In patients with chronic renal failure oral sodium loading fails to increase urine dopamine excretion, while an impaired response of ERPF and GFR to a fixed dose of exogenous dopamine is found. Patients with congestive heart failure exhibit an exaggerated renal haemodynamic and natriuretic response to dopamine infusion.

Finally the therapeutic perspectives of some of the recently developed dopamine agonists in cardiovascular and renal medicine are discussed. Substances like the selective DA_1 dopamine agonist fenoldopam, ibopamine, dopexamine and the selective DA_2 agonist quinpirole are now being evaluated in clinical trials in congestive heart failure, chronic renal failure or as antihypertensives.

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CHAPTER 2

PURPOSE OF THE STUDY

The purpose of this thesis is to describe the effects of exogenous and endogenous dopamine on renal haemodynamic parameters like ERPF, GFR and FF and on natriuresis, and the modification of these effects by pretreatment with blockers of various receptors known to be stimulated by dopamine. Studies have been performed both in normal man and in patients with renal disease. From these studies we attempt to draw conclusions on the physiological role of endogenous renal dopamine:
- is it different from that of exogenously administered dopamine?
- to what extent are various receptor types involved in the studied renal effects of exogenous and endogenous dopamine?
- does the response to dopamine in patients with renal disease differ from that in normal man and is this related to changes in endogenous dopamine formation or sensitivity?

Chapter 3 provides a description of dose-response effects of intravenous infusions of dopamine on renal haemodynamics and electrolyte excretion in normal man. In earlier studies an impaired renal haemodynamic response to a fixed dose of exogenous dopamine had been found in patients with renal disease. In this study the dopamine dose-response effects in a group of patients with renal disease and moderately impaired renal function are compared with those in the healthy volunteers.

In chapters 4 and 5 metoclopramide and sulpiride, respectively, are examined in normal volunteers for their potential as antagonists of dopamine-induced renal effects: dopamine dose-response studies, as described in chapter 3, are compared in the absence and presence of metoclopramide and sulpiride. Confirmation of dopamine antagonist activity of metoclopramide both for renal haemodynamic and natriuretic effects allows some conclusions to be made on the role of endogenous dopamine in the control of renal perfusion and tubular function in normal man and in a group of patients with renal disease and moderately impaired renal function (chapter 4). A discrepancy between the effects of sulpiride on renal haemodynamics and natriuresis in normal man leads to a discussion of possible alpha-antagonist activity of sulpiride.

The latter aspect is addressed in more detail in chapter 6 which describes the influence of alpha-blockade, both with the selective alpha-1-blocker prazosin and the aselective alpha-blocker phentolamine, on dopamine dose-response curves for renal haemodynamics and natriuresis in the absence and presence of sulpiride.

Chapter 7 contains data on plasma and urine dopamine levels and plasma (nor)adrenaline levels in normal man. The reproducibility and the possible influence of our study conditions are tested. The changes during infusions of dopamine and before and during alpha-blockade are studied.

STUDY POPULATION, PROTOCOLS AND METHODS

Study population

Studies were performed in healthy volunteers and in patients with renal disease. All volunteers and patients gave informed verbal consent.

All normal volunteers declared themselves to be healthy and none had suffered from renal disease in the past or was known to have hypertension. None of them used medication other than oral contraceptives. Age ranged between 20 and 58 years, further data on age and sex are given in the following chapters separately for the respective studies. All volunteers were found to have a serum creatinine level below 100 µmol/l.

The patients with renal disease were selected from the patient population visiting the nephrology outpatient clinic of the Department of Medicine of the University Hospital Groningen. Criteria used to select patients were as follows: GFR or calculated creatinine clearance stable and known to be within the 35-90 ml/min range; blood pressure well controlled (with medication), i.e. diastolic blood pressure < 95 mmHg and systolic blood pressure between 110 and 160 mmHg limits; temporary withdrawal of all medication considered possible. The (histological) diagnosis of the renal disease was no exclusion criterium, except when nephrosclerosis was considered to be due to essential hypertension. In all but two patients a histological diagnosis was available. The age range, sex distribution and specific histological diagnoses are given in the relevant chapters. The overall age range was 20-62 years. In all patients medication was withdrawn at least four days before the renal function studies, in the case of beta-blockers at least one week before. If diastolic blood pressure rose above 105 mmHg after withdrawal of medication, patients were excluded from further study.

General remarks on the study protocols

To avoid the inadvertent influence of a high protein load on renal haemodynamics or urine dopamine excretion both healthy volunteers and patients were asked to abstain from animal protein containing foods on the day before and during the renal function studies. They were also asked to adhere to a mildly sodium-restricted diet at least four days before the studies. Some patients instituted on a more rigid sodium restriction, continued their usual diet during the studies. As a control, 24 hour urine sodium excretion was checked before the studies. Urine sodium excretion was not used as an exclusion criterium in any of the studies. 24 hour sodium excretion data are given in the relevant chapters.

Renal function studies were performed with an interval of at least two days to avoid carry-over effects. All studies were performed on an outpatient basis. All participants were asked to drink at least 250 ml/hour of non-sodium containing beverages throughout the study periods. Coffee and tea and a light lunch were permitted but smoking was not allowed. Participants rested in a supine or semi-sitting position during the studies. After the hourly withdrawal of blood by venapuncture they were allowed to void in an upright position if necessary.

The studies usually followed a dose-response protocol. In general after determination of at least two base-line values dopamine was infused in ascending doses (for results of reversed sequences see chapter 3), ranging from 0.25 to 8 μg/kg/min. Each dose was given for a period of one hour. In the metoclopramide, sulpiride and in part of the alpha-blocker studies a period of two hours, during which these substances were infused, preceded the dopamine dose-response curves.

Methods

Measurement of GFR, ERPF and FF

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured simultaneously using ^{125}I-iothalamate and ^{131}I-hippurate respectively, according to the method described by Donker et al [1]. In short, after a priming dose has been given, the radiopharmaceuticals are infused at a constant rate with a Braun-Unita II pump. After an equilibration period of at least one and a half hours, one hour clearances were determined throughout the study. The clearances of ^{125}I-iothalamate and ^{131}I-hippurate were calculated from the urinary tracer excretions and from the mean serum tracer values of two blood samples drawn at the beginning and at the end of each period, using the formulas (I x V)/P and (U x V)/P. The clearance of ^{125}I-iothalamate, which has proven to be a satisfactory substitute for inulin, represents the GFR. The clearance of ^{131}I-hippurate represents ERPF and corresponds to the clearance of p-amino-hippurate as long as the difference in extraction is taken into account. The coefficient of variation of the day to day determinations of the GFR amounts to < 2.2% and for the ERPF to < 5% [1]. Values for GFR and ERPF were corrected for standard body surface area in all studies, unless otherwise indicated. Filtration fraction (FF) represents the ratio GFR/ERPF.

Measurements of serum and urinary electrolytes and enzymes

Urine portions were examined for urinary electrolytes, ß-2-microglobulin and γ-glutamyltransferase (γ-GT). Serum and urinary electrolytes were assayed by routine Technicon SMAc Auto-Analyzer. ß-2-microglobulin concentration in serum and urine portions was determined by a radio-immuno-sorbent technique according to Evrin et al [2]. Urine samples were adjusted to a pH > 5.5 after voiding. Urinary γ-GT enzyme activity measurements were made on a Dupont-ACA using the GPNA-HCL method as described by Szasz with modifications according to Harrison for the Dupont-ACA [3]. Fractional excretions were calculated using the GFR values of the corresponding time intervals instead of the calculated creatinine clearances.

Dopamine and catecholamine assays

Samples for plasma levels were collected at the end of the corresponding hourly periods. Eight milliliters of blood were drawn into a cooled EDTA-containing glass tube (Venoject system). In general blood was obtained by venapuncture; a detailed description of the part of the study comparing plasma levels in blood drawn by routine

venapuncture and by means of an earlier inserted infusion system is given in chapter 7. The tubes were placed in melting ice and subsequently centrifuged in a cooled centrifuge during 10 minutes at 1500 rpm. Plasma was pipetted in a chilled plastic tube and centrifuged at 2000 rpm during 10 minutes. The supernatant plasma was transferred into a plastic tube containing 10 µg of glutathione. Tubes were frozen at -20 ^{0}C until assay.

The solvent extraction procedure for catecholamines in plasma and the subsequent assay using HPLC with electrochemical detection, have been described in detail elsewhere [4]. In short the following procedure was followed. To 2 ml of plasma the following substances are added: 100 µl of an internal standard containing dihydroxybenzylamine 1.25 µg/100 ml; 1 ml of a buffer solution containing 2 M NH_4OH/NH_4Cl, 0.5 % EDTA and 0.2 % diphenylborate-ethanolamine complex, adjusted to a pH of 8.5; 5 ml of an extraction solution, containing 99 % heptane, 1 % n-octanol and 2.5 g tetraoctyl-ammoniumbromide. After shaking for 2 minutes and centrifuging for 5 minutes at 1500 rpm the heptane layer is pipetted into another tube and 2 ml octanol and 250 µl 0.08 N acetic acid are added. After shaking and centrifugation at 1500 rpm the heptane layer is pipetted and discarded. 100 µl of the acetic acid layer is injected with a WISP 710B autosampler on a Brownlee Labs RP-8 spheri 5 column. The eluens consisting of 0.1 % SDS, 0.1 % EDTA, 25 % methanol and 8.6 ml acetic acid per liter is pumped with a flow rate of 1.0 ml/min with a LKB 2150 HPLC pump. A rotating electrochemical detector is mounted with electrodes of carbon paste. Recordings are made on a Philips PM8252 dual-pen recorder using a sensitivity of 10 mV/50 mV and a paper speed of 300 mm/h. As a reference solution 100 µl of a solution of 0.08 N acetic acid is used containing 3 parts of norepinephrine 1.25 µg/100 ml, 3 parts of epinephrine 0.75 µg/ 100 ml, 4 parts of dihydroxybenzylamine 1.25 µg/100 ml and 1 part of dopamine 4.7 µg/100 ml.

Urine dopamine and norepinephrine were assayed by HPLC with electrochemical detection according to the following procedure: after centrifugation for 5 min at 3000 rpm and pipetting the urine, 100 µl DHBA is added as an internal standard to 5 ml of the urine. After addition of 15 ml of EDTA 0.1 % and adjusting the pH to 6.5 with NaOH 0.5 N, the urine samples are injected onto Bio-rad columns (Bio-rad Laboratories GmbH, Munchen) for isolating the catecholamines, which have been preflushed with 0.1 % sodium EDTA (pH 6.5). The catecholamines are eluted from the columns with a 4 % boric acid solution and, after adjusting the pH to 4 with 2 N HCl, injected on the HPLC column. A RP-18 spheri 3 column (Brownlee Labs) was used with a Waters M-6000 A pump at a flow rate of 0.6 ml/min. We used a WISP 710B Autosampler and a Spark Holland 9205 electrochemical detector.

Both assays measure the free dopamine and norepinephrine levels in plasma and urine, respectively. Detection limits are as follows: plasma norepinephrine 10 pg (0.074 pmol), urine norepinephrine 80 pg (0.59 pmol), urine dopamine 0.5 ng (2.6 nmol). For plasma dopamine the mean recovery (+ S.D.) is 112.3 % (+ 5.6 %) (n = 7), for urine dopamine the intra-assay coefficient of variation is 6.7 % (n = 11). Intra-assay coefficient of variation has been found to amount to 4.2 % for plasma norepinephrine and 2.0 % for urine norepinephrine.

Other assays and procedures

Blood samples for plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were obtained by venapuncture with the subjects in a supine position. All individuals had been in a supine position for one hour before withdrawal of blood. PRA and plasma aldosterone concentration were determined by radioimmunoassay [5,6].

Serum metoclopramide levels were determined at 1, 2, 3 and 5 hours after the start of the metoclopramide infusion. Serum sulpiride levels were determined at 1, 2, 3, 5 and 7 hours after the start of the sulpiride infusion. Serum prazosin levels were obtained 4 hours after the first gift of 1 mg (just before the following gift), and one hour later. Reverse phase HPLC was used for serum metoclopramide, sulpiride and prazosin assays. For the prazosin assay a modified version of the method according to Twomey and Hobbs was used [7]. For the metoclopramide assay a modification of the method described by Huizing, Brouwers and Westhuis was used [8].

Heart rate and blood pressure (Riva-Rocci method) were recorded before and during the administration of sulpiride, and before and during the administration of the various doses of dopamine. A standard mercury sphygmomanometer was used.

STATISTICS

As the data were found or expected not to have a normal distribution they are presented as medians and ranges, unless otherwise stated. For the base-line data the mean of the two or three base-line values was used. An exception was made for the renal haemodynamic studies in whom initial plasma tracer levels showed that complete equilibration had not yet been attained. In these cases the second or mean of the second and third base-line value(s) were used. The data given for the infusion of metoclopramide, sulpiride or phentolamine alone are always those of the second hour of infusion.

For statistical analysis Wilcoxon tests for paired data or analysis of variance, using Kruskal-Wallis' non-parametric test were used. The significance of correlations was tested using linear regression analysis. Differences were considered statistically significant at the 5% level.

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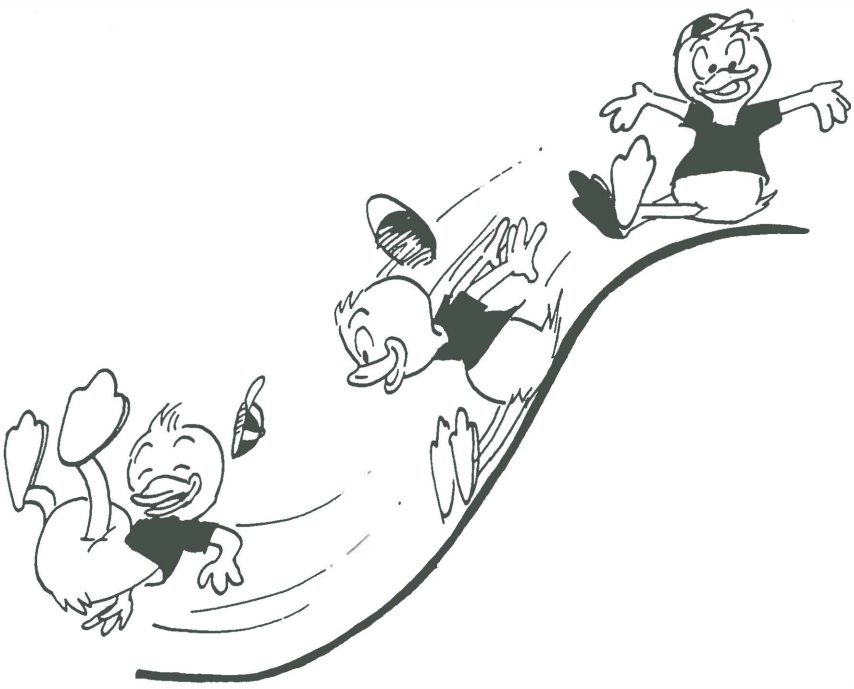
CHAPTER 3

THE DOSE-RESPONSE EFFECT OF INTRAVENOUS DOPAMINE ON RENAL FUNCTION IN NORMAL MAN AND IN PATIENTS WITH RENAL DISEASE



CHAPTER 3

THE DOSE-RESPONSE EFFECT OF INTRAVENOUS DOPAMINE ON RENAL FUNCTION IN NORMAL MAN AND IN PATIENTS WITH RENAL DISEASE

ABSTRACT

Renal function was determined before and during infusion of dopamine in doses ranging from 0.25 to 8 μg/kg/min in healthy volunteers and in patients with renal disease and moderately impaired renal function. In normal volunteers (n = 15) all doses of dopamine resulted in an increase of the ERPF compared to base-line values, which was maximal (47 %) at 8 μg/kg/min dopamine. GFR showed a more modest increase compared to base-line with a maximum of 14.1 % at a dose of 1 μg/kg/min. There was a maximal fall in FF of 24 % at 4 μg/kg/min dopamine.

Control studies with and without dopamine were performed in a subgroup of 5 volunteers: significant changes in ERPF and FF were again observed for doses of 0.25 μg/ kg/min and higher; the increase in GFR, however, was only significant for the 0.5, 4 and 8 μg/kg/min dose.

In patients with moderately impaired renal function (n = 21, base-line GFR ranging from 34 to 85 ml/min), the response of ERPF and FF to dopamine infusion was less outspoken than in the normals: ERPF rose maximally 27.1 % compared to base-line values, and the maximal fall in FF was 13.4 %. Compared to control studies in another group of patients with renal disease, dopamine doses of 0.5 μg/kg/min resulted in significant changes in ERPF and FF; GFR did not increase significantly.

Infusion of dopamine in doses of 2 μg/kg/min and higher resulted in significant increases of the urinary sodium excretion ($Una^+.V$) and the fractional sodium excretion ($FENa^+\%$) compared to base-line values and to control studies without dopamine in both healthy volunteers and patients with renal disease. Base-line $FENa^+\%$ and maximal increase in $FENa^+\%$ during dopamine did not differ between both groups. In both healthy volunteers and patients with renal disease, dopamine infusion was accompanied by an increase in calciuresis (UCa.V) and a fall in tubular reabsorption of phosphate (TRP). An increase in the excretion of ß-2-microglobulin and γ-glutamyl-transferase was observed for higher doses of dopamine in the patients, but not in the healthy volunteers.

The relationship between the dopamine-induced changes in renal haemodynamics and natriuresis, and the mechanisms of the impaired responses of ERPF and FF to dopamine in patients with impaired renal function are discussed.

INTRODUCTION

The beneficial effects of dopamine on renal function are well known and often used in conditions like congestive heart failure and impending acute oliguric renal failure [1-3]. Relatively little is known about the effects of various doses of dopamine on renal function in patients with renal disease [4 5]. In earlier studies of the so-called renal reserve filtration capacity we found that the increase of ERPF and GFR during infusion of a fixed low dose of dopamine (1.5 to 2 μg/kg/min) was much less pronounced in patients with renal disease than in normals, even if the base-line GFR of the patients was in the normal range [6]. A positive correlation was found between the change in GFR, ERPF or FF, and the base-line GFR in these patients. Besides anatomical changes, like a loss of nephrons, functional changes, like an increase in endogenous renal dopamine secretion might also be responsible for this blunted response in patients with renal disease. To acquire more information on the role of some of these factors we extended our observations by comparing dose-response curves of renal function with infusion of various doses of dopamine in normals and in patients with renal disease and moderately impaired renal function.

PATIENTS AND METHODS

We investigated 15 healthy volunteers and 21 patients with renal disease. All normal volunteers had a GFR > 90 ml/min. There were 10 males and 5 females, their ages ranged between 22 and 57 years. Sodium excretion amounted to 139 ± 48 mmol/24 h (mean and S.D.). We selected patients with a mild to moderate impairment of renal function. GFR ranged between 34 and 85 ml/min with a median of 56 ml/min.There were 13 males and 8 females, age was between 20 and 62 years with a median of 44 years. Various diagnoses were represented (Table 1). All medication had been withdrawn at least 4 days before renal function studies were performed, in the case of beta-blockers at least one week before. Sodium excretion was 114 ± 72 mmol/24 h (mean and S.D.).

In the renal function studies, after determining base-line values, dopamine (dopamine hydrochloride) was infused in doses of 0.25, 0.5, 1, 2, 4 and 8 μg/kg/min in the normal volunteers, and 0.5, 2 and 4 μg/kg/min in the patients, each dose for one hour. Doses > 8 μg/kg/min dopamine resulted in palpitations and/or nausea in some of the healthy volunteers and could not be investigated further. Because of marked rises in systolic blood pressure during infusion of 8 μg/kg/min in the healthy controls, this dose was omitted in the patient studies. In some of the normal volunteers reversed sequences of doses of dopamine and base-line infusions were given. In a number of the volunteers (n = 5) with a median age of 33 years, 2 females and 3 males, and a base-line GFR ranging from 108 to 128 ml/min the study was repeated with infusion of glucose (5 %) instead of dopamine. Control studies with an infusion of glucose (5 %) were not done in the same patients who had received dopamine but in another group of 8 patients with renal disease and various histological diagnoses with characteristics comparable to

Table 1: **Characteristics of the patients with renal disease.**

Pat. no.	Diagnosis	Age (years)	GFR (ml/min)	ERPF (ml/min)
1	IgA-nephropathy	22	80	289
2	IgA-nephropathy	25	56	251
3	IgA-nephropathy	34	56	214
4	IgA-nephropathy	44	51	188
5	IgA-nephropathy	45	62	233
6	IgA-nephropathy	51	81	299
7	focal glomerulosclerosis	20	83	398
8	focal glomerulosclerosis	37	45	192
9	focal glomerulosclerosis	50	50	219
10	focal glomerulosclerosis	51	56	300
11	membranous glomerulopathy	47	85	293
12	membranous glomerulopathy	50	69	368
13	membranous glomerulopathy	51	58	250
14	M. Wegener	62	42	173
15	urolithiasis	38	68	285
16	medullary sponge kidneys	24	38	158
17	chronic pyelonephritis	56	46	224
18	membranoproliferative GN	41	34	144
19	local focal GN	37	62	241
20	unknown	26	60	227
21	unknown	48	35	202

those of the study group (median GFR of 58 ml/min, median age 36 years, 4 females). To adjust for the difference in base-line values between the patients who received dopamine and the patients in whom the control studies were performed, changes were expressed as percentages of the base-line values (the first two hours of the study). The results in this subgroup of patients are presented as means and standard deviations and were analyzed by analysis of variance. The same procedure procedure was followed for the control studies in the healthy volunteers for another reason: the small number of healthy volunteers in combination with a rather broad range of base-line values (base-line ERPF ranged from 352 to 560 ml/min, GFR from 108 to 128 ml/min) also supported the use of percentage changes compared to base-line, instead of the medians of the absolute values, for presenting the data. For analysis of absolute values a Kruskal-Wallis test was performed, for the percentage changes normal analysis of variance was used. For other details on the study procedures the reader is referred to chapter 2.

RESULTS

Effects of dopamine infusion on GFR, ERPF and FF

In normal volunteers base-line ERPF amounted to 444 ml/min (range 336-647 ml/min). For all dopamine doses a significant increase of the ERPF was found compared to base-line values (fig 1A). Each dose increment was followed by a significant increase in ERPF compared to that of the previous dose up to 4 μg/kg/min, when a maximum of 661 ml/min was reached. Individual maxima ranged between 415 and 913 ml/min and were all reached at 2, 4 or 8 μg/kg/min dopamine.

GFR showed significant increases compared to base-line values after dopamine doses of 0.5 μg/kg/min and higher. The maximum was already reached at a dose of 1 μg/ kg/min with 126.9 ml/min compared to a base-line value of 111.7 ml/min (range 91-135 ml/min); no significant changes were seen at higher doses (fig 1A). Individual maxima ranged between 106 and 157 ml/min and were reached at dopamine doses varying between 0.5 and 8 μg/kg/min.

The FF fell significantly compared to base-line for all doses of dopamine with a minimum of 0.185 at 4 μg/kg/min compared to a base-line value of 0.24 (range 0.205-0.31). A significant increase in the FF was observed for the 8 μg/kg/min dopamine dose compared to the 4 μg/kg/min dose (fig 1A). The maximal fall in FF was observed at the 2 μg/kg/min dose in 7 volunteers and in 7 others at the 4 μg/kg/min dopamine dose.

Expressed as the percentage change compared to base-line values, the maximal increase for the ERPF was 47 % and was reached at the 8 μg/kg/min dose, for the GFR 14.1 % at the 1 μg/kg/min dose, with a maximal fall of the FF of 24 % at 4 μg/kg/min dopamine (fig 2). The responses were independent of the sequence of administration of dopamine dosages.

In 5 of the volunteers, control studies were performed with infusion of glucose (5 %) instead of dopamine. Comparison of the dopamine-infusion study with the control study revealed significant changes in ERPF and FF for doses of 0.25 μg/kg/min dopamine and higher; for the GFR a significant increase was restricted to the 0.5, 4 and 8 μg/kg/ min dose (fig 3A). No significant change compared to the base-line hours was found for either function during infusion of glucose (5 %).

In patients with renal disease and impaired renal function, all tested doses of dopamine resulted in increases of ERPF and GFR, and a fall in FF compared to base-line values (fig 1B). ERPF rose from a base-line value of 233 ml/min (range 144-398 ml/min) to a maximum of 306 ml/min, GFR from 58.2 to 66.5 ml/min, respectively, both at a dose of 4 μg/kg/min. The maximal ERPF was found at 4 μg/kg/min in 11 patients, in 4 of them at the 2 μg/kg/min dopamine dose. Individual maxima for the GFR in the patients were reached at 0.5 μg/kg/min in 7, at 2 μg/kgmin in 6 and at 4 μg/kg /min in 8 patients. FF fell from a base-line value of 0.238 (range 0.175-0.290) to a minimum of 0.21, also at 4 μg/kg/min. In 19 of the patients the lowest FF was found at the 4 μg/kg/min dopamine dose. For the ERPF and FF, but not for the GFR,

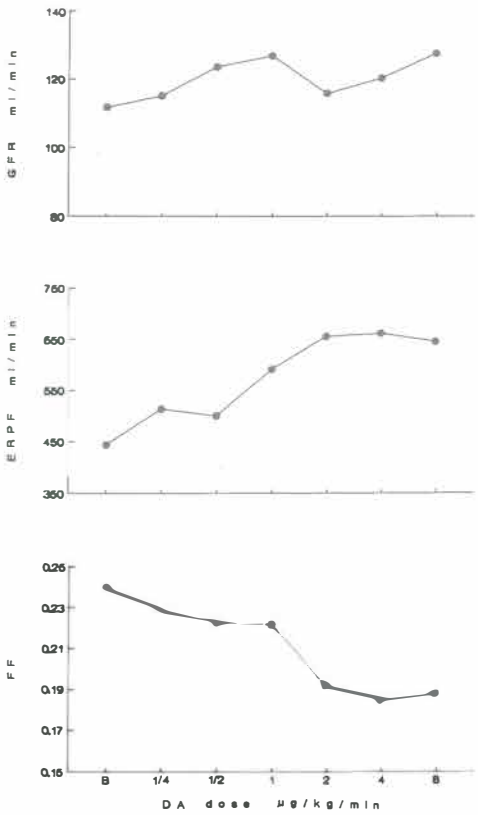


Figure 1A: GFR (ml/min), ERPF (ml/min) and FF before and during infusion of different doses of dopamine in healthy controls (n = 15). The values shown are medians.
B = base-line. All doses of dopamine resulted in a significant change from the base-line values for the ERPF and FF, for the GFR in doses of 0.5 µg/ kg/min and higher ($p < 0.05$ or $p < 0.01$).

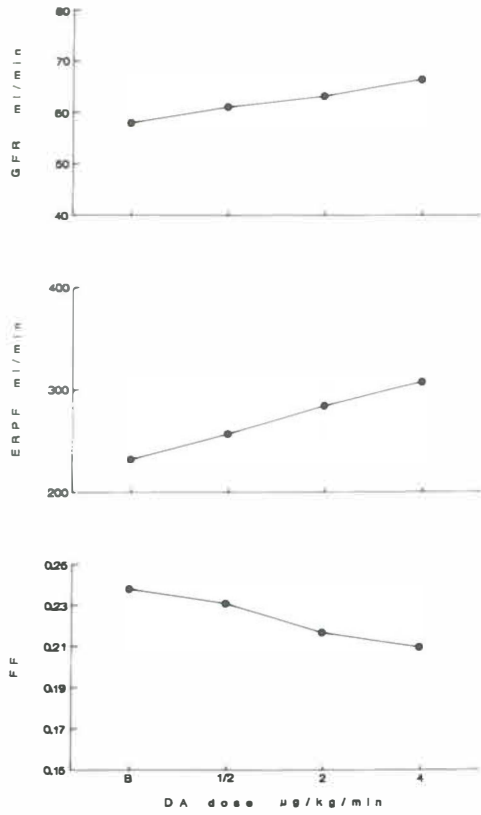


Figure 1B: GFR (ml/min), ERPF (ml/min) and FF before and during infusion of different doses of dopamine in patients with renal disease (n = 21). The values shown are medians.
B = base-line. All doses of dopamine resulted in a significant change from the base-line values for the ERPF, GFR and FF ($p < 0.01$).

each dose increment resulted in significant changes compared to the previous dose. Expressed as the percentage change compared to base-line values the maximal increase for the ERPF was 27.1 % and for the GFR 8.7 % while the maximal fall in FF was 13.4 %, all at the 4 µg/kg/min dopamine dose (fig 2).

Control studies were not done in the same patients but in another group with comparable characteristics for base-line GFR, age and sex. When percentage changes relative to base-line values were compared for identical time periods in the patient groups with and without dopamine, the changes in ERPF and FF were significant for doses of 0.5 µg/kg/min and higher. No significant difference was found between both

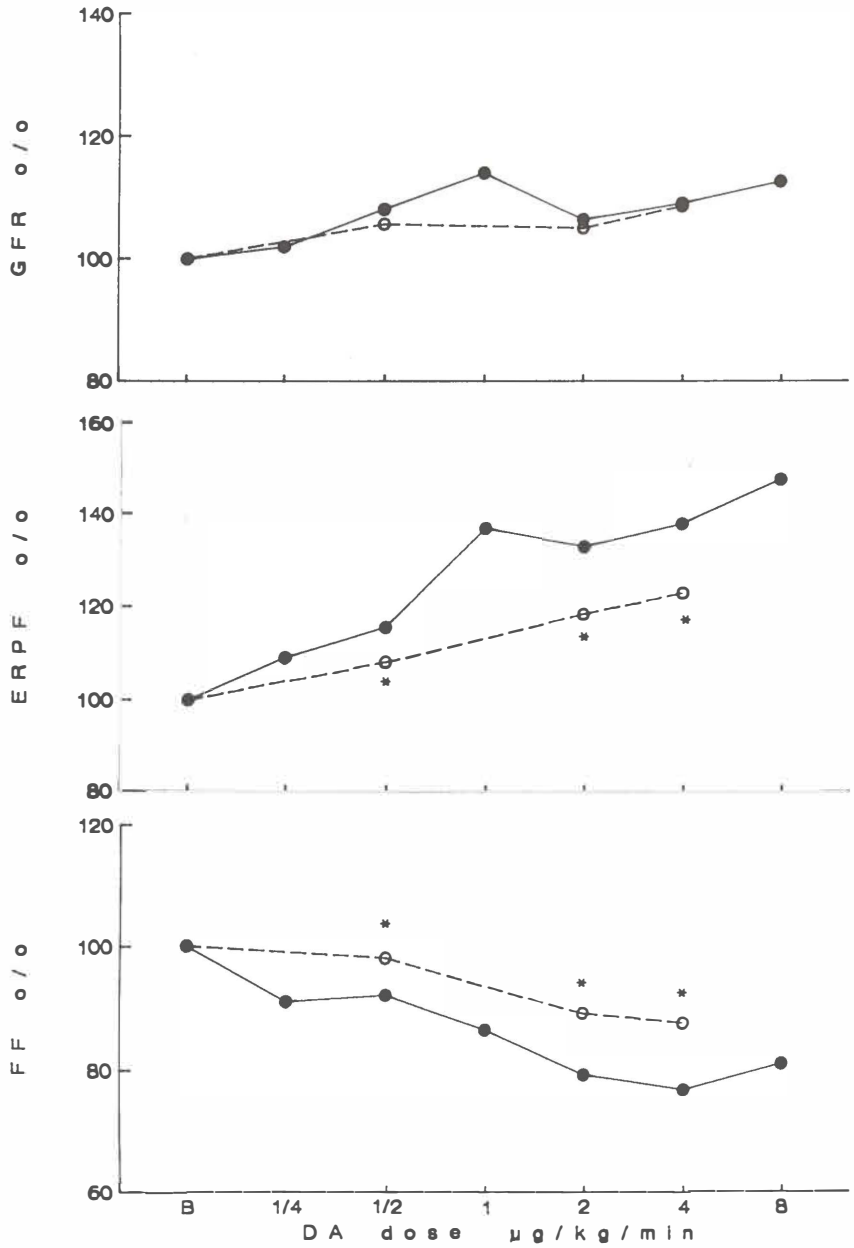


Figure 2: Percentage changes in GFR, ERPF and FF in healthy controls (——) and in patients with renal disease (-------) during infusion of different doses of dopamine. The values shown are medians. B represents the base-line values which were valued at 100 %. For all doses the percentage change in ERPF and FF was less in the patients ($p < 0.05$ for the 0.5 µg/kg/min dose, $p < 0.01$ for the other doses).

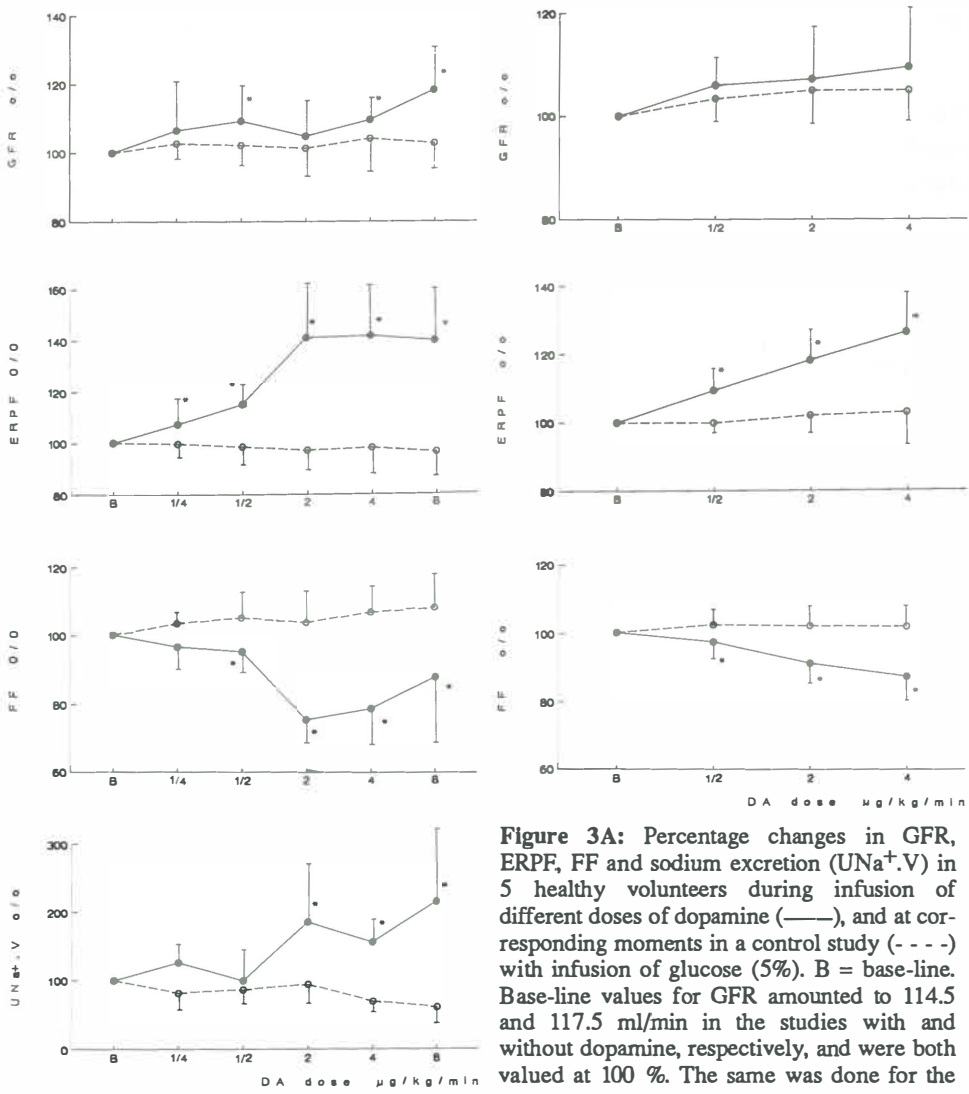


Figure 3A: Percentage changes in GFR, ERPF, FF and sodium excretion ($UNa^+.V$) in 5 healthy volunteers during infusion of different doses of dopamine (——), and at corresponding moments in a control study (- - - -) with infusion of glucose (5%). B = base-line. Base-line values for GFR amounted to 114.5 and 117.5 ml/min in the studies with and without dopamine, respectively, and were both valued at 100 %. The same was done for the ERPF (with base-line values of 439 and 440.5 ml/min respectively) and for the FF (base-line values of 0.265 and 0.275, respectively). Means and standard deviations are represented. * = significantly different from the control study ($p < 0.05$).

Figure 3B: Percentage changes in GFR, ERPF and FF compared between a group of 21 patients with renal disease receiving infusion of different doses of dopamine (——), and a control group of 8 patients not receiving dopamine (- - - -). B = base-line. Base-line values were valued at 100%. Means and standard deviations are represented.
* = significantly different from the control study ($p < 0.05$).

groups for the change in GFR (fig 3B). Again no change in either GFR, ERPF or FF compared to base-line hours occurred during infusion of glucose (5%).

The response of ERPF and FF was significantly less in the patients compared to the healthy controls for all doses of dopamine; for the percentage change in GFR no significant differences were found between both groups (fig 2).

There was a significant correlation between base-line GFR or ERPF and the maximal increase in ERPF during dopamine infusion in the patients. The correlation between base-line ERPF and the maximal change in ERPF during dopamine infusion is described by: ERPF(max - base-line) = 15.60 + 0.2163 base-line ERPF ($r = 0.472$, $p < 0.02$), the correlation between base-line GFR and the maximal change in ERPF is shown in fig 4. There was also a correlation between the base-line ERPF and the maximal fall in FF, described by: FF(min - base-line) = - 0.0034 - 0.000111 base-line ERPF ($r = 0.470$, $p < 0.05$). No relation could be established in our small group of

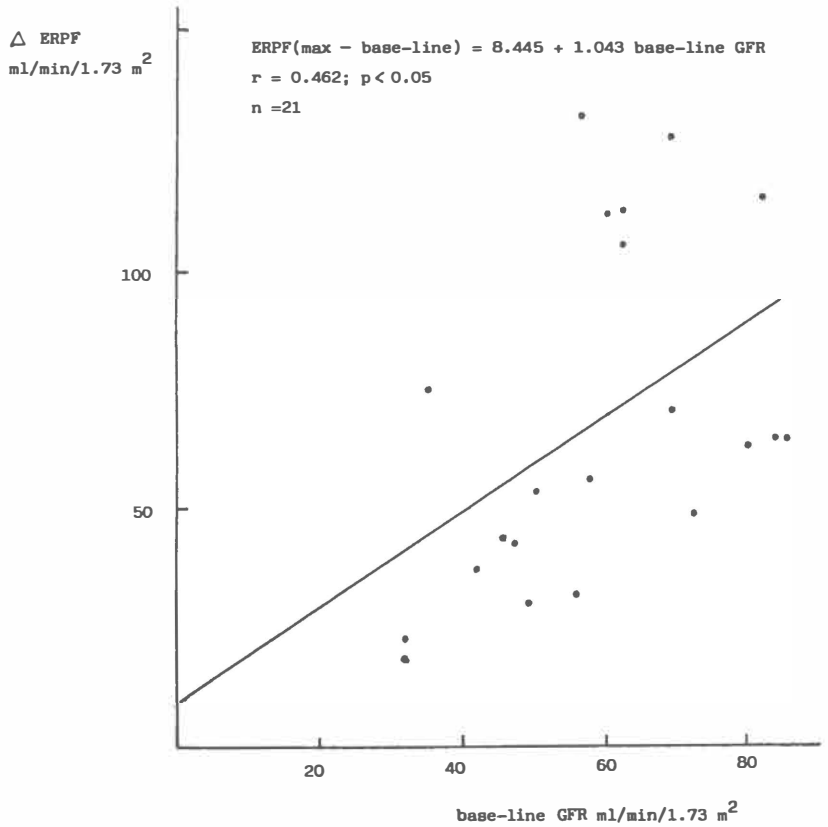


Figure 4: Correlation between the maximal increase in ERPF during dopamine infusion and base-line GFR in patients with renal disease (n = 21).

patients between the response of ERPF or FF and the histological diagnosis. For the healthy controls, but not for the patients, a relation was found between the response of GFR to dopamine and the 24 h sodium excretion ($r = 0.870$; $p < 0.02$). Neither in the patients with renal disease nor in the healthy controls responses of ERPF and FF to dopamine infusion were related to age or 24 h sodium excretion.

Effects of dopamine infusion on the excretion of sodium, other electrolytes and tubular enzymes

Both in healthy volunteers and in patients, a significant and progressive rise in $Una^+.V$ and $FENa^+\%$ compared to base-line values was found during infusion of dopamine in doses of 2 µg/kg/min and higher (fig 5A). In the healthy volunteers, in whom control

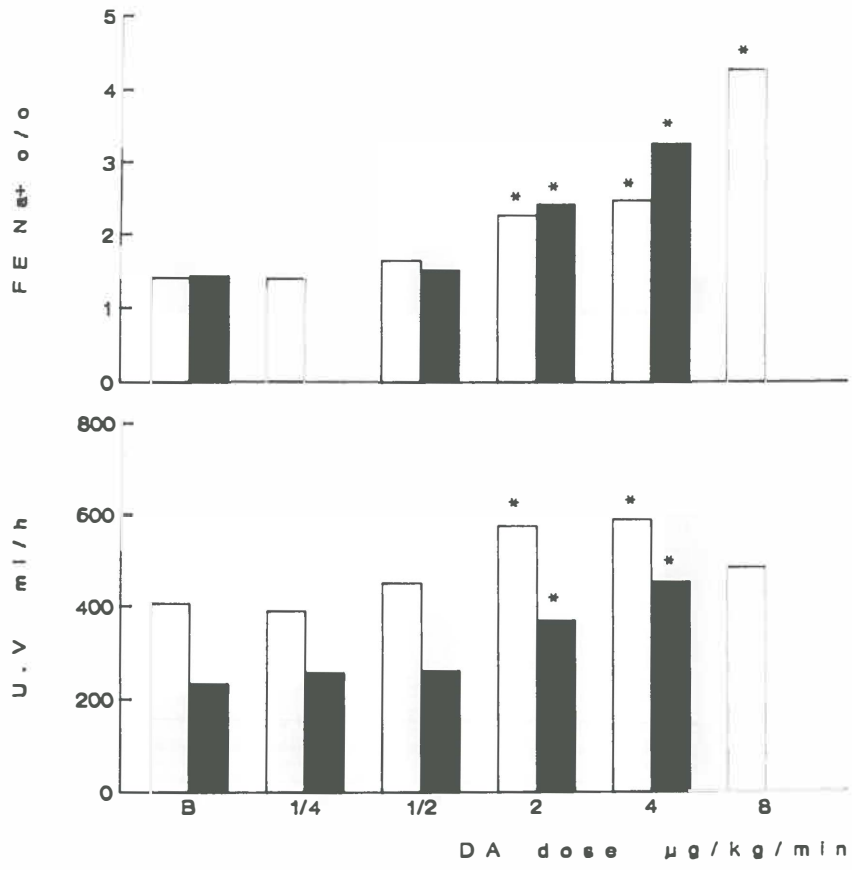


Figure 5A: Fractional excretion of sodium ($FENa^+\%$) and diuresis (U.V)(ml/h) before and during infusion of different doses of dopamine in healthy volunteers (open bars) and in patients with renal disease (closed bars). B = base-line. The values shown are medians. * = $p < 0.05$ compared to base-line.

studies were performed, the increase in $Una^+.V$ and $FENa^+\%$ was also significant compared to the control-study values for doses of 2 µg/kg/min and higher (fig 3A). Base-line $FENa^+\%$ and its maximal percentage increase during dopamine infusion did not differ significantly between the healthy volunteers and the patients. In the patients the increase in $FENa^+\%$ during dopamine was neither related to base-line GFR or ERPF, nor to the maximal percentage change in GFR, ERPF or FF during dopamine.

No changes in kaliuresis or fractional potassium excretion occurred during dopamine infusion. Diuresis rose significantly compared to base-line values and more

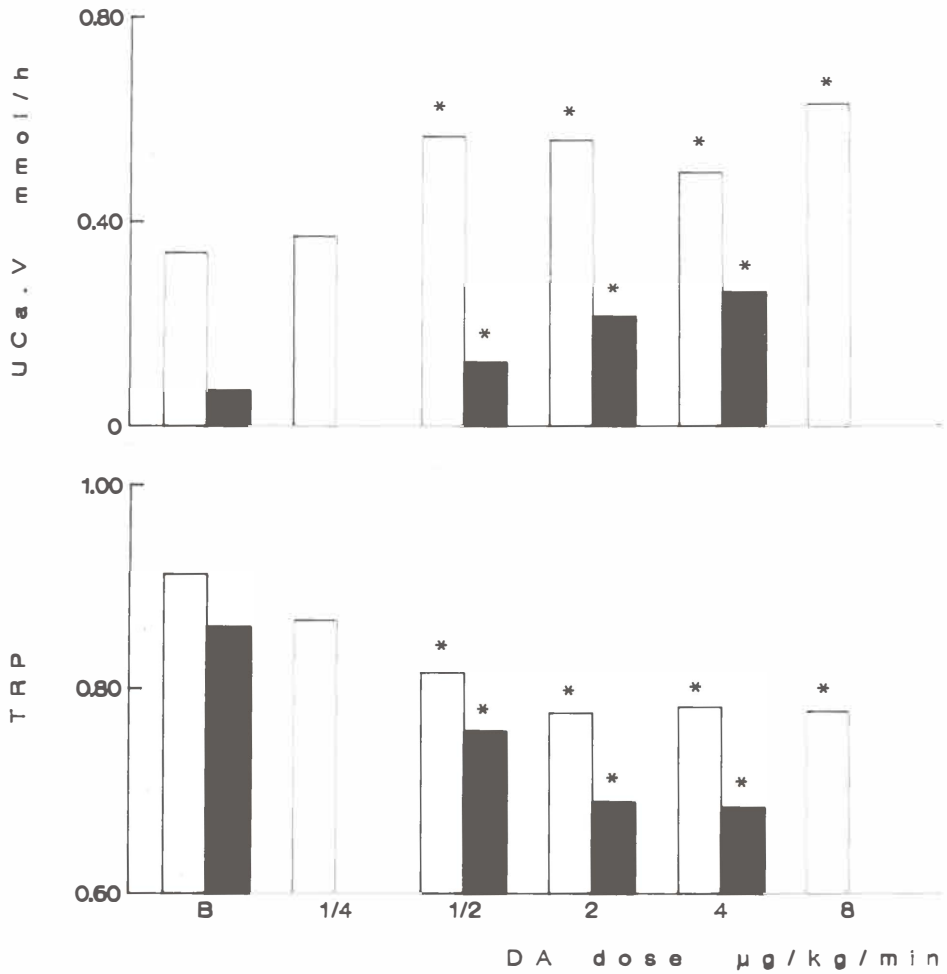


Figure 5B: Calciuresis (UCa.V)(mmol/h) and tubular reabsorption of phosphate (TRP) before and during infusion of different doses of dopamine in healthy volunteers (open bars) and in patients with renal disease (closed bars). B = base-line. The values shown are medians.
* = $p < 0.05$ compared to base-line.

pronounced in the healthy volunteers (fig 5A). An increase in calciuresis compared to base-line was found during dopamine infusion in healthy volunteers for doses of 0.5 µg/kg/min and higher. In the 5 volunteers with control studies, the increase was significant for doses of 2 µg/kg/min and higher. In the patients an increase compared to base-line values was found for all tested doses of dopamine compared both to base-line and to corresponding periods in control studies without dopamine (fig 5B). TRP fell in healthy volunteers and patients for dopamine doses of 0.5 µg/kg/min and higher (fig 5B). A significant increase of the excretion of γ–glutamyltransferase (γ-GT) was

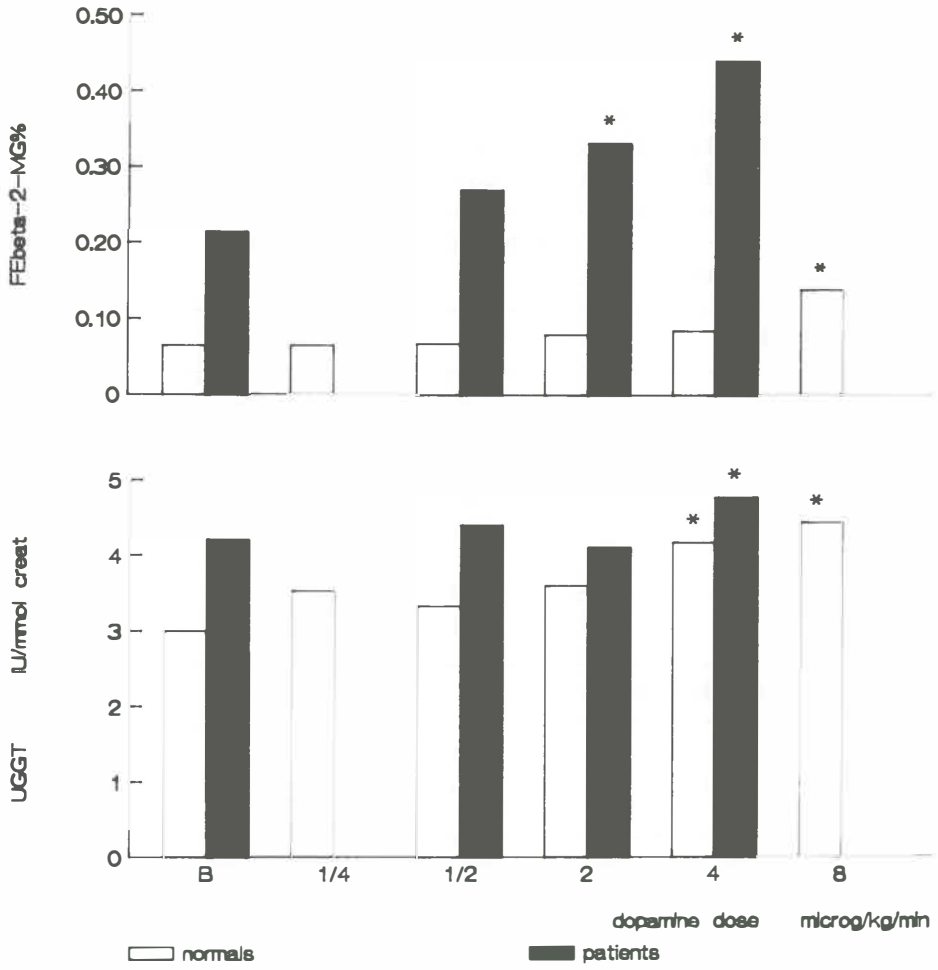


Figure 5C: Fractional excretion of ß-2-microglobulin (FEß-2-MG) and excretion of γ-gamma-glutamyltransferase (UGGT.V)(IU/mmol creatinine) before and during infusion of different doses of dopamine in healthy volunteers (open bars) and in patients with renal disease (closed bars). B = base-line. The values shown are medians. * = $p < 0.05$ compared to base-line.

present in the healthy controls for dopamine doses of 4 and 8 µg/kg/min, from 3 IU/mmol creatinine at base-line (range 1.3-7.1) to 4.2 and 4.4 (range 2.4-8.5) IU/mmol creatinine, respectively; in the patients the increase in γ-GT excretion was small and significance limited to the 4 µg/kg/min dose with a base-line value of 4.2 IU/mmol creatinine (range 1.6-11) and a maximum of 4.8 (range1.2-11.4) (fig 5C). The fractional excretion of ß-2-microglobulin (FEß-2-MG%) rose above base-line values of 0.214 for dopamine doses of 2 µg/kg/min and higher (maximum 0.441 at 4 µg/kg/min) in the patients with renal disease; in the healthy volunteers no significant changes in the FEß-2-MG% were observed during dopamine infusion except for the 8 µg/kg/min dose (base-line value 0.065 and at 8 µg/kg/min 0.139)(fig 5C).

Table 2: **Blood pressure and pulse rate before and during dopamine-infusion in healthy controls and in patients with renal disease.**
Means and S.D. are shown. * = $p < 0.05$, compared with base-line.

	Dopamine dose (microg/kg/min)						
	baseline	0.25	0.5	1	2	4	8
Healthy controls							
systolic RR	124	126	124	124	126	129	148*
(mmHg)	8.2	11.9	8.9	8.6	11.2	11.2	12
diastolic RR	82	81	80	79	81	78*	72*
(mmHg)	5.4	7.6	4.9	5.8	6	7.	6.5
pulse rate	67	66	68	73	71	70	69
(beats/min)	7.1	7.5	7.4	6.6	8.4	9.4	8.6
Patients with renal disease							
systolic RR	134		128		138	158*	
(mmHg)	13.7		16.6		15.8	21	
diastolic RR	91		86*		84*	83*	
(mmHg)	10.3		12.1		11.5	13.3	
pulse rate	68		70		69	68	
(beats/min)	10.9		10.4		9.7	11.3	

Effects on blood pressure and pulse rate

In the healthy volunteers diastolic blood pressure decreased slightly during infusion of dopamine 4 and 8 μg/kg/min, however, systolic blood pressure increased sharply at 8 μg/kg/min (Table 2). In the patients with renal disease, in whom base-line blood pressure was higher than in the healthy volunteers, a significant fall in diastolic blood pressure was observed at 0.5 μg/kg/min dopamine, which was enhanced at 2 μg/kg/min (Table 2). Systolic blood pressure rose at 4 μg/kg/min. Pulse rate remained unchanged throughout the study in both healthy volunteers and patients with renal disease.

DISCUSSION

The endogenous catecholamine dopamine has long been known to exert important renal haemodynamic effects [1 2]. Stimulation of renal dopaminergic receptors, mainly of the DA_1 subtype, leads to renal vasodilation [7]. Direct stimulation of adrenergic receptors by dopamine, presynaptic inhibition of norepinephrine release by dopamine and conversion of dopamine to (nor)epinephrine modulate this response [7]. Most human studies on the effects of various doses of dopamine have been performed in patients with congestive heart failure. In this condition increased circulating levels of catecholamines are found, accompanied by a desensitization or loss of adrenergic receptors [8 9]. Changes in renal function in patients with congestive heart failure during dopamine infusion not only result from direct dopaminergic or adrenergic renal effects of dopamine, but also strongly depend on improvements in cardiac performance. This is evidenced for example by comparing in these patients the renal haemodynamic effects of dopamine and dobutamine, the latter of which does not stimulate renal dopamine receptors [10]. It is therefore not justified to extend observations on renal effects of dopamine in patients with congestive heart failure to normals or other patient groups.

Studies on the effects of dopamine on renal haemodynamics in normal man have mostly been restricted to doses in excess of 1 μg/kg/min. McDonald used a minimum dose of 2.6 μg/kg/min in early studies, resulting in an increase of PAH-clearance of 91 %, while Ramdohr later observed a 48 % increase of PAH-clearance at 175 μg/min [4 5]. Hollenberg did not find an increase in renal blood flow, assessed by xenon washout, at 0.3 and 1.0 μg/kg/min dopamine; however, only two and four normals volunteers were tested for these doses, respectively [11]. This is in contrast with the results of Breckenridge, who for a dose of 1.0 μg/kg/min observed a 77 % increase in renal blood flow measured directly by an indicator dilution technique in the unaffected kidney in 11 hypertensive patients with unilateral renal disease [12].

Our results show that low doses of dopamine indeed have a marked influence on renal function. An evident increase in ERPF occurs at doses of 0.5 μg/kg/min. Although the ERPF continues to rise at higher doses of dopamine, the increase in GFR is already maximal at a dose of 1 μg/kg/min in our study. The differences between the

dopamine-infusion and the control studies, and the comparable results found in those volunteers to whom dopamine doses were given in a reversed sequence indicate that our study conditions, for example the high oral fluid administration, or circadian rhythms cannot be held responsible for the observed changes in renal function in the dopamine-infusion studies.

The dopamine response of ERPF and FF was rather uniform in both the healthy volunteers and the patients since the majority of maximal responses were achieved at the same dose. Renal vasodilation as represented by the FF seems to be maximal at 4 μg/ kg/min. The ERPF also levels off at 4 μg/kg/min. Although this might indicate maximal stimulation of renal dopamine receptors at this dose, it is equally well possible that a balance is reached at higher doses between further vasodilation by dopaminergic stimulation and vasoconstrictive forces by stimulation of alpha-adrenergic receptors by dopamine. The rise of FF at 8 μg/kg/min is compatible with the latter assumption.

The marked rise in ERPF with a rather modest increase in GFR, resulting in a fall in FF, indicates that during dopamine infusion renal vasodilation takes place which is predominantly postglomerular. In our patients with renal disease the changes in ERPF and FF during dopamine infusion are much less outspoken than in our normals and depend on the base-line ERPF. Therefore in patients the capacity for renal vasodilation during dopamine infusion seems to get lost. Besides anatomical changes like a loss or sclerosis of nephrons, functional changes might also be responsible for this blunted response in patients: being a major producer of dopamine, the kidney might in response to damage increase endogenous renal dopamine synthesis with the aim of maintaining renal function. Dopamine has been shown to result in dilation of postglomerular vessels [13]. The fact that the impaired response of ERPF and FF is found for all doses of exogenous dopamine in our patients can be used as an argument in favour of enhanced endogenous renal dopamine generation. A more effective approach to solve this question of increased endogenous dopamine generation in patients with renal disease will be to study the effects of dopamine antagonists selective for renal dopamine receptors in these patients. Unfortunately no studies concerning the urinary excretion of dopamine in patients with renal disease are available to support or refute the hypothesis of enhanced renal dopamine generation in these patients. In chapter 7 we will present data on the urinary excretion of dopamine in patients with renal disease and healthy controls.

Imbs et al concluded from studies in water-loaded and water-deprived dogs that the natriuretic effect of dopamine is secondary to its renal vasodilatory action. An increase in fractional natriuresis was only observed in the water-loaded dogs, in which dopamine led to a fall in filtration fraction with a constant GFR [14]. In our group of healthy volunteers no relation existed between the increase in natriuresis and the fall in FF during dopamine. Despite the blunted response of ERPF and FF to dopamine in patients with renal disease, the increase in natriuresis, $FENa^{+}$% and diuresis in the patients was not different from that in the normal volunteers. This may seem to be in contradiction with the hypothesis formulated above, that the impaired haemodynamic response in the patients is due to enhanced renal dopamine generation; however, a local

vascular increase in endogenous dopamine cannot be excluded. The differences between the dopamine dose-response curves for the GFR, ERPF or FF on the one hand, and the diuresis and natriuresis on the other hand do not provide evidence that the changes in renal haemodynamics during dopamine administration directly influence sodium excretion and diuresis. Other factors like a direct tubular effect of dopamine or a dopamine-induced fall in aldosterone secretion, may be more important in mediating the natriuresis and diuresis during dopamine infusion. The increase in the excretion of calcium, γ-GT and ß-2-microglobulin and the fall in the TRP during dopamine infusion may represent an effect of dopamine on the renal tubule. The reabsorption of phosphate and ß-2- microglobulin occurs mainly or exclusively in the proximal renal tubule; γ-GT excretion also may be regarded as a parameter of proximal tubular function [15-18]. Our results are therefore compatible with an effect of dopamine predominantly at the proximal renal tubule. In agreement with this, dopamine has recently been shown to cause a dose-dependent inhibition of NaKATPase activity in isolated proximal convoluted tubules [19].

Changes in blood pressure were limited to the higher doses of dopamine in both healthy controls and patients with renal disease and agree with those found by other authors [1]. This also illustrates the limited influence of systemic haemodynamics on the observed changes in renal haemodynamics and sodium excretion during dopamine infusion.

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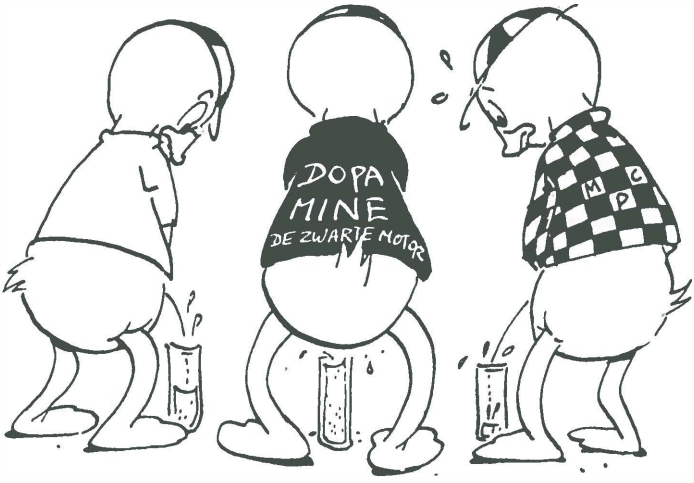
CHAPTER 4

THE EFFECT OF METOCLOPRAMIDE ON DOPAMINE-INDUCED CHANGES IN RENAL FUNCTION IN HEALTHY CONTROLS AND IN PATIENTS WITH RENAL DISEASE



CHAPTER 4

THE EFFECT OF METOCLOPRAMIDE ON DOPAMINE-INDUCED CHANGES IN RENAL FUNCTION IN HEALTHY CONTROLS AND IN PATIENTS WITH RENAL DISEASE

ABSTRACT

The effect of intravenous metoclopramide on base-line values and dopamine dose-response curves for renal haemodynamics and natriuresis was investigated in healthy volunteers and patients with renal disease.

Dopamine infusion alone, in doses ranging from 0.25 to 8 μg/kg/min, resulted in a dose-dependent increase in ERPF and GFR with a fall in FF in eight hydrated healthy volunteers and, to a lesser degree, in twelve patients with renal disease. An increase in natriuresis ($UNa^+.V$), fractional sodium excretion ($FENa^+\%$) and diuresis was found in both groups for doses of 2 μg/kg/min and higher.

Metoclopramide infusion did not alter base-line values of GFR, ERPF or FF, but shifted the dose-response curve for ERPF and FF to dopamine in the healthy volunteers. Metoclopramide induced a fall in $UNa^+.V$ and $FENa^+\%$ in both groups (a fall in base-line $FENa^+\%$ from 1.52 to 0.71 during metoclopramide in the healthy volunteers and from 1.23 to 0.56 in the patients; $P < 0.01$), and blunted the natriuretic response to subsequent dopamine infusion. The fall in $UNa^+.V$ during metoclopramide infusion showed a strong correlation with the base-line GFR ($r = -0.944$). In the patients the response for the fractional excretion of ß-2-microglobulin was comparable to that of $FENa^+\%$.

Dopamine infusion induced a fall, and metoclopramide led to a rise in plasma aldosterone concentration.

We conclude that metoclopramide acts as a dopamine antagonist at the renal level in man. Secondly, endogenous dopamine secretion does not seem to have a role in maintaining ERPF or GFR in hydrated healthy volunteers or in patients with renal disease, but is important for mediating natriuresis. Finally, dopamine-induced changes in natriuresis do not depend on changes in renal haemodynamics.

INTRODUCTION

Infusion of dopamine results in renal vasodilation and an increase in natriuresis and diuresis [1 2]. These changes are attained at pharmacological doses [1]. However, the kidney itself is also an important production site of dopamine [3 4]. In earlier studies we found that the increase in ERPF and the fall in FF during dopamine infusion are much less pronounced in patients with renal disease than in healthy controls, even if base-line renal function of these patients is in the normal range [2 5]. We proposed that besides

anatomical changes an enhanced endogenous renal dopamine generation might be responsible for this phenomenon.

In animal studies dopamine-induced renal vasodilation has been used extensively as a model to investigate peripheral effects of various dopamine antagonists. In man studies on the influence of dopamine antagonists on renal function or dopamine-induced vasodilatation are limited. Recently, Israel et al found that high doses of metoclopramide decreased renal plasma flow in man while Krishna and coworkers reported earlier that much lower doses of metoclopramide blunt the natriuresis after volume expansion [6,7].

In this study we examined the effect of intermediate doses of metoclopramide on dopamine-induced changes in renal function in healthy controls and in patients with renal disease to investigate the possible role of endogenous renal dopamine secretion in maintaining renal function in either group.

PATIENTS AND METHODS

We investigated 8 healthy volunteers and 12 patients with renal disease. The age range of the healthy controls was 22-58 years, median 25 years. There were 5 males and 3 females. Weight ranged from 63 to 82 kg (median 75 kg). GFR ranged from 91 to 135 ml/min with a median of 114 ml/min.

We selected patients with known renal disease with a mild to moderate impairment of renal function (GFR > 35 ml/min), in whom medication could temporarily be withdrawn. The age of the patients ranged from 20 to 62 years, median 40 years. There were 8 males and 4 females. Sodium excretion averaged 97 mmol/24 h. Weight ranged from 59 to 95 kg (median 76 kg). Various diagnoses were represented: IgA-nephropathy in 4 patients, focal glomerulosclerosis in 3 patients and membranous glomerulopathy, local focal glomerulonephritis, Wegener's granulomatosis and chronic pyelonephritis each in one patient, while in one patient no histological diagnosis was available. Base-line GFR ranged from 35 to 80 ml/min with a median of 57 ml/min.

In the first of the two renal function studies dopamine was given in doses of 0.25, 0.5, 2, 4 and 8 µg/kg/min in the normal volunteers, and in doses of 0.5, 2 and 4 µg/kg/min in the patients. In the second renal function study, after determining base-line values, metoclopramide (Primperan®) was given by intravenous infusion: 10 mg was infused in the first 30 minutes, followed by an infusion of 10 mg/h during the rest of the study. Dopamine infusion was started two hours after the beginning of the metoclopramide infusion and was given in the doses mentioned above. Serum metoclopramide levels were determined at 1, 2, 3 and 5 hours after the start of the metoclopramide infusion. Plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were obtained at the end of the base-line period, at the end of the 2 µg/kg/min dopamine-infusion periods (in both studies), and at the end of the infusion of metoclopramide alone. All individuals had been in a supine position one hour before withdrawal of blood.

For further details on the study procedures the reader is referred to chapter 2.

RESULTS

Effects on effective renal plasma flow, glomerular filtration rate and filtration fraction

In the healthy controls infusion of dopamine alone resulted in an increase of the ERPF and a fall in the FF for all doses compared to base-line values (fig 1A). Each dose

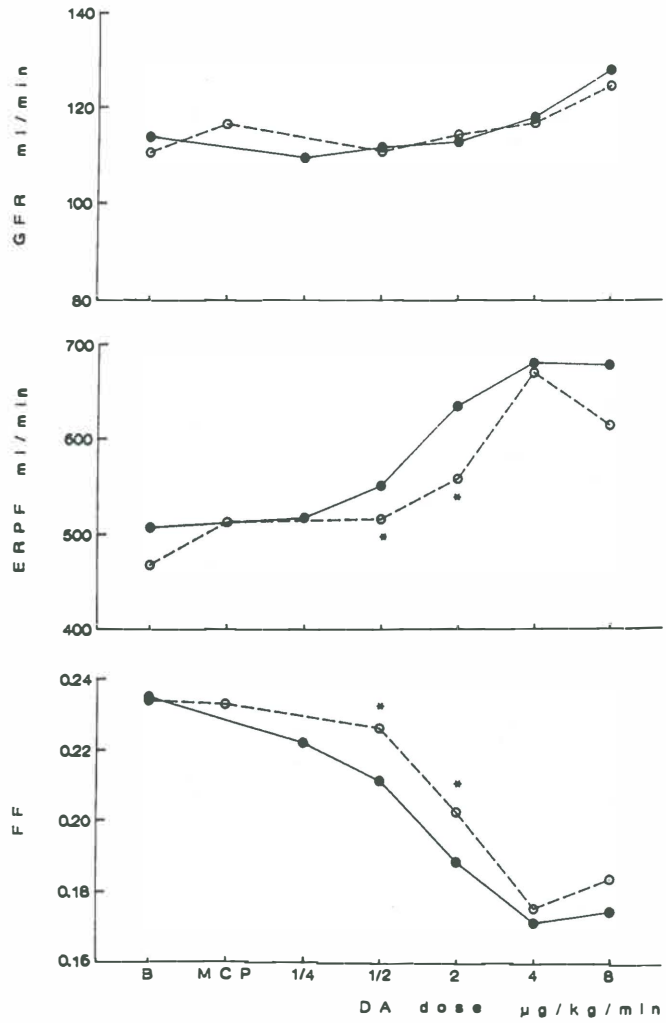


Figure 1A: Dose-response curves for ERPF, GFR and FF before and during infusions of dopamine and metoclopramide and during combined infusion in healthy controls (n = 8). Median values are given.
—— before and during infusion of dopamine alone.
----- before and during infusion of metoclopramide and subsequent infusion of dopamine.
* = significant difference between study without and with metoclopramide ($p < 0.05$).

increment led to a significant further rise in ERPF up to 4 µg/kg/min. In this small group of controls the increase in GFR during dopamine infusion was only significant at the 4 and 8 µg/kg/min dose ($p < 0.02$). In the patients with renal disease, the increase in both ERPF and GFR was significant for all tested doses while the FF fell during infusion of 2 and 4 µg/kg/min of dopamine (fig 1B).

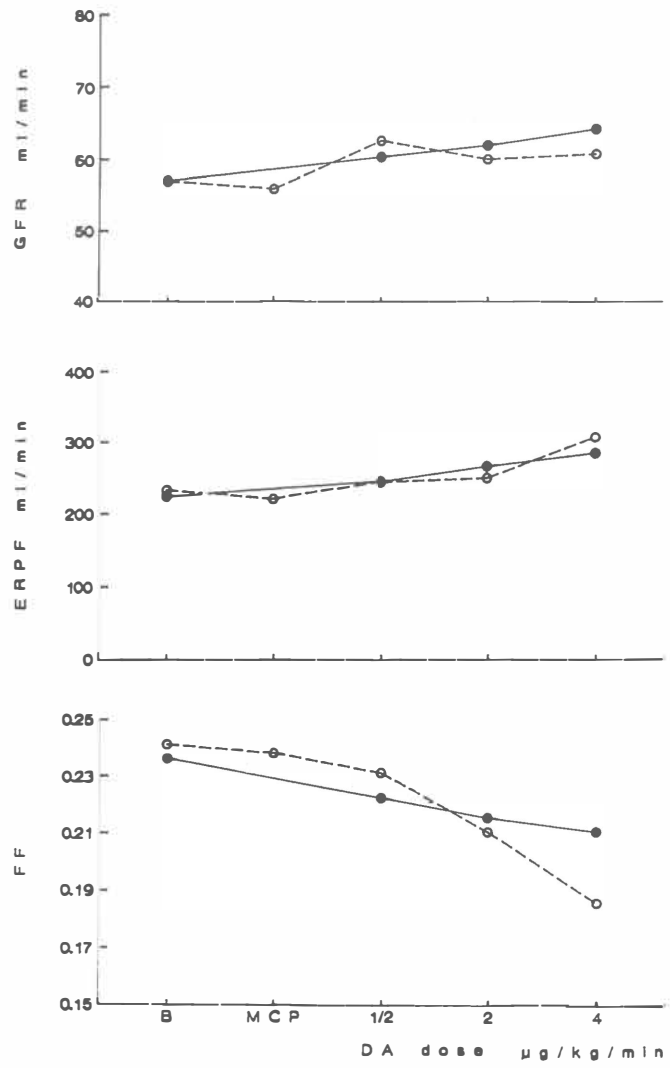


Figure 1B: Dose-response curves for ERPF, GFR and FF before and during infusions of dopamine and metoclopramide and during combined infusion in patients with renal disease (n = 12). Medians values are given.
—— before and during infusion of dopamine alone.
------ before and during infusion of metoclopramide and subsequent.

The base-line values of ERPF, GFR and FF during the second renal function study were not different from those of the first study in either the healthy controls or the patients with renal disease. In the second renal function study the infusion of metoclopramide had no effect on base-line ERPF, GFR or FF in both healthy controls and patients with renal disease (fig 1A and 1B).

In the healthy controls the combined infusion of metoclopramide and dopamine resulted in a significantly blunted response of ERPF and FF to dopamine as compared with the infusion of dopamine alone (fig 1A). A significant difference between both studies was found at the 0.5 and 2 μg/kg/min dose for ERPF and FF. On the other hand, in the patients with renal disease neither the response of ERPF and FF to dopamine, nor that of the GFR did differ between the study with and the one without metoclopramide (fig 1B).

Effects on sodium excretion and diuresis

Base-line ($UNa^+.V$) and diuresis (U.V, ml/h) were higher in the healthy controls than in the patients. No difference was found in base-line $FENa^+$% between both groups. Within the patient group, however, a significant negative correlation was present between base-line $FENa^+$% and base-line ERPF ($r = -0.796$; $p < 0.005$).

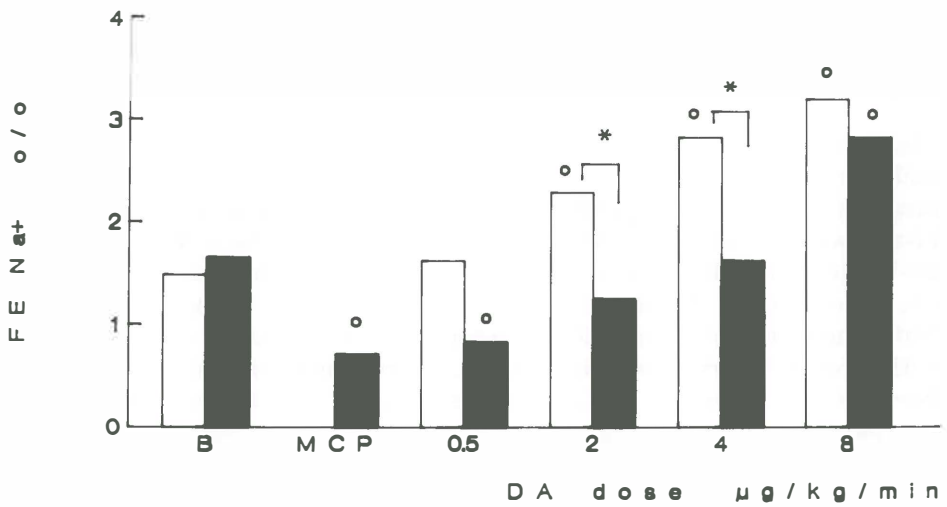


Figure 2A: Dose-response curves for fractional sodium excretion ($FENa^+$%) during infusion of dopamine without and with metoclopramide in healthy volunteers (n = 8). Median values are shown. Open bars represent the values of the renal function study without metoclopramide, closed bars those of the study with metoclopramide.
MCP = during metoclopramide infusion alone.
* = significant difference between studies without and with metoclopramide ($p < 0.05$).
o = significantly different from base-line value ($p < 0.05$).

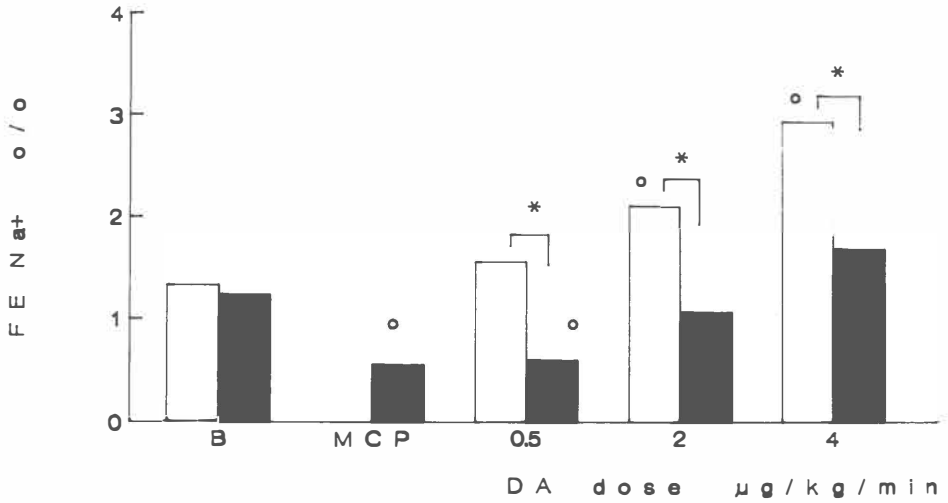


Figure 2B: Dose-response curves for fractional sodium excretion (FENa+%) during infusion of dopamine without and with metoclopramide in patients with renal disease (n = 12). Median values are shown. Open bars represent the values of the renal function study without metoclopramide, closed bars those of the study with metoclopramide. MCP = during metoclopramide infusion alone.
* = significant difference between studies without and with metoclopramide ($p < 0.05$).
o = significantly different from base-line value ($p < 0.05$).

Dopamine infusion alone resulted in an increase in $UNa^+.V$ and $FENa^+\%$ in both healthy controls and patients at doses of 2 µg/kg/min and higher (Table 1, fig 2). The increase in $UNa^+.V$ was comparable in both groups. A pronounced fall in $UNa^+.V$ and $FENa^+\%$ was observed during infusion of metoclopramide. $FENa^+\%$ fell from 1.52 at base-line to 0.71 during metoclopramide in the healthy volunteers and from 1.23 to 0.56, respectively, in the patients ($p < 0.01$). In the patients the percentage fall in $UNa^+.V$ during metoclopramide showed a correlation with base-line GFR which may be described as follows: the ratio of $UNa^+.V$ during metoclopramide infusion and $UNa^+.V$ at base-line = 86.5 - 68.26 base-line GFR; $r = - 0.944$; $p < 0.0001$) (fig 3). Subsequent addition of dopamine led again to an increase in $UNa^+.V$ and $FENa^+\%$ compared to the level during metoclopramide infusion for doses of 2 µg/kg/min and higher in the patients (fig 2B), and for doses of 4 and 8 µg/kg/min in the healthy controls (fig 2A). Comparing both studies, no differences were present between base-line values of $UNa^+.V$ and $FENa^+\%$ but the natriuretic response during dopamine infusion was significantly less in the study with metoclopramide in both normals and in the patients (fig 2A and 2B). This difference was found for all doses in the patients and for the 2 and 4 µg/kg/min doses in the healthy controls (fig 2A-B).

The diuresis was stable in the hours before dopamine was given. Metoclopramide infusion had no effect on diuresis, the increase in diuresis during subsequent dopamine infusion tended to be more marked than in the study without metoclopramide, but this difference did not reach statistical significance.

Table 1: **Sodium excretion and diuresis during infusion of dopamine and metoclopramide.**
Natriuresis ($UNa^+.V$, mmoll/h), and diuresis (ml/h) before and during infusions of dopamine and metoclopramide (MCP) and during combined infusions in healthy controls (n = 8) and in patients with renal disease (n = 12). Medians values are given.
* = significant difference with base-line at a level of $p < 0.05$ or less.
# = significant difference with value during metoclopramide infusion at a level of $p < 0.05$ or less.

			(dopamine µg/kg/min)				
	base-line	MCP	0.25	0.5	2	4	8
Natriuresis (mmol/h):							
Healthy contr.							
-first study	14.5		15.8	13.7	16.7 *	22.1 *	43.2 *
-second study	16.3	10.5 *		9.7	8.9	17.9 #	29.4 #
Patients:							
-first study	6.3			7.0	12.7 *	17.4 *	
-second study	5.7	2.7 *		3.3 *	6.5 #	8.2 *#	
Diuresis (ml/h):							
Healthy contr.							
-first study	362		388	378	468 *	443 *	542 *
-second study	367	315		462	617 *#	575 *#	468
Patients							
-first study	189			232	333 *	493 *	
-second study	203	182		260	432 *#	500 *#	

Effects on the fractional excretion of ß-2-microglobulin and on tubular reabsorption of phosphate

The response of the fractional excretion of ß-2-microglobulin (FEß-2-MG%) was comparable to that of $FENa^+$% in the patients: dopamine infusion resulted in a rise of FEß-2-MG% from a base-line of 0.20 to 0.54 at 4 µg/kg/min. Metoclopramide infusion was accompanied by a fall of FEß-2-MG% from 0.198 to 0.130; FEß-2-MG% rose again after subsequent dopamine infusion to 0.335 at 4 µg/kg/min. In the healthy volunteers no significant changes in FEß-2-MG% were found.

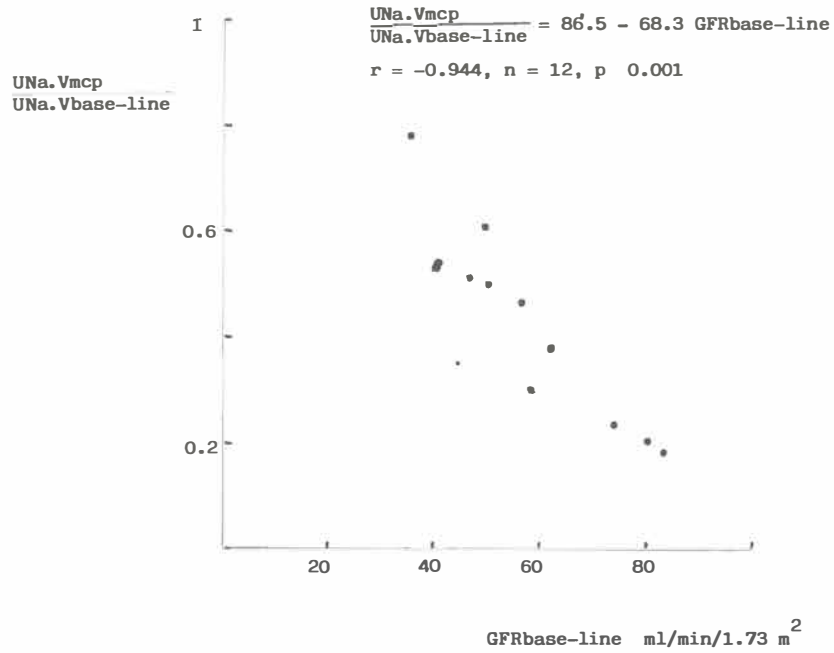


Figure 3: Correlation between the percentage fall in $UNa^+.V$ during metoclopramide infusion and base-line GFR.

Both in healthy volunteers and in the patients, dopamine infusion was accompanied by a fall in tubular reabsorption of phosphate (TRP) for all doses (in the healthy controls from a base-line of 0.92 to a nadir of 0.72 at 8 μg/kg/min; in the patients from 0.84 to 0.65 at 4 μg/kg/min). Metoclopramide infusion did not influence TRP, but attenuated the fall in TRP during subsequent dopamine infusion (in the healthy volunteers a fall from 0.91 to 0.77; in the patients from 0.87 to 0.70). No relation was found between the observed changes in TRP and those in diuresis.

Effects on blood pressure and pulse rate

In both healthy controls and patients there was a rise in systolic and a fall in diastolic blood pressure during infusion of higher doses of dopamine both in the study with and without metoclopramide. In the healthy controls base-line blood pressure was 124/83 mmHg in the study without metoclopramide and changed significantly at 8 μg/kg/min dopamine to 149/71 mmHg. In the study with metoclopramide base-line blood pressure was 123/82 mmHg and during metoclopramide alone 118/80 mmHg (no significant difference), and changed significantly again during the subsequent

dopamine infusion to 165/71 mmHg at 8 μg/kg/min. In the patients with renal disease base-line blood pressures amounted to 133/89 mmHg and 130/87 mmHg for the studies without and with metoclopramide, respectively and 129/85 mmHg during metoclopramide infusion alone, and changed significantly to 160/83 mmHg and 161/80 mmHg, respectively, at 4 μg/kg/min dopamine in both the study without and with metoclopramide. There were no differences in the blood pressure response to dopamine between the healthy controls and the patients with renal disease, nor in their responses between the first and the second study.

Effects on plasma aldosterone and renin

Infusion of dopamine in a dose of 2 μg/kg/min led to a fall in plasma aldosterone concentration (PAC) in both healthy controls and patients (Table 2). PAC was higher in the patients. There were no differences in the base-line PAC between the first and the second study in both groups. Infusion of metoclopramide gave a marked rise in PAC in both groups. During the combined infusion of metoclopramide and dopamine in a dose of 2 μg/kg/min, the PAC fell again; however, only significantly so in the patients. A negative correlation was present between base-line values of PAC and $FENa^+$% ($r = -0.726$; $p < 0.02$ in the patients). No correlation could be established between base-line PAC and serum-potassium, which was also comparable in both groups. In the patients there was also a correlation between the percentage rise in PAC during metoclopramide infusion compared to base-line, and the base-line $FENa^+$% ($r = -0.776$; $p < 0.01$). Infusion of dopamine or metoclopramide alone had no effect on PRA in both groups; the combined infusion of metoclopramide and dopamine led to an increase of the PRA in the patients with renal disease (Table 2).

In the healthy controls the metoclopramide serum levels became stable after the second hour of metoclopramide infusion (after the first hour of infusion a serum level of 101 μg/l; after 2, 3 and 5 hours levels of 181, 212 and 166 μg/l, respectively). In the patients an increase in serum levels was observed during prolonged infusion (metoclopramide serum levels of 76.5, 120, 135 and 167.5 μg/l after 1, 2, 3 and 5 hours, respectively). The differences in the levels between patients and healthy controls were not significant.

Table 2: **Response of PRA and PAC to infusions of dopamine and metoclopramide.** PRA and plama aldosterone concentration in healthy controls and in patients with renal disease. Blood was collected at base-line and during infusion of dopamine 2 μg/kg/min in both studies and during infusion of metoclopramide alone. Median values are given.
* = significant difference compared with base-line, at a level of at least $p < 0.05$.
+ = significant difference compared with metoclopramide alone, $p < 0.05$.

Plasma aldosterone concentrations (nmol/l)

	First study		Second study		
	base-line	dopamine 2 μg/kg/min	base-line	metoclo-pramide	dopamine 2 μg/kg/min
Normals	0.55	0.41*	0.39	0.86*	0.65
Patients	0.90	0.48*	0.90	0.98*	0.89+

Plasma renin activity (nmol A1/l/h)

	First study		Second study		
	base-line	dopamine 2 μg/kg/min	base-line	metoclo-pramide	dopamine 2 μg/kg/min
Normals	1.2	3.0	1.9	2.1	1.8
Patients	1.6	3.4	1.9	2.5	7.4*

DISCUSSION

In animal studies of dopamine-induced renal vasodilation, which is often used to characterise peripheral dopamine receptor interactions, evidence has accumulated that metoclopramide acts as a dopamine antagonist [8-10]. Our study confirms this in man. Metoclopramide infusion resulted in a shift of the dopamine dose-response curve for the ERPF and FF in healthy volunteers. Hahn et al showed, after earlier studies of Day et al in anaesthetized cats and of Goldberg et al with intra-arterial injections of metoclopramide in anaesthetized dogs, that metoclopramide, 1 and 10 mg/kg i.v., resulted in dose-related inhibition of the renal vasodilatory activity to dopamine in dogs [8-10]. Recently, Israel et al reported that administration of metoclopramide in high doses (1 to 2.5 mg/kg) resulted in a decrease of renal plasma flow in patients during intravenous hydration before chemotherapy. They speculated about a role for endogenous dopamine in the maintenance of renal plasma flow in these patients [6]. Our results do not indicate a role for endogenous dopamine secretion in maintaining renal plasma flow in hydrated healthy controls as no fall in ERPF occurred during

metoclopramide infusion. Although the metoclopramide dose in our study was substantially lower than in the study of Israel and coworkers, the shift of the dopamine dose-response curve for the ERPF and FF during metoclopramide shows that our dose was sufficient for demonstrating antagonism of dopamine-induced renal vasodilation.

In earlier studies and in chapter 3, we found an impaired response of ERPF and FF to dopamine infusion in patients with renal disease compared with healthy controls, even if base-line GFR of the patients was in the normal range [5]. Moreover, within the patient group a correlation existed between base-line GFR and the response of ERPF and FF to dopamine infusion. The results of the present study confirm this impaired response in patients and show the same trend for the above-mentioned correlation.

An increase in endogenous dopamine generation in the patients with renal disease is one of the possible mechanisms to explain the impaired response to exogenous dopamine. However, for renal haemodynamics the results in this study are not compatible with increased endogenous dopamine generation in patients, as no fall in ERPF was found during metoclopramide infusion. On the other hand the findings were different in relation to sodium excretion. The marked fall in $UNa^+.V$ and $FENa^+\%$ during metoclopramide infusion alone in both healthy controls and patients with renal disease indeed indicates a role for endogenous dopamine in mediating natriuresis. The concordance between urinary sodium and dopamine excretion is well known [4 11]. An increase in natriuresis during volume expansion is accompanied by a rise in urinary dopamine excretion [12]. In a later study Krishna et al concluded that dopamine might have a causal role in the increase in natriuresis during volume expansion in normal volunteers, as they found that metoclopramide in a dose of 10 mg/h attenuated this increase [7]. The fall in natriuresis during metoclopramide infusion and its blunting effect on the natriuresis induced by subsequent dopamine infusion in our study confirm and extend their conclusions.

The marked changes in natriuresis and $FENa^+\%$ during infusion of metoclopramide alone without concomitant changes in renal haemodynamics in both groups, argue against the assumption that the effects of dopamine on natriuresis are haemodynamically mediated. The differences in renal haemodynamic response to infusions of dopamine and metoclopramide between both groups, which are not accompanied by any differences in the response of sodium excretion, further underline this.

Dopamine has been shown to have direct tubular effects on fluid reabsorption, which can be prevented by dopamine antagonists like haloperidol and metoclopramide [13]. Aperia et al demonstrated in vitro direct inhibition by dopamine of NA/K-ATP-ase in proximal tubules and proposed dopamine as an intrarenal natriuretic hormone [14]. The observed changes in FEß-2-MG% in the patients, and in the TRP in both normals and patients, also may indicate a direct proximal tubular effect of dopamine. The re-absorption of phosphate and ß-2-microglobulin occur mainly or exclusively in the proximal renal tubule [15 16].

However, the influence of dopamine and dopamine antagonists on aldosterone secretion deserves attention in a discussion of the mechanisms by which dopamine

stimulates natriuresis. Dopamine is well known as a modulator of aldosterone release. It has been described to exercise a tonic inhibitory influence on aldosterone secretion [17 18]. Metoclopramide stimulates aldosterone secretion, independently of the renin-angiotensin system [19-21]. In our study infusion of dopamine 2 μg/kg/min led to a fall in PAC, and metoclopramide raised PAC as expected, while no changes in PRA occurred. We cannot explain why an increase in PRA occurred in the patients during the combined infusion of metoclopramide and dopamine. The changes in PAC may at least partly explain the observed changes in natriuresis during metoclopramide and dopamine infusions. The positive correlation between the percentage rise in aldosterone during metoclopramide infusion and base-line $FENa^+$% perhaps also reflects the influence of endogenous renal dopamine on aldosterone secretion: high levels of endogenous renal dopamine, as associated with a high $FENa^+$%, result in progressive inhibition of aldosterone, which can be unmasked by metoclopramide.

The effect of dopamine on aldosterone seems to be mediated via DA_2 dopamine receptors [22 23]. On the other hand dopamine-induced renal vasodilation is considered to be mainly the result of DA_1 dopamine receptor stimulation, although the inhibitory influence of DA_2 dopamine receptors on norepinephrine release may contribute to a vasodilatory response in animals or subjects which, like our volunteers and patients, are not pretreated with an alpha-blocker [24]. The weak effect of metoclopramide on the dopamine-induced renal vasodilation may reflect antagonism at DA_1 dopamine receptors. It is not clear to what extent the more pronounced antinatriuresis during metoclopramide depends on DA_2 receptor antagonism. Studies with selective dopamine receptor agonists and antagonists may shed more light on the contribution of both types of DA receptors to sodium excretion.

Dopamine antagonists are frequently used, often in high doses, as anti-emetics in chemotherapy. The effects of metoclopramide and possibly other dopamine antagonists on tubular function have to be taken into account in studies which evaluate the renal tubular effects of cytostatics like cisplatinum.

In conclusion our study shows that metoclopramide acts as a dopamine antagonist in the human kidney. We did not find evidence that an increased endogenous dopamine generation has a role in maintaining renal plasma flow in hydrated healthy controls or patients with renal disease and moderately impaired renal function. However, endogenous dopamine probably is important in mediating natriuresis. The effects of dopamine on natriuresis were not found to depend on changes in renal haemodynamics. The influence of dopamine on aldosterone secretion may be important for its natriuretic effects.

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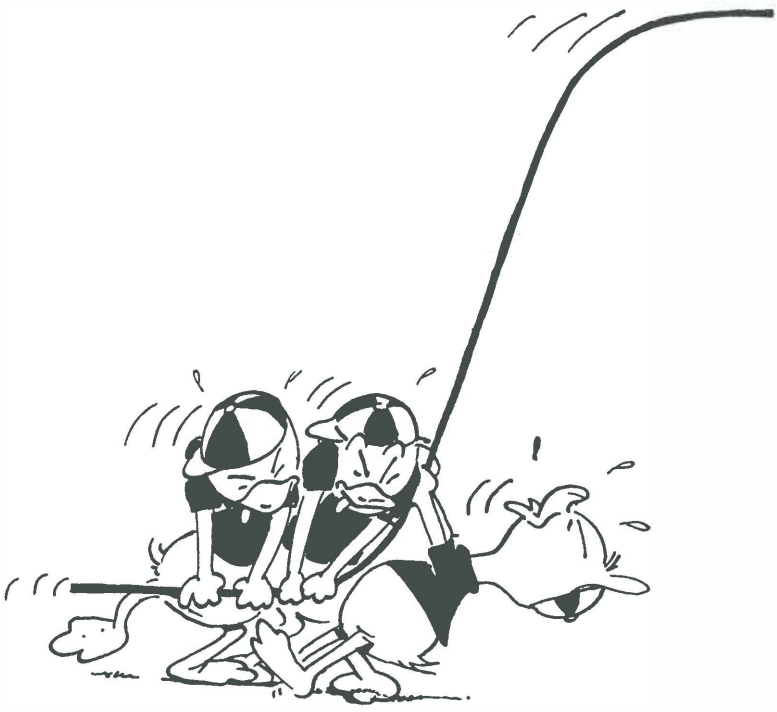
CHAPTER 5

DISSOCIATION OF RENAL VASODILATORY AND NATRIURETIC EFFECTS OF DOPAMINE DURING SULPIRIDE INFUSION IN NORMAL MAN



CHAPTER 5

DISSOCIATION OF RENAL VASODILATORY AND NATRIURETIC EFFECTS OF DOPAMINE DURING SULPIRIDE INFUSION IN NORMAL MAN

ABSTRACT

In 7 normal volunteers dopamine infusions in doses ranging from 0.25 to 8 μg/kg/min resulted in a dose-dependent increase in ERPF and GFR, and a fall in FF. An increase in natriuresis ($UNa^+.V$) and fractional sodium excretion ($FENa^+$%) was found for doses of 2 μg/kg/min dopamine and higher. Sulpiride (200 mg intravenously) had no effect on base-line ERPF and GFR, and did not influence dopamine-induced renal vasodilation in these volunteers. Sulpiride infusion caused a fall in $UNa^+.V$ (from 19.6 to 14 mmol/h) and $FENa^+$% (from 1.7 to 1.38), and shifted the dose-response curve for the natriuretic response to subsequent dopamine infusion. Sulpiride enhanced the fall in diastolic blood pressure during infusion of dopamine.

We conclude that the effects of sulpiride on base-line and dopamine-induced natriuresis in man do not depend on renal haemodynamics and may be the consequence of direct proximal tubular effects of dopamine.

INTRODUCTION

In earlier studies and in chapter 3, we observed a dose-dependent increase in ERPF and GFR during infusions of dopamine in normal volunteers [1 2]. These effects were less pronounced in patients with renal disease in whom the increase in ERPF correlated with base-line renal function. We forwarded the hypothesis that increased endogenous renal dopamine secretion might play a role in the impaired response to exogenous dopamine in patients with renal disease. If this hypothesis is valid, administration of a suitable dopamine antagonist may be expected to result in a fall in renal function in these patients. In dogs, Goldberg and coworkers showed strong antagonist effects of sulpiride on dopamine-induced changes in renal function [3]. Comparable data in humans are lacking. Therefore, we performed renal function studies before and during infusions of various doses of dopamine in normal volunteers, and in some patients with renal disease and moderately impaired renal function. The studies were repeated during intravenous administration of sulpiride.

PATIENTS AND METHODS

Seven healthy volunteers, 5 male and 2 female, were investigated. Their ages ranged from 25 to 50 years. Urinary sodium excretion averaged 129 mmol/24 h (range 64-219 mmol/24 h). GFR was > 95 ml/min in all of them (ranges of GFR and ERPF 99-125 ml/min and 336-529 ml/min, respectively).

Moreover three patients with renal disease were investigated: a 51 year-old female with focal glomerulosclerosis and a GFR of 58 ml/min, a male of 41 years with membranoproliferative glomerulonephritis and a GFR of 38 ml/min, and a 20-year old male with a GFR of 102 ml/min after an acute post-streptococcal glomerulonephritis. Medication in these patients was withdrawn at least 4 days before the studies, in the case of beta-blockers at least one week before. Urinary sodium excretion was 73, 178 and 39 mmol/24 h, respectively. Diastolic blood pressure did not exceed 100 mmHg before or during the studies.

Two renal function studies were performed. After determining base-line values dopamine was given in doses of 0.25, 0.5, 1, 2, 4 and 8 μg/kg/min, each dose during one hour. In the second renal function study, after again determining base-line values, sulpiride (Dogmatil®, a racemic mixture of R- and S-sulpiride) was given by intravenous infusion: 100 mg was given in the first 30 minutes, followed by an infusion of 30 mg/h during the rest of the study. Dopamine infusion was started two hours after the sulpiride infusion and was given in the doses mentioned above.

Serum sulpiride levels were determined at 1, 2, 3 and 6 hours after the start of the sulpiride infusion.

For the presentation of the data of the three patients with renal disease mean values or percentage changes have been used.

For other details of the methods the reader is referred to chapter 2.

RESULTS

Effects on effective renal plasma flow, glomerular filtration rate and filtration fraction

In the normal volunteers dopamine infusion resulted in an increase of both ERPF and GFR for all doses studied. ERPF rose from a base-line value of 419 (median, range 336-529) to a maximum of 655 (range 415-751) ml/min at 2 μg/kg/min (fig 1B). GFR rose from a base-line value of 110 (range 99-125) to a maximum of 127 (range 120-144) ml/min at 0.5 μg/kg/min (fig 1A). FF fell from a base-line value of 0.255 (range 0.205-0.31) to a nadir of 0.196 (range 0.18-0.28) at 4 μg/kg/min (fig 1C).

Infusion of sulpiride in the second renal function study had no significant effect on base-line GFR (109 ml/min with a range from 96 to 140 at base-line and 117 ml/min with a range from 105 to 131 during sulpiride infusion) or ERPF (418 ml/min with a range from 378 to 578, and 449 ml/min with a range from 382 to 544, respectively)(fig 1A-B). FF did not change either with a base-line value of 0.25 (range 0.24-0.32) and 0.26 (range 0.22-0.33) during sulpiride infusion (fig 1C). Sulpiride

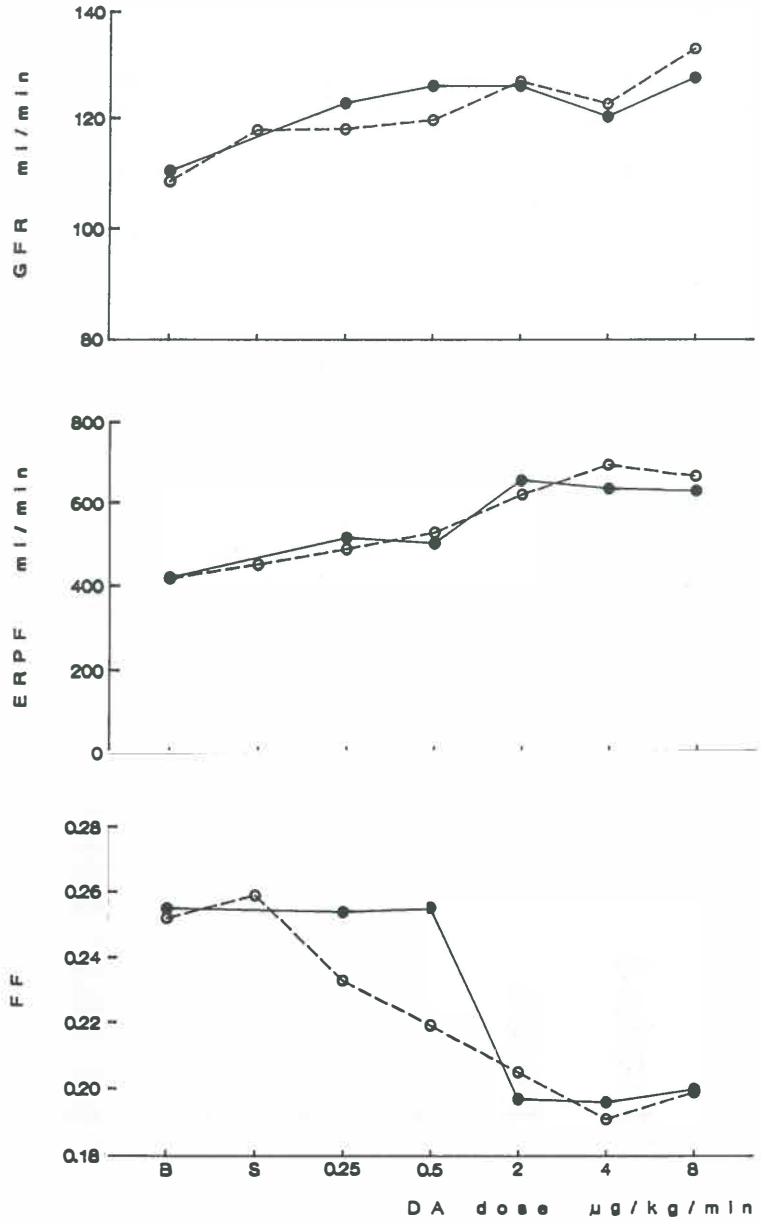


Figure 1A-C: Dose-response curves for GFR (fig 1A), ERPF (fig 1B) and FF (fig 1C) during infusion of dopamine.
B = base-line. S = during infusion of sulpiride alone. Median values are shown.
—— before and during infusion of dopamine alone.
------ before and during infusion of sulpiride and subsequent infusion of dopamine.

caused no shift of the dose-response curve for the dopamine-induced renal vasodilation in either group of normals.

In the patients with renal disease the mean maximal increase in ERPF during dopamine infusion was 29 %, and that of GFR 9 %. Sulpiride had no influence on ERPF or GFR before dopamine infusion (mean ERPF before sulpiride infusion 332 and during sulpiride infusion 330 ml/min, mean GFR being 67 and 65 ml/min, respectively), and did not inhibit the rise in GFR and ERPF during dopamine infusion (mean maximal increase of ERPF and GFR 24 and 11 %, respectively).

Effects on sodium excretion and diuresis

Dopamine infusion resulted in a significant increase in $UNa^+.V$ and $FENa^+\%$ for doses of 2 µg/kg/min and higher: base-line $UNa^+.V$ amounted to 12 mmol /h (range 7.7-31.6) and rose to a maximum of 35 mmol /h (range 28-56) at 8 µg/kg/min. For the $FENa^+\%$ a base-line value of 1.29 (range 0.81-3.46) was established with a maximum of 3.29 (range 2.39-4.83) at 8 µg/kg/min dopamine (fig 2). In the second study sulpiride infusion was associated with a slight but significant fall in both $UNa^+.V$ and $FENa^+\%$: $UNa^+.V$ fell from a base-line value of 19.6 mmol/h (range 8.6-29.9) to 14 mmol/h (range 6.2-28.6) during sulpiride infusion, and $FENa^+\%$ from 1.7 (range 0.72-3.6) to 1.38 (range 0.57-2.8). Sulpiride infusion also significantly impaired the natriuretic

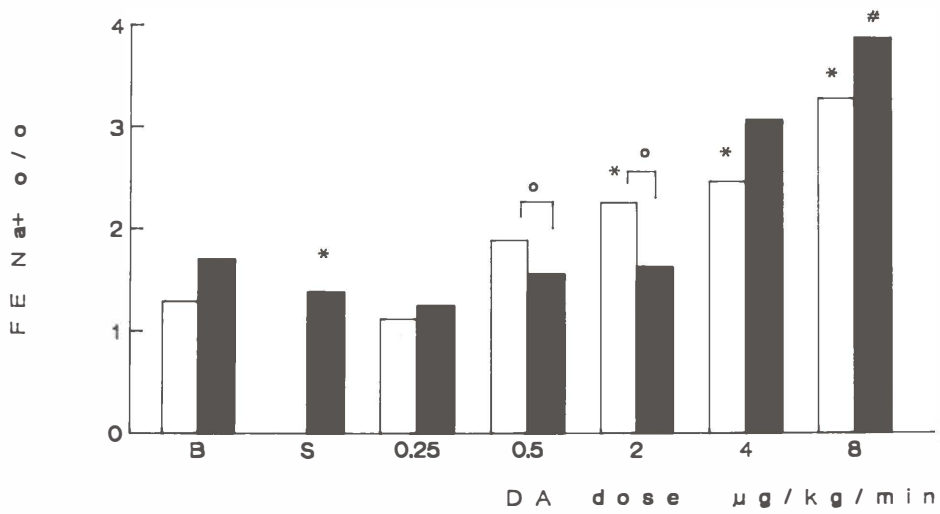


Figure 2: Dose-response curves for fractional sodium excretion ($FENa^+\%$)) during infusion of dopamine in healthy volunteers.
Median values are shown. Open bars represent the values of the renal function study without sulpiride, the closed bars those of the study with sulpiride.
* = significantly different from base-line value or significant difference between studies without and with sulpiride, $p < 0.05$.

response to subsequent dopamine infusion. Testing differences between the study without and with sulpiride for specific doses of dopamine limited significance to the 2 μg/kg/min dose (fig 2).
An increase in diuresis was observed during infusion of dopamine (from a base-line value of 510 (range 238-625) to 775 (range 505-915) ml/h at 2 μg/kg/min dopamine). Sulpiride infusion was accompanied by a fall in diuresis (from 535 ml/h base-line to 295 ml/h), while maximum diuresis during subsequent dopamine infusion now was reached at 4 μg/kg/min (780 ml/h).
In the patients with renal disease sodium excretion and diuresis showed the same trends as in the normals during dopamine and sulpiride infusion (sodium excretion rose from a mean base-line value of 9.4 to 22.2 mmol/h at 4 μg/kg/min dopamine; during sulpiride infusion in the second study $UNa^{+}.V$ fell from 6.8 to 6.1 mmol/h with a rise to 14.4 mmol/h during subsequent dopamine infusion at a dose of 4 μg/kg/min).

Effects on potassium, calcium and ß-2-microglobulin excretion

Dopamine infusion alone did not lead to significant changes in potassium excretion. Sulpiride infusion alone resulted in significantly increased potassium excretion from 8.6 (range 1.4-11.3) to 14.1 (range 8-30.3) mmol/h, with a fall to 3.7 mmol/h (range 2.2-8.1) during subsequent dopamine infusion at the 8 μg/kg/min dose.

An increase in calciuresis ($UCa^{++}.V$) was found for dopamine doses of 2 μg/kg/min and higher with a base-line $UCa^{++}.V$ of 0.317 mmol/h (range 0.04-0.57), rising to 0.655 (range 0.44-0.98) at 8 μg/kg/min. Sulpiride infusion did not change $UCa^{++}.V$ but abolished the increase in $UCa^{++}.V$ during subsequent dopamine infusion (base-line of 0.46 mmol/h, during sulpiride 0.46, maximum during dopamine infusion 0.45).

A significant increase in ß-2-microglobulin excretion (Uß-2-MG.V) was found during infusion of dopamine in doses of 4 and 8 μg/kg/min (base-line 5.2 μg/h, and during dopamine 8 μg/kg/min 12.1 μg/h). Sulpiride infusion had no significant influence on Uß-2-MG.V.

Effects on blood pressure and pulse rate

No changes in blood pressure or pulse rate were observed during dopamine infusion up to a dose of 4 μg/kg/min except for a slight fall in diastolic blood pressure at 2 μg/kg/min dopamine. A rise in systolic and a fall in diastolic blood pressure occurred at 8 μg/kg/min dopamine (Table). Sulpiride did not influence base-line blood pressure or pulse rate. Sulpiride significantly enhanced the fall in diastolic blood pressure during dopamine infusion (Table). In the patients with renal disease dopamine infusion also resulted in a rise in systolic and a fall in diastolic blood pressure for the 4 μg/kg/min dose. Sulpiride did not have any additional effects on the blood pressure in these patients.

Serum sulpiride level in the healthy volunteers was 1.0 mg/l (range 0.6-1.85) one hour after the start of the sulpiride infusion; values at 2, 3 and 6 hours after the start of the

sulpiride infusion were 0.6 (range 0.45-1.28), 0.6 (range 0.22-1.10) and 0.4 (range 0.14-0.96) mg/l, respectively.

Table: **Blood pressure (mmHg) and pulse rate (beats/min) in healthy volunteers (n = 7) before and during dopamine infusion in renal function studies without and with sulpiride.** Median values and ranges are given.
* = significantly different from base-line values ($p < 0.05$).
o = significantly different from first study without sulpiride ($p < 0.05$).

	First study			Second study		
	systolic	diastolic	pulse rate	systolic	diastolic	pulse rate
Base-line	130 (114-134)	84 (76-91)	72 (60-80)	118 (108-132)	78 (76-86)	68 (54-84)
Sulpiride				124 (110-132)	80 (64-90)	76 (60-88)
Dopamine dose µg/kg/min						
0.25	132 (116-138)	78 (68-86)	72 (60-80)	114 (108-128)	78 (72-84)	72 (56-88)
0.5	124 (112-140)	80 (75-88)	72 (60-84)	112o (104-128)	74*o (70-78)	68 (54-84)
1	128 (110-132)	78 (70-88)	72 (64-84)	115 (104-128)	75*o (64-80)	68 (51-92)
2	128 (106-134)	76* (75-85)	76 (64-84)	115 (106-132)	72* (65-84)	72 (48-84)
4	120 (108-144)	78 (68-84)	72 (60-84)	126 (104-138)	76* (64-80)	72 (51-80)
8	148* (118-164)	74* (62-82)	68 (63-80)	166* (138-178)	70*o (56-80)	60 (56-76)

DISCUSSION

In our study sulpiride had neither influence on base-line renal haemodynamics nor on dopamine-induced renal vasodilation. In the studies of Goldberg et al, comparing various dopamine antagonists in the dog, sulpiride was shown to be one of the most potent inhibitors of dopamine-induced renal vasodilation [3]. The results of Bass and Robie support those of Goldberg and coworkers [4]. Schmidt and Imbs also found

sulpiride to have antagonist activity, albeit weak, to dopamine-induced vasodilation in isolated rat kidneys [5]. The dose of sulpiride in our study, leading to serum levels considered to be therapeutical in clinical practice, was lower than in most of the animal studies [6]. However, the effect of sulpiride on sodium excretion in our study argues against the assumption that the dose was anyhow too low to demonstrate dopamine antagonism. Although in some studies the R-enantiomer of sulpiride was found to be more potent in inhibiting dopamine-induced renal vasodilation, the results of Bass et al and Goldberg et al demonstrate that our use of a racemic mixture should not have masked its antagonist activity [3-5 7].

Pretreatment with an alpha-blocker, usually phenoxybenzamine, was used in all the studies discussed above. This is the usual method to eliminate the vasoconstrictory effects of alpha-adrenoceptor stimulation by dopamine. In our study no pretreatment was used. Besides its dopamine antagonist activity, sulpiride has been described to have weak alpha antagonist activity in vitro in rabbits and in vivo in dogs [8]. An alpha-blocking effect of sulpiride would enhance, and dopamine receptor blockade by sulpiride inhibit, a renal vasodilatory response to dopamine. Alpha-antagonist activity of sulpiride may therefore have obscured its dopamine antagonist activity on the renal vasculature. The observed slight fall in diastolic blood pressure during sulpiride infusion and the enhancement of the fall in diastolic blood pressure during subsequent dopamine infusion may also have been due to alpha-antagonist effects of sulpiride.

Dopamine is known to be a potent natriuretic agent [9 10]. Dopamine antagonists have often been used to characterize the natriuretic response to dopamine [11-13]. In several animal models sulpiride was shown to be a potent antagonist of dopamine-induced effects in the kidney [3-5 7 14]. We therefore conclude that the antinatriuretic effect of sulpiride in our study reflects dopamine antagonism in the human kidney. The fall in natriuresis during infusion of sulpiride alone may be the consequence of antagonism of dopamine generated endogenously in the kidney.

It is unclear how and where the effects of dopamine and sulpiride on urinary sodium excretion take place. The increase in sodium excretion during dopamine infusion has often been ascribed to changes in renal haemodynamics [9 15]. The effect of sulpiride on natriuresis without any accompanying change in renal haemodynamics in the present study argue against this assumption. In chapter 3 we found an impaired response of ERPF and FF to dopamine in patients with renal disease compared to normals, while the natriuretic response to dopamine did not differ between both groups. In chapter 4 a fall in natriuresis was observed during infusion of the dopamine antagonist metoclopramide without any change in renal haemodynamics. We therefore conclude that changes in renal haemodynamics seem to be of minor importance in mediating the natriuretic response to dopamine.

Dopamine infusion results in a fall while dopamine antagonists result in a rise in plasma aldosterone concentration (PAC) [16 17]. These changes seem to occur independently of the renin-angiotensin system and may be the result of a direct tonic inhibitory influence by dopamine on aldosterone secretion [18 19]. As we did not measure PAC in this study, it is impossible for us to draw firm conclusions on the role of aldosterone in

dopamine-induced sodium excretion. However, the increase in potassium excretion associated with a fall in sodium excretion during sulpiride infusion is compatible with a rise in PAC and, therefore with an indirect distal tubular effect of dopamine. Beta-2-microglobulin reabsorption takes place almost exclusively in the proximal tubule and its excretion is considered to reflect proximal tubular function [20 21]. The increase in ß-2-Microglobulin excretion during dopamine infusion may indicate that dopamine influences proximal tubular function. This influence probably extends to sodium reabsorption. The observed changes in calciuresis are also compatible with a proximal tubular effect of dopamine. Evidence for direct proximal tubular effects of dopamine furthermore has been collected in in vitro studies [22-24].

Dopamine-induced renal vasodilation is ascribed to stimulation of postsynaptic DA_1 dopamine receptors, although inhibition of norepinephrine release by stimulation of presynaptic DA_2 dopamine receptors may contribute to the vasodilatory response. On the other hand the effects of dopamine on aldosterone release seem to be mediated by DA_2 receptors [25 26]. DA_1 agonists like fenoldopam have been shown to exert direct proximal tubular effects [27]. It is not clear whether DA_2 agonists or antagonists also influence dopamine dependent tubular effects. The discrepancy between the absence of effects of sulpiride on the renal vascular response to dopamine and the presence of antinatriuretic effects might reflect a predominance of DA_2 receptor antagonism for sulpiride in man. However, this would be in clear contrast to the results of the animal studies mentioned earlier, which showed sulpiride to be a potent antagonist of the postsynaptic vascular DA_1 dopamine receptor [4 8].

Studies using more selective dopamine agonists and antagonists may clarify the contribution of both types of dopamine receptors to the natriuretic effects of dopamine in man.

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CHAPTER 6

THE EFFECTS OF ALPHA-BLOCKADE ON DOPAMINE-INDUCED RENAL VASODILATION AND NATRIURESIS

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ABSTRACT

The effects of the selective postsynaptic alpha-1-adrenoceptor antagonist prazosin and the aselective alpha-blocker phentolamine on dopamine-induced renal vasodilation and natriuresis were studied, both in the absence and in the presence of the dopamine antagonist sulpiride, in normal volunteers. Dopamine dose-response curves were also performed without and with pretreatment with prazosin in normal volunteers, and in patients with renal disease and moderately impaired renal function.

In 7 volunteers pretreated with prazosin (1 mg p.o. each 4 hours) subsequent administration of sulpiride (100 mg i.v. in 30 min, thereafter 30 mg/h i.v.) did not influence base-line ERPF, GFR or FF or their response to dopamine infusion, but sodium excretion fell markedly ($FENa^+$% from 1.13 to 0.63).

Phentolamine (20 mg/hour i.v. in the first hour, followed by 10 mg/h i.v.) in 8 volunteers did not influence base-line values of ERPF, GFR, FF or sodium excretion, but completely abolished the natriuretic response to subsequent dopamine infusion. Administration of sulpiride (100 mg i.v. in 30 min, thereafter 30 mg/h i.v.) after phentolamine pretreatment resulted in a fall in sodium excretion without affecting ERPF or FF, and did not shift the dose-response curves for subsequent DA infusion.

Pretreatment with prazosin (1 mg p.o. each 4 hours) in 7 volunteers did not significantly affect base-line values but impaired the response of ERPF, FF and sodium excretion to infusions of DA in doses ranging from 0.5 to 8 μg/kg/min.

In 7 patients with renal disease and a GFR ranging from 38-85 ml/min, pretreatment with prazosin did not affect base-line ERPF, GFR or FF or their response to dopamine infusion, but sodium excretion and its reponse to dopamine infusion were reduced ($FENa^+$% at base-line 1.78 without, and 0.89 with prazosin pretreatment).

We conclude that the lack of effect of sulpiride on dopamine-induced renal vasodilation in man cannot be ascribed to its possibe alpha-antagonist activity. Both selective and aselective alpha-blockade abolish dopamine-induced natriuresis Prazosin also impairs the renal vasodilatory action of dopamine. Possible mechanisms are discussed.

INTRODUCTION

Dopamine, besides being a precursor of norepinephrine, is well known to stimulate adrenergic receptors on its own account [1 2]. The fact that dopamine possesses its own receptors was obscured in early studies by the overriding adrenergic activity of high

doses of dopamine. To abolish any possible adrenergic influences of dopamine agonists and antagonists in experimental structure-activity studies of such drugs, pretreatment with an alpha- and to a lesser degree beta-blocker is frequently used. In such studies in dogs, sulpiride was found to be a potent antagonist of dopamine-induced renal vasodilation [3]. In our study described in chapter 5 sulpiride was found to lack any effect on dopamine-induced changes in ERPF and FF in normal man. Sulpiride has been described to have alpha-antagonist activity [4]. Whereas a dopamine antagonist would be expected to oppose dopamine-induced renal vasodilation, an alpha-antagonist might, on the contrary, block a renal vasoconstrictory response to alpha-adrenergic stimulation by dopamine. It is conceivable that sulpiride with, albeit weak, alpha-blocking activity would fail to shift a dopamine dose-response curve for renal vasodilation when no pretreatment with an alpha-blocker is given.

In the present study the dopamine dose-response curves in healthy volunteers with and without sulpiride have therefore been repeated after pretreatment with prazosin as a selective postsynaptic alpha-blocker, and with phentolamine as an aselective alpha-adrenergic antagonist.

The results of these studies redirected our attention to the effects of alpha-blockade on the relation between dopamine-induced renal vasodilation and natriuresis. Dopamine dose-response curves during prazosin pretreatment were also compared to those without pretreatment in healthy volunteers and in patients with renal disease.

PATIENTS AND METHODS

The study consisted of several parts. All studies except those mentioned in the third part were done in normal volunteers. All volunteers were asked to adhere to a 150 mmol sodium diet for at least 4 days before the studies. GFR was > 100 ml/min in all of them.

1- In the first part, the effect of pretreatment with prazosin on dopamine dose-response curves with and without sulpiride was investigated in 7 healthy volunteers. There were 4 males and 3 females, ages ranged from 21 to 50 years with a median of 22 years and sodium excretion was 141 mmol/24 h (median, range 99-219 mmol/24 h). Half a milligram of prazosin was used orally late in the evening before the study while 1 mg orally was given at the start of the study and 4 and 8 hours later. After two base-line values had been obtained, dopamine (dopamine hydrochloride) infusion was started in the first study. Dopamine was given in doses of 0.25, 0.5, 2, 4 and 8 µg/kg/min, each dose during one hour. In the second renal function study, after determining base-line values, sulpiride (Dogmatil®, a racemic mixture of R- and S-sulpiride) was given by intravenous infusion: 100 mg was infused in the first 30 minutes, followed by an infusion of 30 mg/h during the rest of the study. Dopamine infusion was begun two hours after the start of the sulpiride infusion and was given in the doses mentioned above except the 0.25 µg/kg/min dose.

2- In the second part, 8 healthy volunteers received an infusion with phentolamine after determining base-line values and before subsequently performing dopamine dose-response curves with and without sulpiride. There were 7 males and the median age was 27 years, range 21-44 years. Sodium excretion was 166 mmol/24 h (median, range 81-224 mmol/24 h). After measuring two base-line levels, phentolamine was infused at a rate of 20 mg/h during the first hour and at a rate of 10 mg/h during the rest of the study. The subsequent dopamine dose-response curves in the absence and presence of sulpiride were performed as described above.

3- In the third part of the study dopamine dose-response curves were performed both without and with prazosin pretreatment in 7 volunteers and in 7 patients with renal disease and moderately impaired renal function. Of the volunteers 5 were male and their ages ranged from 21 to 50 years with a median of 29 years. Sodium excretion was 147 mmol/24 h (median, range 53-219 mmol/24 h). For the patients with renal disease, the age range was from 24 to 51 years, the median was 45 years and there were 3 females and 4 males. Urinary sodium excretion ranged from 21 to 197 mmol/24 h with a median of 107 mmol/24 h. GFR ranged from 38 to 85 ml/min with a median of 68 ml/min. Diagnoses varied: in 3 patients IgA nephropathy, in 2 patients membranous glomerulopathy, and adult polycystic kidney disease and urolithiasis, respectively, in the two remaining patients. In the study with prazosin pretreatment, prazosin was administered as described above in the first part of the study.

Serum sulpiride levels were determined at 1, 2, 3, 5 and 7 hours after the start of the sulpiride infusion. Serum prazosin levels were obtained 4 hours after the first dose of 1 mg (just before the following dose), and one hour later.

For further details on the study procedures the reader is referred to chapter 2.

RESULTS

Part 1: Dopamine dose-response curves without and with sulpiride in healthy volunteers pretreated with prazosin

Dopamine infusion resulted in an increase of the ERPF for all doses compared to base-line in the 7 normal volunteers. ERPF rose from 498 (range 379-660) to a maximum of 780 (range 579-1082) ml/min at 8 µg/kg/min (fig 1B). GFR rose from a base-line value of 115 (range 90-137) ml/min to 130 (range 117-152) ml/min at 0.5 µg/kg/min (fig 1A). The rise in GFR was significant compared to base-line for all doses. FF fell significantly compared to base-line for all doses with a base-line value of 0.225 (range 0.20-0.29) and a minimum of 0.1625 (range 0.12-0.19) at 8 µg/kg/min (fig 1C).

Infusion of sulpiride in the second renal function study had no significant effect on base-line GFR (118 ml/min at base-line and 117.4 ml/min during sulpiride infusion), ERPF (458 and 484 ml/min, respectively) and FF (0.22 and 0.23, respectively).

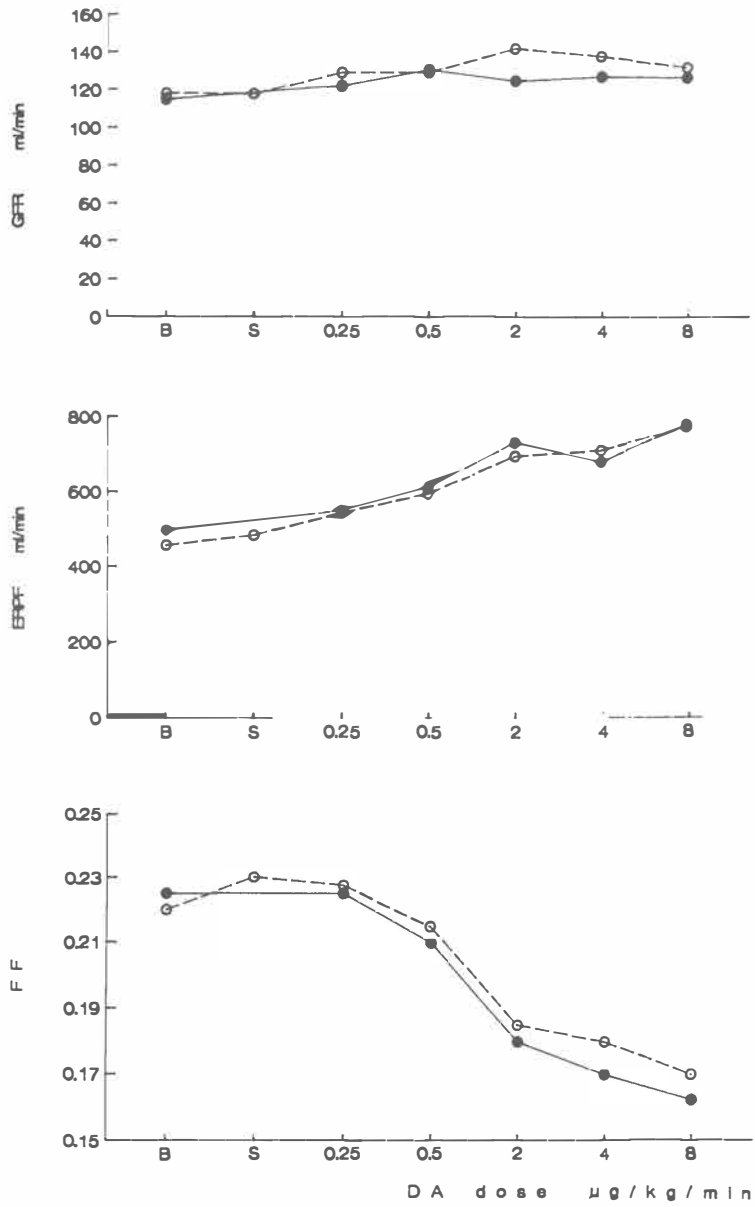


Figure 1A-C: Dose-response curves for GFR (ml/min) (fig 1A), ERPF (ml/min) (fig 1B) and FF (fig 1C) in 7 healthy volunteers pretreated with prazosin before and during infusion of dopamine or sulpiride, or the combined infusion of sulpiride and subsequently dopamine. B = base-line value. S = during infusion of sulpiride alone. Median values are shown.
The increase in ERPF and GFR and the fall in FF were significant compared to the base-line value for all doses of dopamine ($p < 0.05$).

Sulpiride caused no shift of the dose-response curve for the dopamine-induced renal vasodilation (fig 1).

Dopamine infusion did not significantly increase sodium excretion. Base-line value of $UNa^{+}.V$ amounted to 14.9 (range 4.3-26.5) mmol/h and remained at 16.6 (range 4-25.7)mmol/h at 8 µg/kg/min. For the $FENa^{+}$% no significant changes were observed either, with a base-line value of 1.39 (range 0.56-2.37) and 1.7 (range 0.39-2.58) at 8 µg/kg/min dopamine (fig 2). Sulpiride infusion was associated with a fall in both $UNa^{+}.V$ from a base-line value of 10.6 (range 2-20.9) mmol/h to 5.9 (range 1.6-13.1) mmol/h during sulpiride infusion and $FENa^{+}$% from 1.13 (range 0.2-2.1) to 0.63 (range 0.15-1.23). During subsequent dopamine infusion sodium excretion eventually returned to the base-line values.

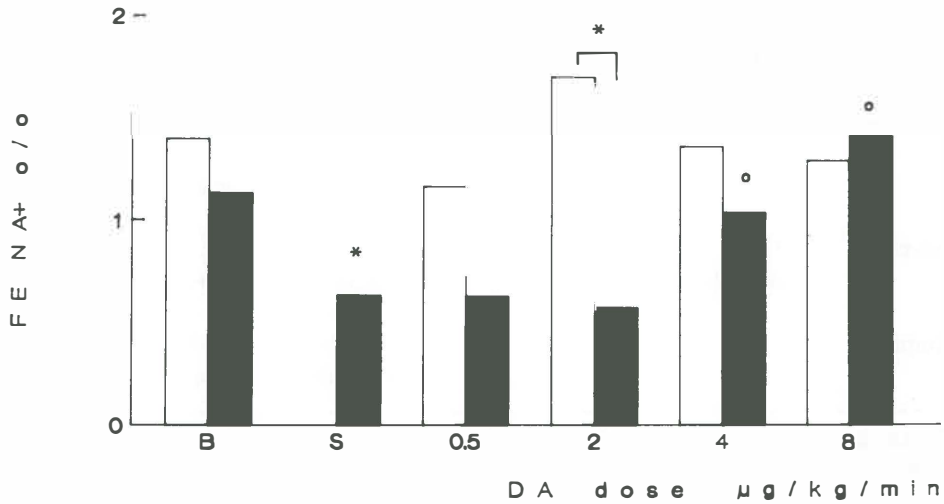


Figure 2: Dose-response bargraphs for fractional excretion of sodium ($FENa^{+}$%) in 7 healthy volunteers pretreated with prazosin before and during infusion of dopamine or sulpiride, or the combined infusion of sulpiride and subsequently dopamine. B = base-line value. S = during infusion of sulpiride alone. Median values are shown.
* = significantly different from base-line value or significant difference between both studies ($p < 0.05$).
o = significant different from values during infusion of sulpiride alone ($p < 0.05$).

Dopamine infusion alone did not lead to significant changes in potassium excretion. Sulpiride infusion alone resulted in a significantly increased potassium excretion from 5.9 (range 2.9-15) to 8.3 (range 4.8-14.5) mmol/h.

Dopamine infusion did not result in changes in calcium excretion ($UCa^{++}.V$). Sulpiride caused a fall in $UCa^{++}.V$ from 0.42 (range 0.26-0.72) to 0.30 (range 0.22-0.44) mmol/h. After subsequent dopamine infusion $UCa^{++}.V$ returned to base-line values. No significant changes were observed for the urinary excretion or the fractional excretion of γ-glutamyltransferase or ß-2-microglobulin.

No changes in blood pressure or pulse rate were observed during dopamine infusion up to a dose of 4 μg/kg/min. A rise in systolic and a fall in diastolic blood pressure occurred at 8 μg/kg/min dopamine (Table 1). Sulpiride caused a slight increase in pulse rate. It did not influence base-line blood pressure or its response to dopamine.

Table 1: **Blood pressure (mmHg) and pulse rate (beats/min) in 7 healthy volunteers pretreated with prazosin (n = 7) in renal function studies without (first study) and with (second study) sulpiride (100 mg in the first 30 minutes; 30 mg/h during the rest of the study) before and during dopamine infusion (in doses ranging from 0.25 to 8 μg/kg/min).**
Medians and ranges are represented.
* = significantly different from base-line values ($p < 0.05$).

	First study			**Second study**		
	sys-tolic	dia-stolic	pulse rate	sys-tolic	dia-stolic	pulse rate
Base-line	117 (108-128)	74 (58-78)	76 (60-92)	118 (108-130)	76 (66-84)	72 (56-76)
Sulpiride				120 (108-136)	74 (60-84)	80* (64-96)
Dopamine dose μg/kg/min						
0.25	116 (108-130)	70 (62-82)	72 (66-84)	114 (108-132)	79 (68-92)	78 (64-92)
0.5	114 (105-126)	70 (55-78)	72 (60-80)	110 (106-126)	74 (54-86)	80 (60-96)
2	116 (106-126)	69* (58-78)	72 (68-96)	110 (108-126)	69* (56-74)	80 (64-96)
4	116 (108-132)	68* (52-74)	76 (60-92)	137* (118-144)	68* (50-72)	82 (64-92)
8	142* (130-152)	58* (50-68)	76 (68-84)	154* (124-170)	61* (40-66)	78 (60-112)

Serum sulpiride levels were stable throughout the study period: median sulpiride levels of 0.53 (range 0.32-0.71), 0.54, 0.5, 0.5 and 0.5 mg/l at 1, 2, 3, 5 and 7 hours after the start of the sulpiride infusion. Trough serum prazosin level 4 hours after the previous dose amounted to 4.7 µg/l (range 2.5-7.8). One hour after the administration of prazosin, a median level of 9.5 µg/l (range 5.0-21.5) was reached.

Part 2: Phentolamine infusion

Phentolamine-infusion did not influence base-line ERPF and GFR. ERPF was 519 ml/min (range 418-610) at base-line and 482 ml/min (range 391-553) during phentolamine infusion in the first study, and 556 (range 447-594) and 521 (range 396-591) ml/min, respectively, in the second study (fig 3B). The GFR base-line values during the first and second study were 118 (range 99-127) and 117 (range 101-126) ml/min and during phentolamine infusion 119 (range 98-125) and 121 (range 93-130) ml/min, respectively (fig 3A). FF was 0.22 (range 0.19-0.30) at base-line in the first study and did not change during phentolamine infusion (0.23). In the second study a significant change from 0.205 (range 0.20-0.26) at base-line to 0.233 (range 0.205-0.25) during phentolamine infusion was observed (fig 3C).

Dopamine infusion led to an increase of the ERPF for the 2, 4, and 8 µg/kg/min doses; the maximum was reached at the latter dose with 676 ml/min (range 578-819) (fig 3). GFR did not change significantly for either of the dopamine doses. FF fell for the 4 and 8 µg/kg/min dopamine doses to 0.18 and 0.178, respectively.

Sulpiride infusion was associated with a slight fall in the ERPF from 521 during phentolamine infusion to 487 ml/min during the combined phentolamine and sulpiride infusion, while GFR and FF did not change compared to the values during phentolamine infusion (fig 3).

Sulpiride infusion did not shift the dopamine dose-response curves for ERPF, GFR or FF.

Phentolamine infusion had no effect on sodium excretion. Base-line $FENa^+$% was 1.08 at base-line in the first, and 1.007 in the second study; respective values during phentolamine administration were 0.894 and 1.09 (fig 4). Dopamine infusion did not influence sodium excretion either. Sulpiride infusion was associated with a fall in $FENa^+$% to 0.534. During subsequent dopamine infusion $FENa^+$% immediately returned to levels found previously and comparable to those in the first study during dopamine infusion.

No significant changes in diuresis were observed during phentolamine, sulpiride or dopamine infusions.

The potassium excretion rose in both studies during phentolamine infusion from base-line values of 5.8 mmol/h (range 2.2-14.7) and 5.6 mmol/h (range 1.5-14.8) in the first and second study, to 10.4 mmol/h (range 1.7-17.7) and 7.6 mmol/h (range 2.5-34.5), respectively. Subsequent dopamine infusion resulted in a fall of potassium excretion to 4.1 (range 2.4-15.2) mmol/h and 3.2 (range 2-8.6) mmol/h, respectively, in the first and second study. Sulpiride infusion did not affect potassium excretion.

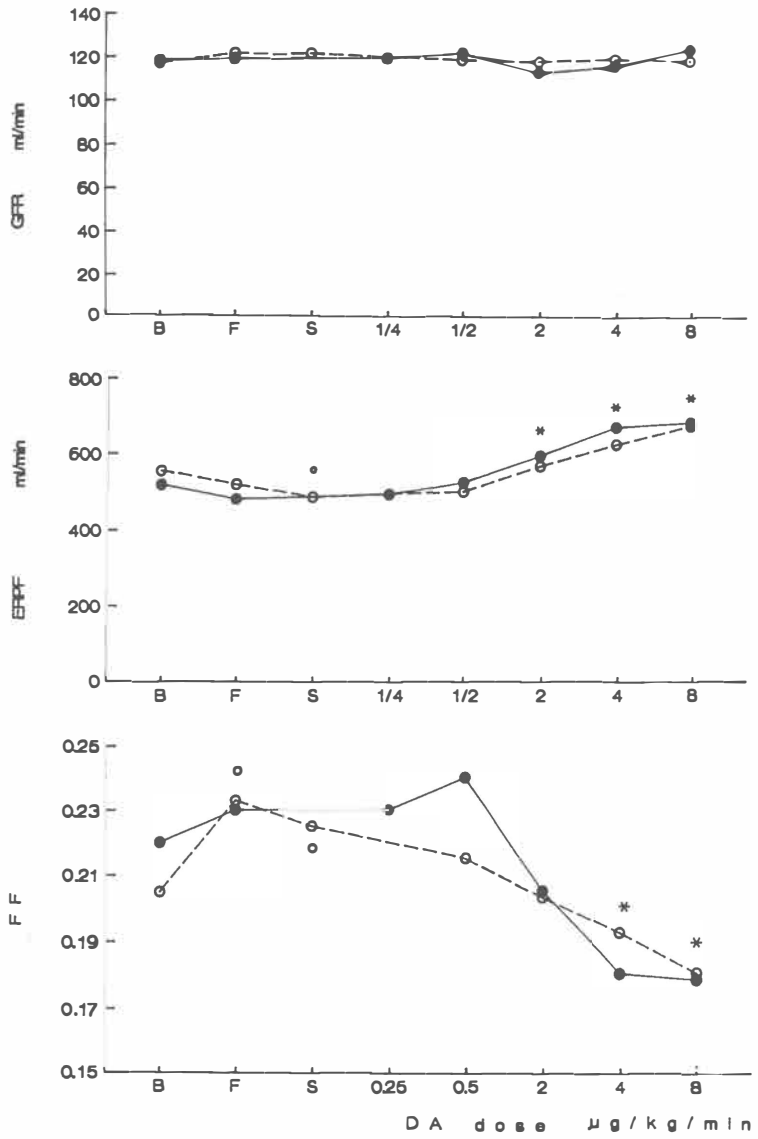


Figure 3A-C: Dose-response curves for GFR (ml/min) (fig 3A), ERPF (ml/min) (fig 3B) and FF (fig 3C) in 8 healthy volunteers before and after infusions of phentolamine followed by a dopamine dose-response curve, or by a combined infusion of sulpiride and subsequently dopamine. B = base-line value. F = during infusion of phentolamine. S = during subsequent infusion of sulpiride. Median values are shown.

* = significantly different from base-line value ($p < 0.05$). For the FF there was also a significant difference compared to values during infusion of phentolamine or sulpiride for the 4 and 8 µg/kg/min dopamine doses ($p < 0.05$).

o = significantly different from value during infusion of phentolamine alone.

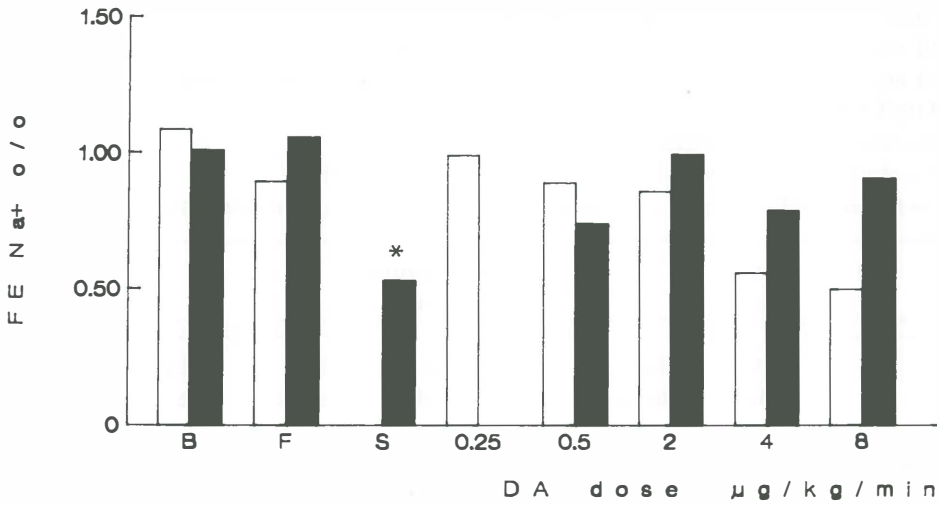


Figure 4: Dose-response bargraphs for fractional excretion of sodium ($FENa^+$%) in 8 healthy volunteers before and after infusions of phentolamine followed by a dopamine dose-response curve, or by a combined infusion of sulpiride and subsequently dopamine.
B = base-line value. F = during infusion of phentolamine. S = during subsequent infusion of sulpiride. Median values are shown.
* = significantly different from value during infusion of phentolamine ($p < 0.05$).

Phentolamine infusion did not change base-line calcium excretion (amounting to 0.35 and 0.4 mmol/h in the first and second study). Sulpiride infusion resulted in a fall from 0.48 (range 0.28-1.23) mmol/h during phentolamine infusion to 0.287 mmol/h (range 0.17-1.03) after addition of sulpiride. Although dopamine infusion did not increase calcium excretion in the first study, in the second study the calcium excretion returned to the values observed at base-line and during phentolamine upon infusion of dopamine in doses > 0.5 µg/kg/min.

Tubular reabsorption of phosphate (TRP) was not affected by phentolamine or sulpiride infusions. TRP fell during infusion of dopamine in doses > 0.5 µg/kg/min (from base-line values of 0.96 (range 0.87-1) and 0.95 (range 0.91-1) to minima of 0.88 (range 0.76-0.99) and 0.84 (range 0.73-0.95) in the first and second study, respectively, for dopamine doses of 4 µg/kg/min.

The excretion of γ-glutamyltransferase was not altered by phentolamine or sulpiride infusions; dopamine infusion in doses > 2 µg/kg/min led to an increase from a base-line value of 4.4 IU/mmol creatinine (range 3.2-7.2) to maxima of 6.5 (range 4.6-9.6) in the first and 5.5 IU/mmol creatinine (range 4.4-8) in the second study, at the 4 and 8 µg/kg/min doses, respectively.

Absolute and fractional excretion of ß-2-microglobulin did not change during phentolamine or sulpiride infusions; subsequent dopamine infusion was accompanied by an increase of ß-2-microglobulin excretion from 3.1 µg/h at base-line to a maximum

Table 2: **Blood pressure (mmHg) and pulse rate (beats/min) in 8 healthy volunteers before and during infusions of phentolamine (20 mg/h in the first hour; 10 mg/h during the rest of the study), sulpiride (100 mg in the first 30 minutes, 30 mg/h during the rest of the study) and dopamine (in doses ranging from 0.25 to 8 μg/kg/min).**
Median values and ranges are given.
* = significantly different from base-line values ($p < 0.05$).
^{0} = significantly different from values during phentolamine infusion ($p < 0.05$).

	First study			**Second study**		
	systolic	diastolic	pulse rate	systolic	diastolic	pulse rate
Base-line	130 (110-138)	86 (76-102)	64 (60-88)	122 (98-146)	68 (56-92)	68 (56-92)
Phentolamine	132 (114-152)	78* (72-90)	76* (64-136)	126 (114-136)	80* (60-100)	80* (72-128)
Sulpiride				139 (108-156)	82* (68-96)	82* (72-116)
Dopamine dose μg/kg/min						
0.25	144 (118-148)	84* (60-92)	80* (72-84)			
0.5	132 (92-152)	82* (72-96)	76* (68-124)	138 (114-154)	79* (68-96)	84* (68-104)
2	128 (112-154)	82* (64-89)	88* (76-124)	144*^{0} (115-154)	80* (60-97)	92* (80-108)
4	138 (112-162)	74*^{0} (55-87)	92*^{0} (68-132)	152*^{0} (114-178)	71*^{0} (64-82)	90* (72-116)
8	156*^{0} (126-172)	68*^{0} (48-85)	92*^{0} (68-136)	159*^{0} (154-166)	66*^{0} (50-75)	92* (80-116)

of 7 μg/h at the 8 μg/kg/min dopamine dose in the first study, and from 3.7 to a maximum of 11.3 μg/h at 4 μg/kg/min dopamine in the second study.

Phentolamine infusion was associated with an acceleration of the pulse rate and a fall in diastolic blood pressure (Table 2). Addition of sulpiride did not affect blood pressure or pulse rate. Dopamine infusion in doses of 4 and 8 μg/kg/min resulted in a further increase in pulse rate and a fall in diastolic blood pressure while systolic blood pressure rose.

Sulpiride levels in μg/l were 0.4 (range 0.2-0.93), 0.54 (range (0.2-0.94), 0.6 (range 0.28-1.10) and 0.7 (range 0.3-1.24) at 1, 2, 3 and 5 hours after the start of the sulpiride infusion, respectively (no significant changes).

Part 3: Dopamine dose-response curves without and with prazosin in healthy volunteers and in patients with renal disease

Healthy volunteers

In 7 healthy volunteers dopamine infusion without previous prazosin treatment resulted in an increase of ERPF compared to base-line for dopamine doses of 2 μg/kg/min and higher, with a base-line value of 478 (range 419-545) ml/min and a maximum of 728 (range 627-742) ml/min at 8 μg/kg/min (fig 5B). GFR rose for the 4 and 8 μg/kg/min dopamine doses from a base-line value of 116 ml/min (range 103-128) to a maximum of 127 (range 120-146) ml/min at 8 μg/kg/min. FF fell significantly compared to base-line for all dopamine doses, from a base-line value of 0.24 (range 0.205-0.297) to a minimum of 0.187 (range 0.165-0.233) at 8 μg/kg/min (fig 5C).

Prazosin pretreatment did not affect the base-line values of ERPF, GFR or FF. Although during subsequent dopamine infusion ERPF rose compared to base-line values for dopamine doses of 2 μg/kg/min and higher, the response of the ERPF to dopamine was blunted compared to the study without prazosin pretreatment (fig 5). The response of the GFR to dopamine infusion was not significantly influenced by prazosin pretreatment). FF fell compared to base-line values during dopamine infusion in doses of 2 μg/kg/min and higher; the fall in FF tended to be impaired in comparison to the study without prazosin; however, this was significant for the 2 μg/kg/min dose only (fig 5).

It should be noted that during prazosin pretreatment ERPF at the 8 μg/kg/min dopamine dose was significantly higher than the ERPF at 4 μg/kg/min; this pattern was not observed in the study without prazosin.

Dopamine infusion resulted in an increase in sodium excretion for doses of 2 μg/kg/min and higher (fig 6). $FENa^+$% rose from a base-line value of 1.275 (range 0.585-2.689) to a maximum of 3.02 (range 0.56-4.8) at 8 μg/kg/min dopamine. This natriuretic response to dopamine was completely abolished by pretreatment with prazosin although prazosin did not affect base-line $FENa^+$%.

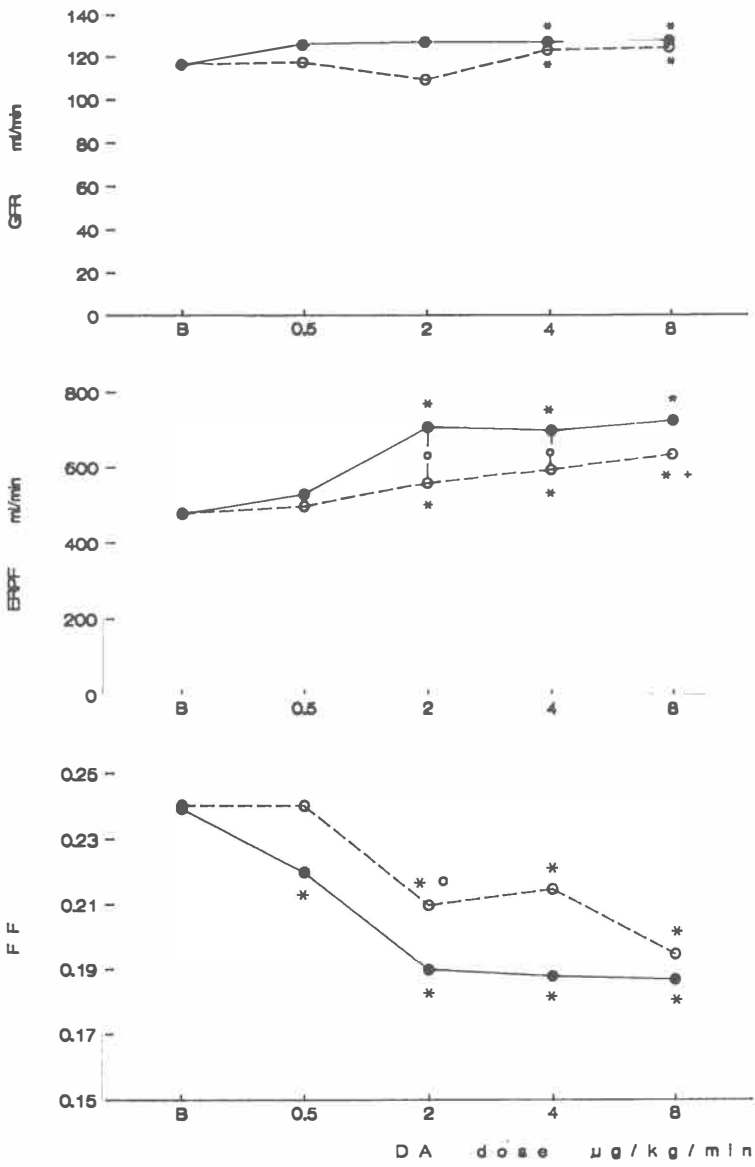


Figure 5A-C: Dopamine dose-response curves for GFR (ml/min) (fig 5A), ERPF (ml/min) (fig 5B) and FF (fig 5C) in 7 healthy volunteers in studies without and with pretreatment with prazosin.
B = base-line value. Median values are shown.
* = significantly different from base-line value ($p < 0.05$).
o = significant difference between study without and with prazosin ($p < 0.05$).
+ = significantly different from 4 µg/kg/min dopamine dose value in the study with prazosin ($p < 0.05$).

Comparing the studies without and with prazosin, no differences in potassium or calcium excretion were found. Calcium excretion rose during dopamine infusion from 0.411 (range 0.04-0.89) mmol/h at base-line to a maximum of 0.567 (range 0.21-1.23) at the 8 µg/kg/min dopamine dose. No significant differences were observed between both studies for the tubular resorption of phosphate (which fell during infusion of dopamine in doses of 2 µg/kg/min and higher) or for the absolute or fractional excretions of γ-glutamyltransferase and ß-2-microglobulin (which did not rise significantly during dopamine infusion).

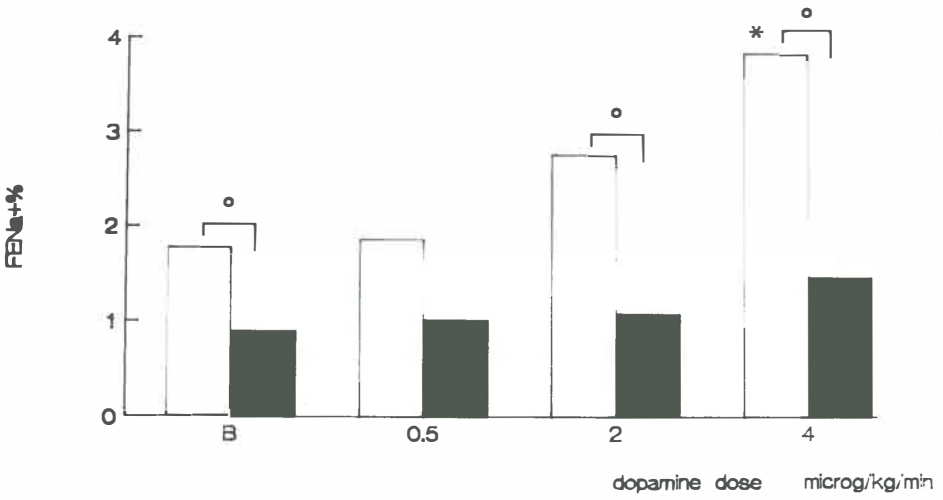


Figure 6: Dopamine dose-response bargraphs for fractional excretion of sodium ($FENa^+$%) in 7 healthy volunteers in studies without and with pretreatment with prazosin.
B = base-line value. Median values are shown.
* = significantly different from base-line value ($p < 0.05$).
o = significant difference between study without and with prazosin ($p < 0.05$).

Although in the study with prazosin blood pressure was not significantly different from that in the study without prazosin, pulse rate was higher in the prazosin study (Table 3). Comparing base-line and each dopamine dose separately, significant differences for the pulse rate were restricted to the 0.5 and 4 µg/kg/min dopamine dose. Dopamine infusions resulted in a rise in systolic blood pressure for the 8 µg/kg/min dose, and in a fall in diastolic blood pressure and an increase in pulse rate for the 4 and 8 µg/kg/min dose.

Prazosin levels amounted to 7 µg/l (range 3-9 µg/l) 4 hours after the start of the infusion, i.e. just before the following prazosin administration, and to 13 µg/l (range 4-23 µg/l) one hour after a dose of prazosin.

Table 3: **Blood pressure (mmHg) and pulse rate (beats/min) in 7 healthy volunteers without (first study) and with (second study) pretreatment with prazosin before and during dopamine infusions (in doses ranging from 0.5 to 8 μg/kg/min).**
Median values and ranges are given.
* = significantly different from base-line values ($p < 0.05$).
o = significantly different from values for the first study ($p < 0.05$).

	First study			**Second study**		
	sys-tolic	dia-stolic	pulse rate	sys-tolic	dia-stolic	puls rate
Base-line	130 (108-142)	84 (58-88)	68 (56-92)	120 (103-146)	82 (72-89)	76 (64-84)
Dopamine dose μg/kg/min						
0.5	126 (105-138)	82 (55-90)	68 (60-80)	126 (95-154)	84 (68-88)	72^{o} (72-84)
2	130 (106-148)	80 (58-96)	72 (60-88)	128 (108-148)	74 (64-92)	80 (68-88)
4	128 (114-148)	80* (52-90)	72 (60-92)	122 (108-158)	74* (60-86)	84*^{o} (72-88)
8	152* (140-164)	76* (48-82)	68 (64-96)	148* (130-156)	68* (45-84)	80* (68-96)

Patients with renal disease and impaired renal function

In the patients with renal disease and impaired renal function pretreatment with prazosin did neither influence base-line ERPF, GFR or FF, nor their response to dopamine infusion (fig 7). Dopamine infusion resulted in a rise of the ERPF compared to base-line from 285 (range 158-367) to a maximum of 357 ml/min (range 234-498) at 4 μg/kg/min dopamine in the first study, and from 300 to 350 ml/min, respectively, in the second study. GFR did not change during dopamine infusion compared to the base-line values of 68 (range 38-85) and 64 ml/min respectively, in both studies. FF fell from base-line values of 0.24 (range 0.19-0.29) and 0.242 (range 0.18-0.28) to a minimum of 0.215 in the first and 0.20 in the second study.

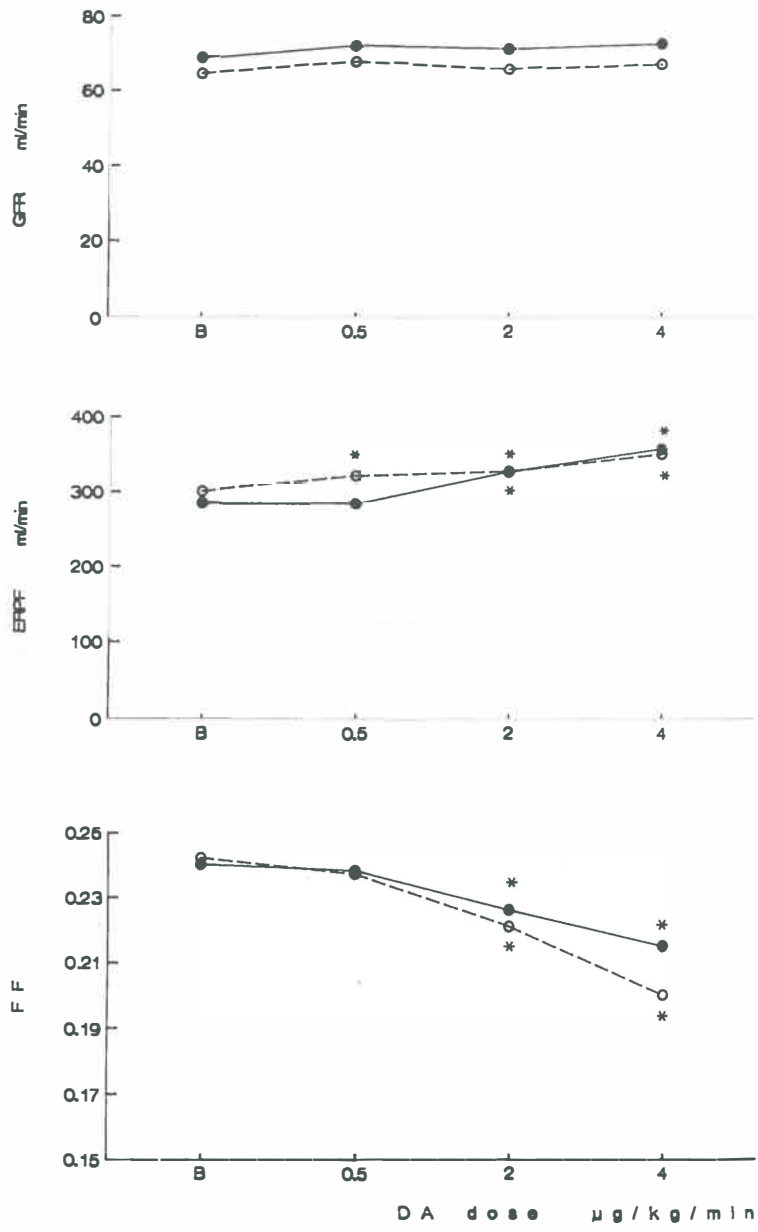


Figure 7A-C: Dopamine dose-response curves for GFR (ml/min) (fig 7A), ERPF (ml/min) (fig 7B) and FF (fig 7C) in 7 patients with renal disease in studies without and with pretreatment with prazosin.
B = base-line value. Median values are shown.
* = significantly different from base-line value ($p < 0.05$).

Both base-line sodium excretion and the natriuretic response to dopamine were significantly impaired by prazosin pretreatment. In the study without prazosin FENa+% rose significantly from a base-line value of 1.78 (range 0.07-3.33) to 3.83 (range 0.25-6.9) during dopamine infusion at a rate of 4 µg/kg/min; in the study with prazosin these values were 0.89 (range 0.1-1.53) and 1.46 (range 0.17-2.82), respectively (no significant change during dopamine)(fig 8).

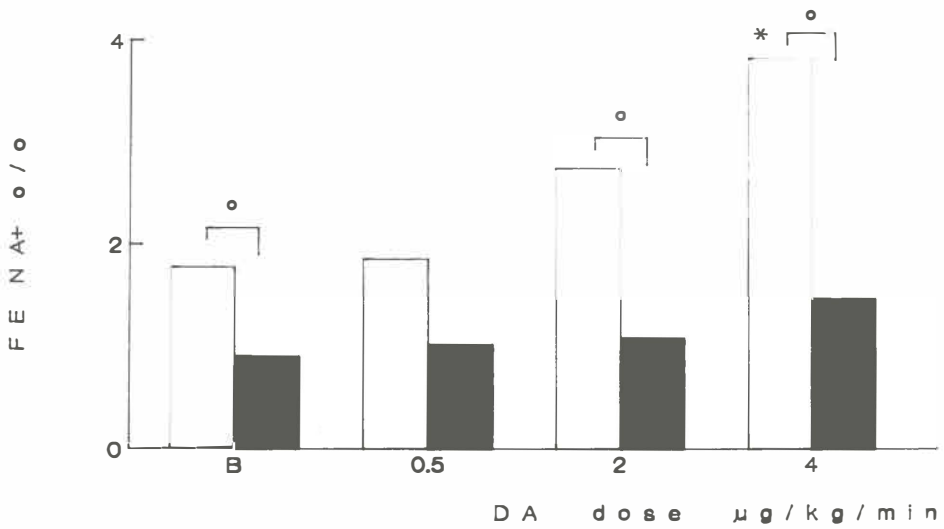


Figure 8: Dopamine dose-response bargraphs for fractional excretion of sodium ($FENa^{+}$%) in 7-patients with renal disease in studies without and with pretreatment with prazosin.
B = base-line value. Median values are shown.
* = significantly different from base-line value ($p < 0.05$).
o = significant difference between study without and with prazosin ($p < 0.05$).

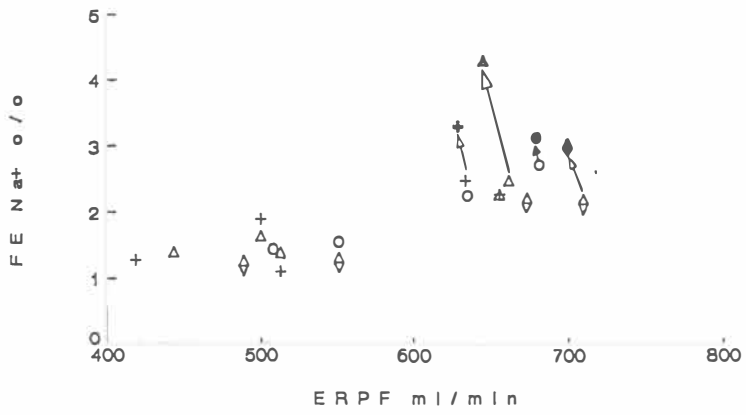
Diuresis was not influenced by prazosin pretreatment and did not change significantly during dopamine infusion, except for the 4 µg/kg/min dose with an increase from 235 (range 154-505) to 405 (range 263-785) ml/h. Prazosin pretreatment did not change potassium or calcium excretion compared to the study without prazosin. Calcium excretion rose from 0.15 (range 0.06-0.55) mmol/h at base-line to 0.42 (range 0.2-0.69) mmol/h at 4 µg/kg/min dopamine in the study without prazosin, and from 0.0.165 to 0.33 mmol/h in the second study. Pretreatment with prazosin did not influence base-line urinary γ-glutamyltransferase excretion with base-line values of 4.2 (range 1.4-6.3) and 4.5 (range 1.3-6) IU/mmol creatinine in the studies without and with prazosin, respectively, but abolished the small increase in Uγ-GT.V during dopamine infusion with maximal values of 5.3 (range 3.1-10) and 4.5 (range 1.5-6.7) IU/mmol creatinine for both studies during dopamine infusion, respectively. An increase in the excretion of ß-2-microglobulin during dopamine infusion from a base-line of 4 to 6.1 µg/l was abolished by pretreatment with prazosin (with values of 4.7 and 3.45 µg/l, respectively).

No significant differences in the base-line blood pressure or the response of blood pressure to dopamine were found between the studies without and with prazosin; however, the pulse rate was higher in the study with prazosin (Table 4).

Prazosin levels 4 hours after the administration of prazosin amounted to 4.6 μg/l (range 1.9-10.4).

Table 4: **Blood pressure (mmHg) and pulse rate (beats/min) in 7 patients with renal disease and impaired renal function without (first study) and with (second study) pretreatment with prazosin before and during dopamine infusions (in doses ranging from 0.5 to 4 μg/kg/min).**
Median values and ranges are given.
* = significantly different from base-line values ($p < 0.05$).
o = significantly different from values for the first study ($p < 0.05$).

	First study			**Second study**		
	sys-tolic	dia-stolic	pulse rate	sys-tolic	dia-stolic	pulse rate
Base-line	136 (114-148)	88 (73-108)	64 (48-84)	124 (104-140)	82 (72-104)	76^{o} (64-96)
Dopamine dose μg/kg/min						
0.5	120 (92-145)	75 (66-104)	64 (56-84)	126 (96-138)	86 (64-96)	76 (64-88)
2	126 (114-142)	78* (66-94)	64 (56-84)	128 (106-132)	78 (65-92)	80^{o} (68-92)
4	150* (130-160)	78* (60-94)	68 (56-88)	140* (126-145)	76* (50-96)	76^{o} (64-112)


FE Na+ o/o
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3
2
1
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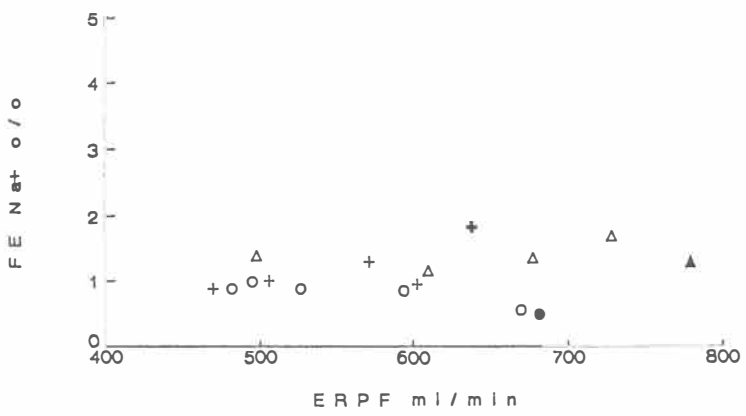
500
600
700
800
ERPF ml/min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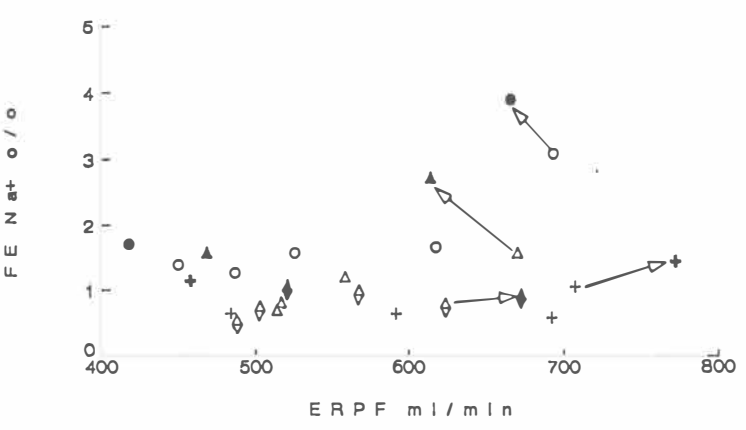
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ERPF ml/min

DISCUSSION

The subject of the present studies was to evaluate the influence of alpha-blockade on the renal effects of dopamine in man. The studies were initiated in an attempt to answer questions formulated in previous chapters, i.e. is the lack of effect of the dopamine antagonist sulpiride on dopamine-induced renal vasodilation in healthy volunteers due to additional alpha antagonist activity of sulpiride (see chapter 5)?

In the study of dopamine-induced renal vasodilation, maximal effects on ERPF and FF seemed to be reached in the upper part of our dose-response range: does this really represent maximal dopaminergic stimulation or is a shift in the balance between vasodilating dopaminergic and vasoconstrictive alpha-adrenergic effects of dopamine responsible (see chapter 2)? The results of the experiments designed to answer these questions prompted us to extend our observations on the influence of alpha-blockade on dopamine-induced renal effects.

In chapter 5 we failed to show any activity of sulpiride on dopamine-induced renal vasodilation. This was in marked contrast to the results of several animal studies which were further discussed in chapter 5 [3 5 6]. Pretreatment with an alpha-blocker, generally phenoxybenzamine, was used in all these studies to eliminate the alpha-adrenergic effects of dopamine. In the study in chapter 5 no such pretreatment was used. Besides

Figure 9A-C: Relation between the median values of ERPF (ml/min) and FENa+% before and during dopamine infusions in the studies in healthy volunteers described in chapters 3-6.
Open symbols represent the values before and during infusion of dopamine except for the 8 µg/kg/min dose for which corresponding closed or bold symbols are used.

Figure 9A gives the results of the studies without pretreatment with a dopamine antagonist or alpha-blocker. The arrows illustrate the leftward shift, after the dopamine dose is increased from 4 to 8 µg/kg/min.
Δ = study described in chapter. 3.
o = study described in chapter 4. + = study described in chapter 5.
ϴ = study described in this chapter, part 3.

Figure 9B shows the results of the studies in which pretreatment with an alpha-blocker was used. No leftward shift is observed for the 8 µg/kg/min dopamine dose.
Δ = study with prazosin, described in this chapter, part 1.
o = study with phentolamine, described in this chapter, part 2.
+ = study with prazosin described in this chapter in part 3.

Figure 9C shows the results of the studies in which dopamine antagonists in the absence or presence of alpha-blockade were used. In this figure the closed and bold symbols on the left side of the panel show values measured before administration of a dopamine antagonist, on the right side of the panel they again represent the 8 microg/kg/min dose. The presence of a dopamine antagonist does not prevent the leftward shift at the 8 microg/kg/min dose, however, combined dopamine and alpha-adrenoceptor blockade does.
Δ = study with metoclopramide, described in chapter 4.
o = study with sulpiride described in chapter 5.
+ = study with both prazosin and sulpiride, described in this chapter, part 1.
ϴ = study with both phentolamine and sulpiride described in this chapter, part 2.

its dopamine antagonist activity, sulpiride has been described to have weak alpha-antagonist activity in vitro in rabbits and in vivo in dogs [4 7]. An alpha-blocking effect of sulpiride would enhance, and dopamine blockade by sulpiride inhibit, a renal vasodilatory response to dopamine. Alpha-antagonist activity of sulpiride might therefore have obscured its dopamine antagonist activity on the renal vasculature in chapter 5. However, in the present studies both pretreatment with the selective postsynaptic alpha-1-adrenoceptor antagonist prazosin and the aselective alpha-blocker phentolamine did not result in a dissociation of the subsequent dopamine dose-response curves for the ERPF and FF without and with sulpiride and thus failed to reveal any dopamine antagonist activity of sulpiride on the renal vasculature.

Our results in normal volunteers remain in marked discrepancy with the animal studies mentioned above. The dose of sulpiride in our study, leading to serum levels considered to be therapeutic in clinical practice, was lower than in most of the animal studies [8]. However, the effect of sulpiride on sodium excretion in our study argues against the assumption that the dose was too low to demonstrate dopamine antagonism, as discussed before in chapter 5.

One might object that the dose of alpha-blockers in our study was too low to compete with the alpha-antagonist activity of sulpiride. However, the alpha-antagonist activity of sulpiride in other studies was quite weak. As for prazosin, the measured serum levels were low indeed and we did not check the adequacy of alpha-blockade by performing e.g. phenylephrine infusions; However, the observed increase in pulse rate and fall in diastolic blood pressure indicate the occurrence of alpha antagonist effects while serum levels after oral prazosin have been found to have a poor relation to clinical effects of alpha-blockade [9]. The observed systemic response in our phentolamine-pretreated volunteers is also a reflection of some degree of alpha-blockade. We, therefore, conclude that sulpiride, being inactive under adequate alpha-blockade, in our studies does not exert any significant effect on the renal vascular dopamine receptor.

To our knowledge the acute renal effects of prazosin in man have not been reported before. Our findings of a stable GFR and ERPF, and an unaltered $FENa^+$% during the first day of administration of a relatively low dose of prazosin correspond to the observed effects of chronic prazosin treatment on renal function. Preston et al observed no significant changes in PAH-clearance, creatinine clearance or filtration fraction after one month of prazosin treatment in patients with essential hypertension, although total renal vascular resistance fell [10]. Comparable results were found after eight weeks and after three weeks of treatment by Koshy et al and Bauer et al, respectively, in hypertensive patients [11 12]. Bauer and coworkers observed no change in $FENa^+$% either. The published effects of phentolamine on renal function in man are less uniform and probably depend on the route of administration. Grüninger et al found no significant changes in GFR, renal plasma flow and $FENa^+$% after intravenous phentolamine, while Reubi observed a small fall in GFR with an unchanged PAH-clearance [13 14]. In our study in normal volunteers no significant changes of ERPF, GFR and $FENa^+$% were observed despite a fall in (diastolic) blood pressure. However, intrarenal infusion of phentolamine in man is associated with an increase in renal blood flow and a fall in

renal vascular resistance [15]. It seems that the fall in blood pressure is balanced by some fall in renal vascular resistance. All studies mentioned above were performed in patients with essential hypertension.

Pretreatment with prazosin led to a significant increase of ERPF for the 8 μg/kg/min dopamine dose compared to the 4 μg/kg/min dose. Whereas the maximal response of ERPF and FF to dopamine infusion was reached at the 4 μg/kg/min dose in the normal volunteers of chapter 2, pretreatment both with prazosin or phentolamine resulted in maximal response of ERPF and FF for the highest dose used in our studies, i.e. the 8 μg/kg/min dose. This might lead to the conclusion that alpha-adrenergic stimulation by dopamine blunts the vasodilatory response to dopamine receptor stimulation and that the apparent maximal response of ERPF and FF to dopamine, as found in chapter 2, cannot be assumed to represent maximal dopaminergic stimulation. However, caution is needed with such a conclusion because prazosin was also found to impair the renal vasodilatory response to dopamine in the third part of the present studies in the lower dose ranges.

When performing the dopamine dose-response studies with and without sulpiride in our volunteers pretreated with prazosin, we found that the natriuretic response to dopamine was absent, in clear contrast to the results described in chapter 5. We, therefore, decided to repeat the dopamine dose-respose studies in a group of volunteers before and after pretreatment with prazosin. The abolishment of the natriuretic response to dopamine was confirmed while prazosin also impaired the renal vasodilatory response to dopamine. In the group of volunteers pretreated with phentolamine, we also observed the absence of a natriuretic response to dopamine infusion. Thus, both selective postsynaptic and aselective alpha-adrenergic blockers inhibit the natriuretic response to dopamine. To our knowledge no other authors have addressed the subject of sodium excretion during dopamine administration combined with alpha-blockade in human studies. Although numerous in vitro and in vivo animal studies have been performed using dopamine infusions in combination with an alpha-blocker to antagonize the alpha-adrenergic effects of dopamine, most of these were concerned with the effects on renal haemodynamics, but little attention was given to sodium excretion [3 5 6]. As discussed above, another problem concerns the doses of the alpha-blockers, generally phenoxybenzamine or phentolamine, and of the other drugs in such studies, usually far exceeding those in our and other human studies. Therefore, neither the impaired response of ERPF and FF to dopamine during prazosin pretreatment nor the abolishment of dopamine-induced natriuresis during alpha blockade can easily be placed in the perspective of comparable studies.

Although we realize that an extrapolation of mechanisms operative at the receptor level to effects observed in vivo in man is speculative, our first hypothesis to explain the prazosin-induced inhibition of the natriuretic response to dopamine involves receptor modification by prazosin. In the kidney both alpha-1- and alpha-2-adrenoceptors are present with a preponderance of the alpha-2 type [16]. Activation of renal alpha-adrenergic receptors by administration of alpha-adrenoceptor agonists or by low-level renal nerve stimulation (RNS) induces vasoconstriction and sodium retention,

while renal denervation causes sodium and water excretion [17-19]. The alpha-1-adrenergic receptor seems to be responsible for the sodium retention which occurs after RNS [20]. However, prazosin-induced alpha-1-adrenergic receptor blockade is not associated clinically with natriuresis; in fact an increase in sodium retention occurs during prazosin therapy [12 21]. Smyth et al observed an increase in the renal density of alpha-2-adrenergic receptors during prazosin treatment in the rat [22]. They also found that the sodium-retaining effects of RNS are reversed by acute administration of prazosin, but that after prazosin pretreatment combined alpha-1- and alpha-2-blockade is necessary to abolish the sodium retention of RNS. This implicates that during prazosin pretreatment alpha-2-adrenoceptors also become involved in the RNS-antinatriuresis. Evidence exists that alpha-2-adrenoceptors in the kidney are predominantly located outside the synaptic cleft in contrast to the alpha-1-adrenoceptors which are located postsynaptically [23 24]. Preferential activation of alpha-1-adrenoceptors by RNS and alpha-2-adrenoceptors by circulating catecholamines would correspond with such a difference in location. In our volunteers prazosin pretratment did not affect base-line sodium excretion but reversed the natriuretic response to dopamine infusion. A prazosin-induced increase in extrajunctional alpha-2-adrenoceptors would be compatible with the effects observed in our volunteers: such extrajunctional receptors would not yet be stimulated under base-line circumstances but would be sensitive to the high level of circulating dopamine during dopamine infusion and mediate vasoconstriction and an antinatriuresis. However, the fact that identical effects on sodium excretion are observed during infusion of phentolamine which also blocks alpha-2-adrenoceptors, is in contradiction to this theory.

The second hypothesis is based on the effects of dopamine and alpha-adrenoceptor stimulation on renal phospholipase-C. DA_1 dopamine receptors have recently been found to stimulate phospholipase-C selectively in renal cortical membranes [25] Phospholipase-C is like other phospholipases under marked stimulatory influence of the alpha-1 adrenergic receptor, while alpha-2 adrenoceptors lack any effect on these enzymes [26]. As was mentioned in chapter 1, phospholipase-C is involved in sodium transport mechanisms in the proximal tubule by alterations in cytosolic calcium. One might assume that stimulation of both the DA_1 dopamine receptor and the alpha-1 adrenoceptor is needed for a phospholipase-C mediated natriuresis to occur. Blockade of the alpha-1 adrenoceptor by prazosin or phentolamine would prevent the natriuretic response to dopamine infusion. The more marked fall in sodium excretion during prazosin than during phentolamine might be accounted for by the following: blockade of presynaptic alpha-2 adrenoceptors by phentolamine (but not by prazosin) facilitates postganglionic release of norepinephrine which will compete with phentolamine for the postsynaptic alpha-1 adrenergic receptor. Inhibition of the alpha-1 receptor and thereby phospholipase-C will be more complete with prazosin, thereby resulting in a more marked blockade of sodium excretion. This tubular antinatriuretic effect of prazosin might also be responsible for the observed blunted response of the ERPF to dopamine during prazosin: the rise in peritubular sodium concentration will favour a fall in perfusion with a constant filtration pressure, reflected by an impaired response of the

ERPF with a constant GFR. In fact, a synergistic effect of alpha-1 adrenoceptor and DA_1 dopamine receptor stimulation on phospholipase-C mediated sodium excretion might explain the disproportionate rise in sodium excretion during infusion of dopamine in doses at which ERPF starts to fall due to the vasoconstrictory effect of alpha-adrenergic stimulation. This divergence between the vasodilatory and natriuretic effects of dopamine infusion is illustrated in fig 9. However, it is hard to reconcile this theory with the above-mentioned sodium-retaining effects of alpha-agonists and RNS [17-20]. Perhaps differences in systemic responses and activation of the renin-angiotensin-aldosterone system are responsible for such discrepancies.

If one considers the results of the study without and with prazosin pretreatment, as depicted in fig 5-6, one might be inclined to think that prazosin has dopamine antagonist activity: the dopamine-induced vasodilation and natriuresisi are both inhibited by prazosin. However, the fall in sodium excretion after sulpiride in the volunteers pretreated with prazosin, as depicted in fig 2, discredits the hypothesis that prazosin has dopamineantagonist activity. Furthermore, no support for such a hypothesis could be found in studies of other authors. Finally, the strict conformational requirements for agonists and antagonists for especially the DA_1 dopamine receptors make it hard to believe that prazosin, a drug completely unrelated to the known groups of dopamine antagonists, fortuitously has dopamine antagonist activity.

A fourth hypothesis involves the systemic effects of alpha-blockade: both prazosin and phentolamine induce a reflex increase of sympathetic nervous system activity reflected in an increase in circulating norepinephrine levels and clinically in a tachycardia [27 28]. It is questionable whether this response is even more enhanced during phentolamine than during prazosin, as phentolamine blocks presynaptic alpha-2-adrenoceptors which inhibit the stimulated release of norepinephrine from postganglionic sympathetic nerve terminals [29]. Stimulated norepinephrine release might result in beta-adrenoceptor mediated activation of the renin-angiotensin-aldosterone axis. Activation of the renin-angiotensin-aldosterone axis will result in renal vasoconstriction and sodium retention [30-32]. Although such an indirect effect of alpha-blockade might account for part of the impaired dopamine response in our volunteers during pretreatment with alpha-blockers, it does not explain why base-line values did not change during alpha-blockade. One might assume that under base-line conditions no changes occur because a balance exists between the vasoconstrictory and antinatriuretic effects of an activated renin-angiotensin axis and the vasodilatory and possibly natriuretic effects of alpha-blockade. However, blockade of the alpha-adrenergic effects of higher doses of dopamine would be expected to favour a shift of such a balance in the direction of renal vasodilation and natriuresis. The strong inhibitory influence of dopamine on aldosterone release would also enhance natriuresis.

Finally, a fifth hypothesis assumes a stimulated endogenous renal dopamine generation during alpha-blockade. The fall in natriuresis during sulpiride infusion in the volunteers without prazosin pretreatment in chapter 5 was ascribed to inhibition of endogenous renal dopamine. This fall seems to be far more pronounced in the prazosin- or phentolamine-pretreated volunteers than in the volunteers without preceding alpha-

blockade in chapter 5, possibly implicating that natriuresis is even more dependent on endogenous renal dopamine during alpha-blockade. If alpha-blockade would indeed be associated with an increase in renal dopamine generation, this might explain the impaired renal vasodilatory and natriuretic response to infusion of exogenous dopamine. Under base-line conditions a balance might exist between the direct antinatriuretic action of prazosin described above and the natriuretic effect of enhanced dopamine generation. However the teleological background of such an enhanced renal dopamine generation during alpha-blockade remains obscure.

In chapters 3 and 4 we discussed the possibility of an enhanced renal dopamine generation in patients with renal disease. In the present study we also performed dopamine dose-response curves before and after pretreatment with prazosin in patients with renal disease and moderately impaired renal function. The effects of prazosin on dopamine-induced natriuresis were comparable to those in the normal volunteers. On the other hand in contrast to the results in the normal volunteers prazosin pretreatment led to a decrease in base-line sodium excretion but did not affect base-line ERPF or FF, or their response to dopamine infusion. These results again illustrate the dissociation of natriuretic and renal vasodilatory effects of dopamine discussed before in chapters 3 and 4. Although the antinatriuretic effect of prazosin can be explained in the terms of the first two hypotheses offered above, a synthesis of the theory claiming enhanced renal dopamine generation in patients with renal disease, and of the hypothesis of stimulated dopamine secretion during alpha blockade, better fits the results in our patients. If enhanced renal dopamine generation is already present in these patients, the response to exogenous dopamine will be impaired and any influence of prazosin, supposed to stimulate dopamine secretion, will be limited. The fall in baseline sodium excretion after prazosin in the patients might be due to the direct antinatriuretic action of prazosin, which now is not balanced by an increase in natriuresis-inducing endogenous dopamine as it was supposed to occur in the normal volunteers. We want to stress that this theory is highly speculative and should merely be considered as an attempt to reconcile a set of apparently contradictory results in our studies. We also want to remind the reader of the doubts raised by the results of the metoclopramide studies in chapter 4 on the validity of the hypothesis of enhanced dopamine generation in patients with renal diseaese. Detailed data on dopamine levels in normal man and in patients with renal disease under various conditions, e g. before and during alpha blockade, may help to support or discredit such a theory. Of course the use of recently developed selective dopamine agonists or antiagonists will allow one te define more precisely the effects of dopaminergic stimulation, avoiding the pretreatment with alpha- or beta- blockers.

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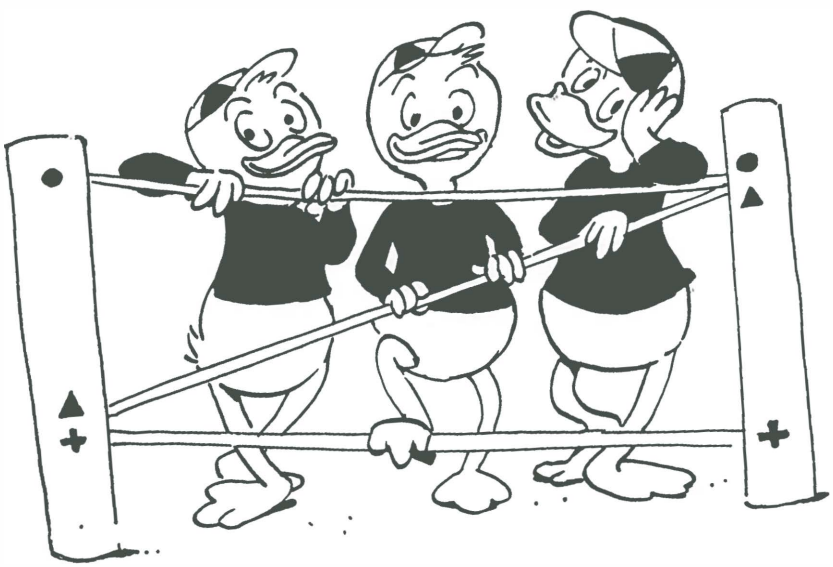
CHAPTER 7

PLASMA AND URINE DOPAMINE LEVELS



CHAPTER 7

PLASMA AND URINE DOPAMINE LEVELS

ABSTRACT

We measured free dopamine levels in plasma and urine in healthy volunteers under base-line conditions, during dopamine infusions, and after alpha-adrenoceptor and dopamine blockade (with phentolamine and prazosin, and sulpiride, respectively). A solvent extraction procedure using complex formation between diphenylborate and catechol-groups in alkaline medium was performed to isolate catecholamines, which were subsequently assayed by HPLC with electrochemical detection. Plasma norepinephrine levels were also measured and related to the expected changes during dopamine infusion or alpha-blockade.

Base-line level of free dopamine was 0.1 nmol/l (median, range 0.04-0.47) in plasma, while urine excretion of free dopamine amounted to 71.7 nmol/h (median, range 6.22-145.6) in 15 healthy volunteers on a 150 mmol sodium diet. The median of the percentual change from hour to hour was 25.6 and 28.4 % for plasma and urine free dopamine, respectively, while the median of the percentual change from day to day was 30 and 31.7 %, respectively. The procedure for withdrawal of blood, by venapuncture or from an indwelling intravenous needle, was found not to influence dopamine levels.

Infusion of dopamine in doses ranging from 0.25 to 8 μg/kg/min led to marked increases in both plasma and urine free dopamine levels. At an infusion rate of 0.5 μg/kg/min dopamine plasma free dopamine was 13 nmol/l and at 8 μg/kg/min 140 nmol/l. Urine free dopamine excretion amounted to 382 (range 92-590) and 1747 nmol/h (range 396-3342), respectively. Alpha-blockade with both phentolamine and prazosin resulted in a small increase in plasma dopamine levels, which increase was significant for phentolamine only (from 0.1 to 0.165 nmol/l). Phentolamine nor prazosin influenced urine free dopamine levels. Dopamine blockade, using sulpiride, did not influence plasma or urine dopamine levels.

Using our assay procedure and study conditions, plasma norepinephrine (NE) levels were 1.81 nmol/l (median, range 0.78-6.24 nmol/l), the hour-to-hour variation being 20.8 and the day-to-day variation 26.9 %. Plasma NE level was not higher when venapuncture was used for withdrawal of blood instead of an indwelling intravenous needle. Alpha-blockade with phentolamine, 10 mg/h i.v., led to a rise in NE levels from 1.35 to 3.55 nmol/l. Prazosin, 1 mg orally, did not significantly increase plasma norepinephrine. Infusion of dopamine, both combined with and without alpha-blockade, in doses ranging from 0.25 to 8 μg/kg/min, did not change plasma NE levels, except when under alpha-blockade pretreatment with sulpiride had been given. Sulpiride did not influence NE levels during phentolamine pretreatment.

Urinary clearance of dopamine amounted to 11327 ml/min (median, range 902-38387) under base-line conditions, fell to 6141 ml/min (median, range 2902-

15762) during phentolamine infusion and ranged during dopamine infusions from 269 (67-526) at 0.25 μg/kg/min to 675 (154-6080) at 8 μg/kg/min. Urinary clearance of norepinephrine was 91.6 ml/min (range 28.3-484 ml/min) at base-line, and did not change during infusion of phentolamine (median 121.4 ml/min, range 76-184).

We conclude that with our assay procedure reproducible results are attained and changes during pharmacological interventions are easily detected. Dopamine levels in both plasma and urine rise sharply during administration of exogenous dopamine, even when administered in doses which do not affect renal function. Alpha-blockade does not influence urine dopamine levels, but in the case of phentolamine resulted in a fall of the urinary clearance of dopamine. Phentolamine increased plasma norepinephrine levels. The calculated urinary clearances of dopamine indicate that dopamine is produced in the kidney and actively secreted into the urine.

INTRODUCTION

Practically all of the dopamine circulating in plasma does so in a conjugated form [1]. It has been estimated that 99% of circulating dopamine is conjugated; sulphoconjugates predominate [2]. Dopamine is conjugated to a much higher degree than the other two main catecholamines epinephrine and norepinephrine. Conjugated dopamine is biologically inactive [3 4]. In urine too, most dopamine is conjugated [5].

Up to a few years ago, plasma free dopamine was considered undetectable. However, the advent of newer extraction and analysis techniques like HPLC has provided new possibilities in this respect. Reliable assays for catecholamines based on these techniques are now in general use and the applicability of such assays in an adapted form for plasma free dopamine has meanwhile been confirmed [4 6 7]. Several authors have reported on the changes in dopamine levels under physiological circumstances like exercise or sodium loading. However, systematic studies of the plasma and urine dopamine levels attained under pharmacological conditions like exogenous administration of dopamine in man using these new assays are scarce. Because conclusions on the physiological role of dopamine are often based on the results of such pharmacological interventions, it seems of interest to acquire more data on dopamine levels under these circumstances.

In the present study we first determined plasma and urine dopamine levels under the conditions which had been used in the studies described in the preceding chapters, their hour-to-hour variation with special attention to a possible circadian rhythm, and their day-to-day variation. The levels attained during infusion of various doses of dopamine (as used in the preceding chapters), and during alpha-blockade and dopamine blockade were measured for plasma and urine dopamine combinedly. This also allowed us to calculate urinary clearances of dopamine during these pharmacological interventions.

PATIENTS AND METHODS

Studies were performed in healthy volunteers and patients on an outpatient basis. The study procedures conformed to those described in chapter 2. The following remarks and additions are to be made: to avoid the inadvertent influence of a high protein load on urine dopamine excretion, both healthy volunteers and patients were asked to abstain from animal protein containing foods on the day before and during the studies, like in our other studies [8]. They were also asked to adhere to an approximately 150 mmol sodium containing diet at least four days before the studies. In the part of the study examining the influence of prazosin administration on plasma catecholamine and urine dopamine levels, the volunteers had a breakfast consisting of clear fluids only.

The study consisted of several parts. In all instances plasma and urine dopamine levels, and plasma norepinephrine levels were determined. In some parts of the studies results of plasma epinephrine and urine norepinephrine levels have been added. Urinary clearances of dopamine or norepinephrine were calculated using the formula (U x V)/P, where for the plasma level the sample drawn after the urine collection was used.

1- In the first part of the study, base-line catecholamine levels were measured to test their variability under the study conditions described above and used in the previous chapters. The hour-to-hour variation and day-to-day variation were investigated in 15 volunteers. In all of them dopamine levels were measured under base-line conditions on two separate days, and on both these days one hour apart. There were 5 females and 10 males, median age being 28 years, range 21-56 years. In eight of these volunteers (median age 28 years, 4 females) plasma dopamine and norepinephrine levels obtained by venapuncture were compared with those obtained by withdrawal of blood from a previously inserted infusion needle (for a detailed description of methods see below). Changes due to a posssible circadian rhythm or due to the possible influence of our study conditions during the periods used in the previous chapters, were monitored by measuring plasma and urine levels for a period of 8 hours in 5 volunteers (median age 34 years and ranging from 21 to 51 years; 2 females) receiving a glucose (5 %) infusion .

2- In the second part of the study, the effect of infusion of dopamine in doses of 0.5 and 2 μg/kg/min on plasma and urine catecholamine levels was observed in 8 volunteers (median age 27 years with a range of 21-56; 5 females).

3- In the third part of the study the effects of alpha-blockade with either phentolamine or prazosin on plasma and urine catecholamine levels were assessed. In 8 volunteers (median age 27 years, range being 21-44 years; 7 males), after measuring two base-line levels, phentolamine was infused at a rate of 20 mg/h during the first hour and at a rate of 10 mg/h during the rest of the study. Plasma and urine were obtained 1 and 2 hours after the start of the phentolamine infusion. In a prazosin-treated group of 7 volunteers (median age 27 years, range being 21-56 years; 3 females), after determining 2 base-line values, prazosin 1 mg orally was given. Plasma was obtained two hours later, and urine from the second hour after the prazosin dose. Results were compared with those of identical moments in a control study during which no prazosin was given.

The effect of subsequent dopamine infusion in doses of 0.25, 0.5, 2 and 8 μg/kg/min was observed in the volunteers subjected to phentolamine infusion described above, and also in another group of 6 volunteers (median age 27 years, range 23-38 years; 2 females) in whom prazosin pretreatment had been given in the following fashion: 0.5 mg prazosin orally was given the evening before the study at bedtime, 1 mg orally was given at the start of the study and 4 and 8 hours later. Dopamine infusion was started after 4 hours; two plasma and urine catecholamine samples being collected 3 and 4 hours after the start of the study.

4- In the fourth part of the study in both the group with phentolamine pretreatment and the latter described group with prazosin pretreatment, the effect of the dopamine antagonist sulpiride was tested. Sulpiride was administered by infusion at a rate of 200 mg/h in the first half hour, after which the infusion was continued at 30 mg/h. Catecholamine levels were obtained 2 hours after the start of the sulpiride infusion.

5- Finally, in a preliminary study of dopamine levels in patients with renal disease and moderately impaired renal function, base-line dopamine levels in plasma and urine are reported for 7 patients. Their median age was 53 years, range 26-61 years. There were 2 females and 5 males. GFR ranged from 26 to 81 ml/min with a median of 56 ml/min. Histological diagnosis was diverse. Use of centrally-acting antihypertensives, adrenergic receptor blocking or stimulating agents, and ACE-inhibitors was prohibited.

In the parts of the studies in which a dopamine dose-response protocol was followed, after determination of at least two base-line values, dopamine was infused in ascending doses ranging from 0.25 to 8 μg/kg/min. Each dose was given for a period of one hour. For the dopamine dose-response curves in the alpha-blocker study with phentolamine and sulpiride, two adjacent periods of two hours during which infusion of these substances was started, preceded these dopamine dose-response curves.

Samples for plasma catecholamine levels were collected at the end of the corresponding hourly periods. Generally blood was obtained by routine venapuncture; however, in the part of the study comparing plasma levels obtained by venapuncture versus that obtained by means of an earlier inserted intravenous needle the following procedure was used. An infusion needle (20 gauge) was inserted in an arm vein and flushed with glucose (5 %) at 1 liter/24h. At least half an hour later, blood was collected from the infusion needle via a three-way cock, connected directly to the infusion needle. Immediately afterwards blood was obtained from the contralateral arm by routine venapuncture. For the catecholamine assay procedures, the reader is referred to chapter 2.

Serum sulpiride levels were determined at 1, 2, 3 and 5 hours after the start of the sulpiride infusion. Serum prazosin levels were obtained 2 hours after the administration of prazosin in the part of the study in which the acute effect of prazosin on plasma and urine catecholamines was compared with a control study. In the other group of

volunteers that received prazosin followed by a dopamine dose-response curve (see above), prazosin levels were determined 4 hours after the first administration of 1 mg (just before the following dose), and one hour later. In the patients with renal disease, a prazosin serum level was determined 4 hours after the first administration of 1 mg.

Day-to-day and hour-to-hour coefficient of variation were calculated as follows: for each individual the percentual change in the second hour compared to the first hour, or for the second day compared to the first day (using the corresponding moments), respectively, was calculated. Medians and ranges of these percentual changes are represented.

RESULTS

Base-line conditions

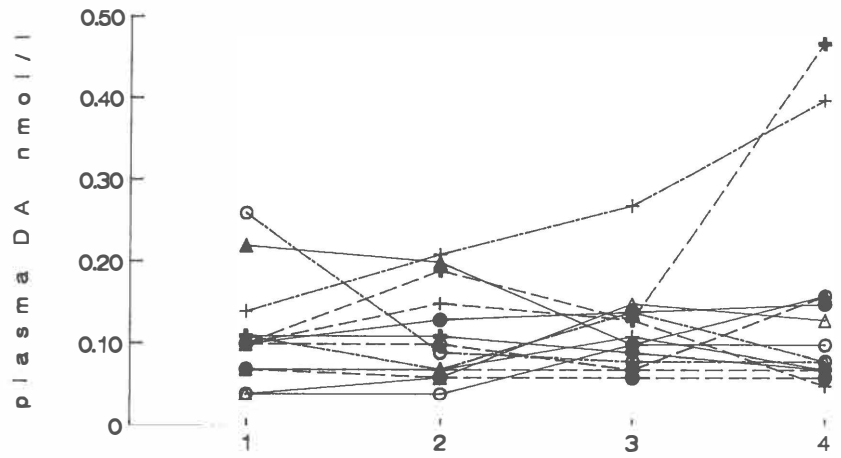
In the first part of the study, base-line dopamine levels in plasma and urine were measured four times one hour apart and on two separate days in 15 healthy volunteers with a median age of 28 years, range 21-56, of whom 5 were female. Sodium excretion amounted to 166 mmol/24 h (range 71-224). Plasma dopamine amounted to 0.1 nmol/l with a range from 0.04 to 0.47. The median of the percentual changes for samples taken one hour apart was 25.6 % (range 0-262), the median of the percentual changes from day to day was 30 % (range 0-275) (fig 1A-B). Urine dopamine was 71.7 nmol/h, with a range from 6.2 to 145.6. The hour-to-hour variation was 28.4 % (range 2-187), the day-to-day variation was 31.7 % (range 0.7-137.4) (fig 1C). No significant changes from hour to hour, or from day to day, were observed for both plasma and urine dopamine.

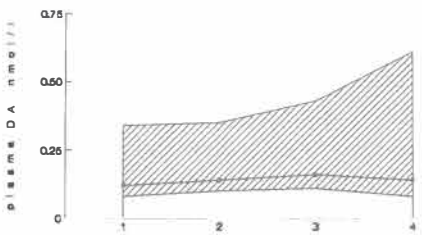
In 8 volunteers, 4 females and 4 males, median age 28 years with a range from 21 to 56, plasma dopamine levels obtained by withdrawal of blood from an indwelling intravenous infusion needle amounted to 0.1 nmol/l (median, range 0.03-0.34), while levels obtained by routine venapuncture were 0.1 nmol/l (median, range 0.04-0.28)(no significant difference)(fig 2A).

In 5 volunteers (median age 34 years, range 21-51, 2 females) medians of plasma dopamine levels after 2, 3, 4, 5 and 8 hours of glucose (5 %) infusion were 0.08, 0.1, 0.08, 0.09 and 0.06 nmol/l, respectively (no significant changes). Urine dopamine excretion amounted to 72.4, 63.3, 52.1, 55.6, and 55.9 nmol/h, respectively (no significant changes).

Plasma norepinephrine levels at base-line were 1.81 nmol/l with a range from 0.78 to 6.24. The hour-to-hour variation was 20.8 % (range 0-186) and the day-to-day variation 26.9 % (range 5.5-162)(fig 1D). No significant changes occurred from hour to hour, or from day to day.

Venapuncture did not result in higher plasma norepinephrine levels than withdrawal of blood from an indwelling intravenous needle: 2.64 nmol/l (range 1.23-6.24) using


plasma DA nmol/l
0.50
0.40
0.30
0.20
0.10
0
1
2
3
4


plasma DA nmol/l
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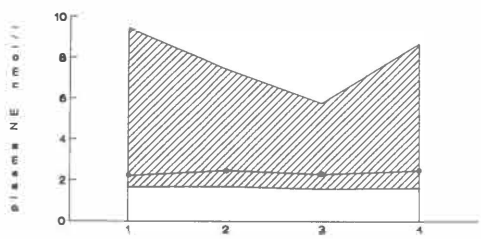
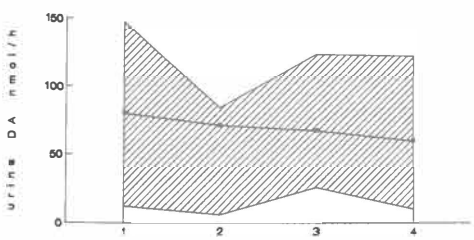
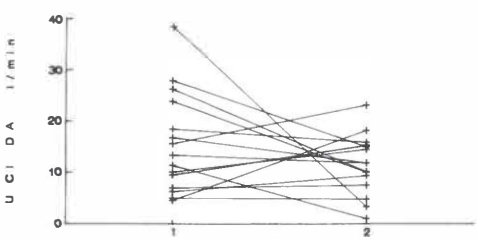
0.50
0.25
0
1
2
3
4


plasma NE nmol/l
10
8
6
4
2
0
1
2
3
4


urine DA nmol/h
150
100
50
0
1
2
3
4


UCl DA l/min
40
30
20
10
0
1
2

venapuncture versus 3.62 nmol/l (range 1.12-7.22) using the indwelling intravenous needle (fig 2B). During prolonged (for 8 hours) observation, plasma norepinephrine levels remained stable (1.52, 2.0, 1.93, 1.67 and 1.56 nmol/l after 2, 3, 4, 5 and 8 hours, respectively).

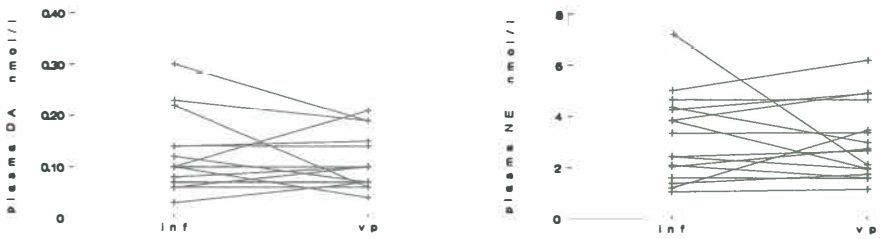


Figure 2A-B: Plasma dopamine levels (nmol/l) (fig 2A) and plasma norepinephrine levels (nmol/l) (fig 2B) in 8 healthy volunteers from whom blood was collected by venapuncture (vp) or from an indwelling needle (inf). Each line represents an individual volunteer.

Plasma epinephrine levels in 7 of the volunteers under base-line conditons were 0.17 nmol/l (range 0.13-0.36); the hour-to-hour variation was 42.8 % (range 0-500), the day-to-day variation was 62.6 % (range 0-383)(no significant changes from hour to hour, or from day to day).

Urinary clearance of dopamine was 11327 ml/min (range 902-38387) under base-line conditions. The day-to-day variation was 53.4 % (range 2.7-459) (fig 1E). No significant changes were observed from day to day.

Infusion of dopamine

Infusion of dopamine in a dose of 0.5 µg/kg/min resulted in a rise in plasma dopamine from a base-line level of 0.1 nmol/l (range 0.04-0.31) to 12.97 nmol/l (range 8.6-23.9) in 8 subjects with a median age of 27 years, range 21-56, 5 females and 3 males. Urine dopamine rose from 70.7 nmol/h at base-line to 382.2 nmol/h (range 92.3-590) at

Figure 1A: Hour-to-hour and day-to-day variation of plasma dopamine levels (nmol/l) in 15 healthy volunteers.
The range is indicated by the shaded area; the line connects the medians.
1 = day 1, time 1. 2 = day 1, one hour later. 3 = day 2, time 1. 4 = day 2, one hour later.

Figure 1B: Individual curves for the hour-to-hour and day-to-day variation of plasma dopamine levels (nmol/h) in 15 healthy volunteers.
1 = day 1, time 1. 2 = day 1, one hour later. 3 = day 2, time 1. 4 = day 2, one hour later.

Figure 1C-D: Hour-to-hour and day-to-day variation of urine dopamine levels (nmol/h) (fig 1C) and of plasma norepinephrine levels (nmol/l) (fig 1D) in 15 healthy volunteers.
The range is indicated by the shaded area; the line connects the medians.
1 = day 1, time 1. 2 = day 1, one hour later. 3 = day 2, time 1. 4 = day 2, one hour later.

0.5 μg/kg/min. At a dose of 2 μg/kg/min, plasma and urine dopamine levels of 43.7 nmol/l (range 9.4-71) and 1747 nmol/h (range 396.2-3342) nmol/h), respectively, were found.

Plasma norepinephrine was 3.4 nmol/l (range 1.45-6.94) at base-line, 2.38 nmol/l (range 0.89-5.0) during infusion of 0.5 μg/kg/min dopamine, and 3.32 nmol/l (range 1.91-10.2) at 2 μg/kg/min dopamine (no significant changes). Urine norepinephrine excretion ranged from 4.7-21.4 nmol/h at base-line (median 11.6) and did not change significantly during dopamine infusion with 13.4 and 20 nmol/h at the 0.5 and 2 μg/kg/min dopamine doses.

In this group of volunteers urinary clearance of dopamine fell from a base-line level of 9927 ml/min (range 2265-34789) to 423.8 (range 79.6-923) ml/min and 776 ml/min at the 0.5 and 2 μg/kg/min dopamine doses, respectively. Urinary clearance of norepinephrine did not change during dopamine infusion with a base-line level of 92 ml/min (range 37.4-484) and 79 and 108 ml/min at the 0.5 and 2 μg/kg/min dopamine doses, respectively.

Alpha-blockade and dopamine blockade

Infusion of phentolamine in 8 volunteers with a median age of 27 years, range 21-44, 1 female and 7 males, all investigated twice, resulted in a significant rise of plasma dopamine from 0.1 nmol/l (range 0.04-0.22) to 0.165 nmol/l (range 0.09-0.41) (fig 3A). Urine dopamine excretion did not change: 55.9 nmol/h (range 48.7-145.6) at base-line and 74.4 nmol/h (range 11.7-126.4) during the second hour of phentolamine infusion (fig 3B).

Prazosin administration 1 mg orally in 7 volunteers, age range 21-56 years, median 28, 4 females and 3 males, did not influence plasma dopamine levels: 0.14 nmol/l (range 0.06-0.47) at base-line and 0.19 nmol/l (range 0.06-0.28) after prazosin, while in a control study in the same volunteers levels at comparable moments were 0.1 nnmol/l (0.04-0.21) and 0.1 nmol/l (range 0.04-0.31), respectively (fig 4A). Urine dopamine did not change either: 62.2 nmol/h (range 27-95) at base-line and 77.6 nmol/h (range 43.7-284) in the second hour after prazosin administration, while the excretions amounted to 53.6 and 70.6 nmol/h in the control study.

Subsequent dopamine administration in the group of volunteers pretreated with phentolamine led to marked increases in plasma and urine dopamine levels: plasma dopamine was 13.5 (range 9.9-39.7) nmol/l at the 0.25 μg/kg/min dopamine dose and rose dose-dependently to 140.2 (range 28.8-445) nmol/l at the 8 μg/kg/min dose. Urine dopamine was 191 (range 118-465) nmol/h at the 0.25 μg/kg/min dopamine dose and 6248 nmol/h (range 2813-11868) at the 8 μg/kg/min dose. Urinary clearance of dopamine was 269 ml/min (range 67-526) during infusion of 0.25 μg/kg/min dopamine and rose to 338, 580 and 675 ml/min at doses of 0.5, 2 and 8 μg/kg/min dopamine, respectively. Urinary clearance of norepinephrine did not change significantly during dopamine infusion.

In another group of 6 volunteers (age range 23-38 years, median 27, 2 females and 4 males) pretreated with prazosin dopamine infusions in doses ranging from 0.25 to

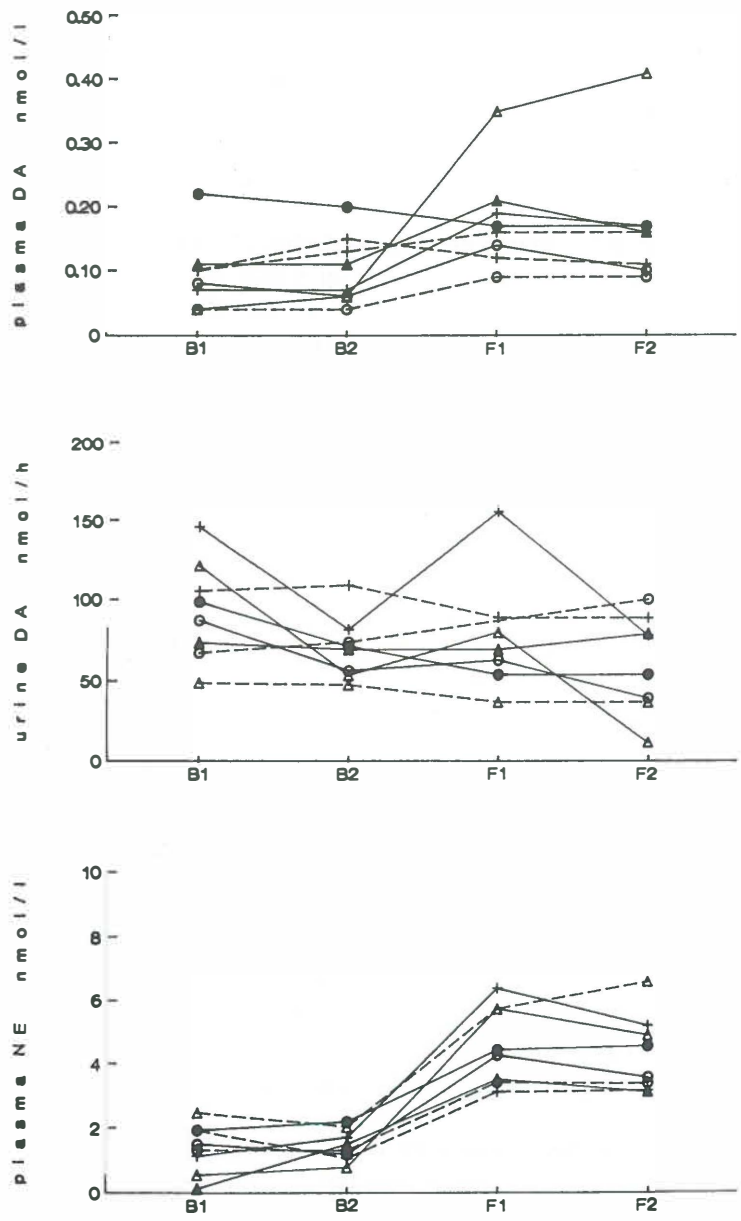


Figure 3A-C: Plasma dopamine levels (nmol/l) (fig 3A), urine dopamine levels (nmol/h) (fig 3B) and plasma norepinephrine levels (nmol/l) (fig 3C) before and during infusion of phentolamine in 8 healthy volunteers. Individual data are shown for the first study day.
B1 = before infusion of phentolamine, time 1. B2 = before infusion of phentolamine, time 2.
F1 = after the first hour of phentolamine infusion (at a rate of 20 mg/h). F2 = after the second hour of phentolamine infusion (at a rate of 10 mg/h).

8 μg/kg/min resulted in increases in plasma and urine dopamine levels which did not differ from those in the phentolamine group nor from the previously described group of 7 volunteers in whom the 0.5 and 2 μg/kg/min doses of dopamine had been tested without alpha-blocker pretreatment (see above). Therefore data on the plasma and urine dopamine levels during infusion of dopamine in these three groups have been pooled and summarized in the table.

Table **Plasma and urine dopamine during infusion of various doses of dopamine. Data have been pooled of the volunteers in whom no pretreatment (n = 7), simultaneous infusion of phentolamine (at a rate of 10 mg/h) (n = 8), or pretreatment with prazosin 1 mg p.o. (n = 6) had been given. Median and range (between brackets) are given.**

Dopamine infusion rate: (μg/kg/min)	plasma dopamine (nmol/h)	urine dopamine (nmol/h)
0.25	13.6 (9.9-40)	191 (118-465)
0.5	19.6 (4-51)	473 (92-920)
2	61.2 (9.4-105)	2325 (898-3541)
8	166.7 (28.8-465)	8262 (2722-13204)

Infusion of sulpiride during alpha-blockade with phentolamine had no effect on plasma dopamine levels: 0.155 nmol/l (range 0.09-0.41) during phentolamine and 0.215 nmol/l (range 0.08-0.54) during subsequent sulpiride infusion. Sulpiride infusion was not associated with a change in urine dopamine excretion: 63 nmol/h (range 28.3-98) before and 83 nmol/h (range 34.9-118) during sulpiride infusion.

Plasma norepinephrine rose from 1.35 nmol/l (range 0.55-3.15) at base-line to 3.55 nmol/l (range 2.06-6.58) during infusion of phentolamine (fig 3C). Prazosin administration did not significantly increase plasma norepinephrine: 2.69 nmol/l (range 1.33-6.21) before and 4.4 nmol/l (range 2.7-6) two hours after prazosin, while in the control study levels of 3.04 nmol/l (range 1.66-6.24) and 3.4 nmol/l (range 1.45-6.94) were found.(fig 4B). In the phentolamine-treated group neither dopamine nor sulpiride infusion changed plasma norepinephrine levels; however, the combined infusion of sulpiride and dopamine was associated with a rise of plasma norepinephrine from 3.52 (range 3.25-5.28) to 6.43 nmol/l (range 3.67-14.6).

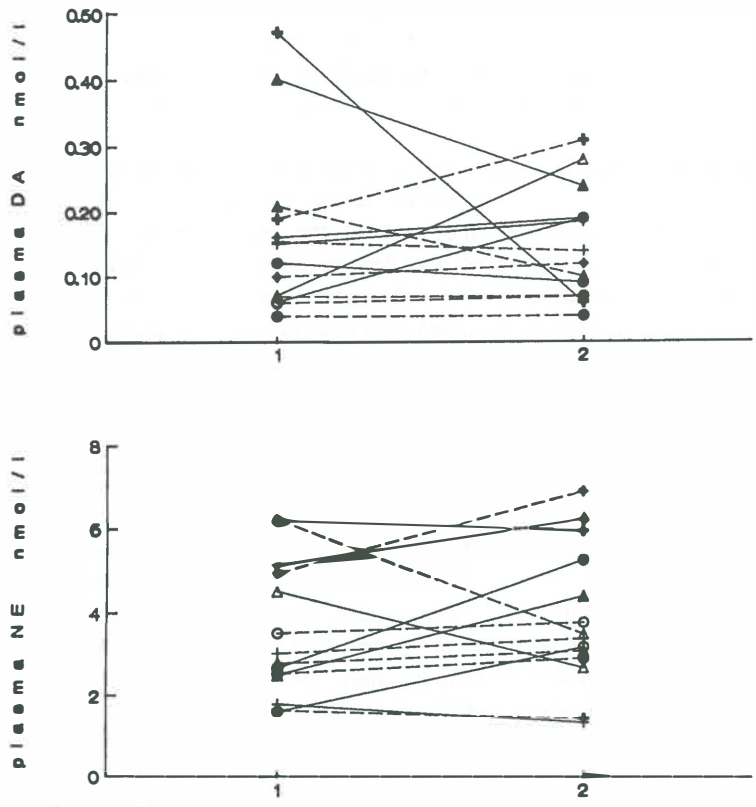


Figure 4A-B: Plasma dopamine levels (nmol/l) (fig 4A) and plasma norepinephrine levels (nmol/l) (fig 4B) before and after the administration of 1 mg prazosin (——), and in a control study (----) in 7 healthy volunteers. Individual data are shown.
1 = just before the administration of prazosin. 2 = two hours after the administration of prazosin in the prazosin study, and two hours after 1 in the control study, respectively.

Urinary clearance of dopamine and norepinephrine was measured in the group of volunteers receiving phentolamine infusions. In these volunteers urinary clearance of dopamine fell significantly from 14534 ml/min (range 4537-38387) to 6141 ml/min (range 2902-15762); urinary clearances of norepinephrine were 114 ml/min (range 75-252) and 121 ml/min (range 76-184), respectively (no significant changes).

Patients with renal disease

In the patients with renal disease plasma dopamine ranged from 0.03 to 0.96 nmol/l, median 0.18. Urine dopamine ranged from 2.9 to 36 nmol/h, median 27.1. Corrected for a GFR of 110 ml/min urine dopamine excretion ranged from 12.4 to 66.1 nmol/h,

median 41.7. Plasma norepinephrine ranged from 1.29 to 3.75 nmol/l, median 2.51 nmol/l. Urine norepinephrine excretion ranged from 0.9 to 10.5 nmol/h (median 6.3). Urine dopamine and norepinephrine excretion were both significantly correlated to the GFR with $r = 0.80$ and 0.83, respectively ($p < 0.02$).

Infusion of phentolamine was associated with an increase in pulse rate from 64 (range 60-88) to 76 (range 64-128) beats/min; blood pressure was 130/84 before and 132/78 mmHg two hours after the start of the phentolamine infusion. Prazosin administration did not change pulse rate: before prazosin 76 beats/min (range 56-88) and 80 (range 64-104) beats/min two hours after prazosin; blood pressure fell from 109/79 before to 98/70 mmHg two hours after administration of prazosin (in the control study medians of systolic and diastolic blood pressures were 108/74 and 114/79 mmHg at comparable moments). Sulpiride and dopamine infusion were not associated with changes in pulse rate or blood pressure except for the 8 µg/kg/min dose of dopamine (increase in pulse rate to 92 beats/min, rise in systolic and fall in diastolic blood pressure to 156/68 mmHg at the 8 µg/kg/min dopamine dose).

Prazosin levels in the group of volunteers, in whom prazosin had been started the evening before the study amounted to 7 µg/l (range 3-9) 4 hours after the start of the infusion just before the following prazosin dose, and to 13 µg/l (range 4-23) one hour after the administration of prazosin. In the group of volunteers to whom prazosin had been given after determining base-line values, prazosin level was 6.7 µg/l (range 1.0-10) 2 hours after the administation of prazosin. In the patients with renal disease prazosin levels 4 hours after the early morning dose amounted to 4.6 µg/l (range 1.9-10.4). Sulpiride levels in µg/l were 0.4 (range 0.2-0.93), 0.54 (range (0.2-0.94), 0.6 (range 0.28-1.10) and 0.7 (range 0.3-1.24) at 1, 2, 3 and 5 hours after the start of the sulpiride infusion, respectively (no significant changes).

DISCUSSION

We restricted our studies to measurements of free dopamine. Although in vitro dopamine-3-sulphate has been shown to inhibit aldosterone secretion and early studies of Unger et al in vivo also suggested a role for dopamine conjugates exceeding that of a metabolite, this has not been borne out by later studies which failed to show any significant biological activity of dopamine conjugates in vivo [3 4 9-11]. Urinary excretion of conjugated dopamine also has no relation to the neurotransmitter function of peripheral catecholamines [5]. Conjugated dopamine also shows marked variability which may be due to dietary influences: the body is protected against food-derived catecholamines by an efficient sulphoconjugation barrier in the intestine [1].

A few years ago Hjemdahl reviewed methods for plasma catecholamine assays and reported values for plasma free dopamine of 0.1-0.2 nmol/l (= 15-30 pg/ml) when HPLC assays were used [12]. In our study comparable values are found. In studies of

Cuche and Wang free dopamine levels assayed with a radio-enzymatic method, amounted in normal volunteers to 36 ± 8 and 50 ±10 pg/ml, respectively, while plasma sulphoconjugated dopamine levels varied around 5568 and 2490 pg/ml [13]. Van Loon reported base-line plasma dopamine levels of 53 ± 6 pg/ml for men and 51 ± 8 pg/ml for females [14]. The higher levels reported in these studies were found with radio-enzymatic methods which usually show considerable scatter. Deconjugation of dopamine may be a greater problem with enzymatic methods than with HPLC that is only preceded by an extraction procedure and involves no enzyme treatment. Urine dopamine excretion has been reported to amount to 44.8 ± 7.1 nmol/h in healthy volunteers on an unrestricted diet [15]. This is in reasonable agreement with the results observed in the present study.

Little is known of a possible circadian rhythm of free dopamine in plasma; for urine dopamine no circadian changes were found [5]. In our study no changes in plasma or urine dopamine levels were found during the eight hours of the control studies from early in the morning till late in the afternoon. This also implicates that some of the study conditions which have been described and used in previous chapters do not influence dopamine levels: moderate oral hydration with non-sodium containing beverages, necessary in our studies to facilitate collection of spontaneously voided hourly urine portions, did not influence plasma or urine dopamine in our normal volunteers on a mildly sodium-restricted diet. Being in a supine position for one hour after having voided in the upright position also assured reasonably stable levels. No differences between our male and female volunteers were found for plasma and urine dopamine levels. This is in agreement with published data [14]. Plasma dopamine has been reported to rise in reaction to exercise, assuming the upright position and acute (surgical) stress [14]. We did not find any effect of venapuncture, a procedure possibly eliciting a minor stress reaction. However, it must be stressed that plasma norepinephrine levels were also almost identical in blood obtained by venapuncture and withdrawal from an indwelling intravenous infusion needle, respectively. The plasma free norepinephrine levels in our study were comparable with those reported in other laboratories [12].

As for the influence of several physiological manoeuvers on urine dopamine excretion, most studies stem from the group of Lee, Ball and Oates. Administration of oral or intravenous sodium chloride resulted in an increase in urine dopamine [15]. In healthy volunteers Oates et al found no changes in plasma dopamine during an increase in dietary sodium intake from 20 to 220 mmol/day; urine dopamine rose from approximately 1.2 to 1.8 µmol/day [16]. An increase in dietary sodium from 10 mmol to 200 mmol in a study of Alexander et al was accompanied by an approximately 50% increase in urine dopamine [17]. Carey et al also investigated the influence of sodium restriction on plasma and urine dopamine. Sodium restriction from 150 to 10 meq Na^+ led to a small fall in plasma dopamine from 58 ± 10 to 43 ± 12 pg/ml on day 4 of the 10 meq Na^+ period. Urine dopamine decreased from 12 ± 2 to 8 ± 1 µg/h, while plasma NE increased and urine NE remained unchanged [18]. Plasma dopamine levels correlated inversely with sodium excretion in an older study of Romoff et al [19]. In conclusion,

urine dopamine exhibits an approximately 50 % rise after transition from a low- to a high-sodium diet. Ingestion of a protein-meal leads to a comparable increase in urine dopamine excretion [8]. The volunteers and patients in our studies were therefore asked to abstain from animal-derived proteins on the day before and during the studies. Pregnancy is also associated with an increase in urine dopamine [20]. The results on plasma dopamine levels in some of these studies can hardly be considered reliable due to the methodological assay problems mentioned above. Therefore, no undisputed relation has been established between plasma dopamine and the supposed physiological renal effects of dopamine.

In the second part of our study we investigated the dopamine levels attained in plasma and urine during administration of dopamine in doses which we used in previous studies. As expected even infusion of low doses of dopamine resulted in huge increases in plasma dopamine, while urine dopamine rose less markedly. Levinson et al, using liquid chromatography with electrochemical detection for dopamine assays, found in normal volunteers base-line free dopamine levels < 30 pg/ml which rose to 690 ± 120 ng/ml during infusion of 0.03 µg/kg/min dopamine and to 38400 ± 3800 pg/ml at 3 µg/kg/min. Urine dopamine excretion rose from a control value of 107 ± 12 ng/min to 192 ± 14 and 6280 ± 1590 ng/min, respectively [21]. Os et al, using a radioenzymatic technique, reported base-line dopamine levels of approximately 200 pg/ml, which rose to approximately 4000 pg/ml upon infusion of 0.5 µg/kg/min of dopamine, and to 16000 pg/ml at 2 µg/kg/min dopamine [22]. Considering the high base-line plasma levels reported in the latter study, the authors do not state the specificity of their method in separating free from conjugated dopamine, nor did they measure urine dopamine. Ball et al observed an increase of plasma dopamine levels, assayed with a modified version of the radioenzymatic method of Da Prada and Zürcher, from 0.77 ± 0.14 nmol/l at base-line to 1.4 ± 0.2 nmol/l at a dopamine infusion rate of 0.16 nmol/kg/min (= 0.03 µg/ kg/min) and to 1655 ± 106 nmol/l at 160 nmol/kg/min (= 30 µg/kg/min) in conscious dogs [23]. Gundert-Rémy et al investigated the pharmacokinetics of dopamine in doses ranging from 200 to 800 µg/min in healthy volunteers [24]. Steady state plasma dopamine levels, measured with a double isotope technique, were reached within 15 to 30 minutes after the start of the infusion, and amounted to 36.5 µg/l at 200 µg/min dopamine, 73.5 µg/l at 400 µg/min and 207 µg/l at 800 µg/min dopamine.

Ball and coworkers also performed dopamine clearance studies. They determined plasma and urine free dopamine simultaneously in normals and calculated a urinary clearance for dopamine of 1996 ml/min (range 402-3844 ml/min) [15]. Considering these very high levels, the calculation of fractional urinary excretions instead of a urinary clearance might have been more appropriate. However, we also decided to use urine dopamine clearances, in the first place to be able to compare our results with those of Ball et al and secondly, because we did not have GFR values of simultaneous time periods available for all the volunteers of this study while the usually low urine creatinine values did not allow calculation of reliable creatinine clearance values. The discrepancy between the results of Ball et al and those of our study which show markedly higher (five- to sixfold) values for the urinary clearances of dopamine, is

probably due to differences in the dopamine assay methods. Ball et al used a radioenzymatic method (according to Da Prada and Zürcher) resulting in plasma levels of free dopamine four- to fivefold those of our volunteers, while urine free dopamine excretion was somewhat lower than in our volunteers. Interestingly, at low dopamine infusion rates, e.g. 0.25 or 0.5 μg/kg/min, the steep rise in plasma free dopamine is associated with a modest increase of urine free dopamine excretion, reflected in an approximately fiftyfold fall in urinary clearance of dopamine to levels of roughly the same order of magnitude as the ERPF. Remarkably some increase in urinary dopamine clearance occurrs at higher dopamine infusion rates which is largely determined by an augmentation of the urine dopamine excretion. The urinary clearance of dopamine approaches the level for the renal blood flow in these volunteers, possibly implicating that all circulating dopamine passing the kidney is extracted and secreted into the urine. These observations may also be in accordance with an extrapolation of the observation made under different, physiological, circumstances that the renal effects of dopamine are closely related to the urine dopamine excretion and hardly or not to plasma dopamine levels. The huge increase in plasma levels reached during infusions of dopamine in doses which did not affect renal function in our previous studies had a limited effect on urine dopamine excretion. On the other hand, infusion of dopamine at rates known to augment renal blood flow and sodium excretion is associated with a rise in urine dopamine excretion which is disproportionately large in relation to the rather small increase in plasma dopamine levels within this dose range.

An alternative explanation for the observed levels of calculated urinary clearance of dopamine and their changes during infusion of exogenous dopamine involves renal deconjugation of circulating dopamine. As has been discussed above, the major part of dopamine circulates in a conjugated form. The ratio of conjugated versus free dopamine in the urine is lower, perhaps implicating that part of the circulating conjugated dopamine is deconjugated by the kidney and subsequently secreted into the urine as free dopamine [5 11]. This would result in high levels of calculated urinary clearance of free dopamine. During infusion of exogenous dopamine plasma free dopamine will rise and its clearance (which only in this instance represents a real clearance) might be responsible for the moderate increase in urinary free dopamine excretion. However this theory also fails to explain the disproportionate rise in urine dopamine we found at high dopamine infusion rates.

Gundert-Rémy and others found an increase of plasma norepinephrine and epinephrine levels in healthy volunteers during infusion of dopamine in doses ranging from 200 to 800 μg/min. However, they state that with their double-isotope method for plasma catecholamines high dopamine concentrations resulted in false levels for plasma norepinephrine and epinephrine, which they tried to correct by a linear regression calculation of the measured levels. In a study of Levinson et al of dopamine infusions in normal men at rates of 0.03, 0.3 and 3 μg/kg/min stimulation of plasma norepinephrine levels was found for the highest dose only [21]. At dopamine infusion rates comparable to the higher doses in the Levinson study, no changes in plasma norepinephrine were observed in our study. We also did not find an increase of urine

excretion of norepinephrine. Our results are in agreement with those of a study of Ball et al in dogs, in which no changes in plasma norepinephrine or epinephrine were found during infusion of dopamine in doses ranging from 0.03 to 30 μg/kg/min [23]. Dopamine may be converted to norepinephrine after uptake into neural tissue and subsequently released into plasma [25]. On the other hand, stimulation of DA_2 dopamine receptors may result in an inhibition of the release of norepinephrine from sympathetic nerve terminals [26 27]. Interestingly, a significant increase in plasma norepinephrine occurred during the combined infusion of sulpiride and 8 μg/kg/min dopamine. It is tempting to speculate that presynaptic DA_2 dopamine receptor blockade with sulpiride may have been responsible for the rise in norepinephrine levels, perhaps further enhanced by the conversion of dopamine to norepinephrine.

It is remarkable that the low doses of phentolamine used in our study were able to influence plasma norepinephrine and dopamine levels. The increase in plasma norepinephrine during alpha-blockade with phentolamine is in agreement with the results of other authors, who usually administered much higher doses [28 29]. It seems that systemic vasodilation, due to postsynaptic alpha-adrenoceptor blockade, induces reflex sympathetic nerve stimulation resulting in an enhanced norepinephrine release. The fact that prazosin, a selective postsynaptic alpha-blocker, did not significantly increase plasma norepinephrine levels might be due to stimulation of presynaptic alpha-adrenergic receptors which inhibit norepinephrine release from sympathetic nerve terminals. The fall in blood pressure or the increase in pulse rate during phentolamine or prazosin administration probably reflect the systemic response in our volunteers. Perhaps this systemic response is, like the reaction of plasma dopamine to standing or stress mentioned earlier, also reponsible for the otherwise unexplained rise in plasma dopamine.

Sulpiride did not influence dopamine or norepinephrine levels in the volunteers which had all been pretreated with phentolamine. As explained above the rise in plasma norepinephrine levels during combined sulpiride and dopamine infusion may be due DA_2 receptor inhibition. Sulpiride has been described to possess some alpha-2-antagonist activity. Blockade of presynaptie alpha-2 adrenoceptors may also result in disinhibition of norepinephrine release. However, this alternative explanation for the observed rise in plasma norepinephrine does not seem probable because phentolamine, already blocking alpha-2 adrenoceptors, was simultaneously administered [30].

The dopamine levels in the patients with impaired renal function were slightly higher than those in the normal volunteers, while the urine excretion of dopamine was lower even when corrected for the GFR. Although our data are of course preliminary and concern a very small number of patients, they do not give any support to the hypothesis of an enhanced renal dopamine generation, which should have been reflected in a relatively increased urine dopamine secretion. Clearly more data from patients in different GFR ranges and diagnosis categories are needed to draw any firmer conclusions. Plasma and urine norepinephrine levels in this small group of patients were not different from those in healthy volunteers. This norepinephrine pattern was also found in the recent study of Laederach and Weidmann, who found that

a chronic reduction in excretory kidney function has no relevant impact on circulating norepinephrine and epinephrine levels, and that their urinary excretion is not altered before GFR falls below 20 ml/min [31].

In conclusion our results show that our study conditions and our assay method for plasma and urine free dopamine allow us to identify the effects on these levels of pharmacological manipulations like alpha-blockade, even with the low doses of phentolamine and prazosin used in our studies, and dopamine infusions. Physiological manoeuvers were not investigated except that no circadian rhythm within the studied time period, and no effect of the possible stress of venapuncture could be defined. The pattern of plasma and urine dopamine levels during infusion of dopamine at various rates indicates that the renal effects of dopamine are more closely related to the attained levels of urine than plasma dopamine.

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SUMMARY AND CONCLUSIONS.

Dopamine is generally considered as a valuable drug in clinical medicine. The expression "renal dose" of dopamine is often used and known to most physicians. This illustrates not only that they are familiar with the idea of a specific dose range to obtain the desired effects but also the value attributed to these renal effects of the drug. Infusion of dopamine results in an increase in renal blood flow, glomerular filtration and sodium excretion. This is the result of stimulation of specific dopamine receptors. At higher dopamine infusion rates, renal blood flow and glomerular filtration fall again which is usually ascribed to stimulation of adrenoceptors, resulting in renal vasoconstriction. The link between the effects of dopamine and stimulation of specific receptors has been based on pharmacological studies using dopamine and adrenergic receptor agonists and antagonists.

The facts that dopamine is present in the kidney and in even much larger amounts in urine, that specific dopamine receptors exist within the kidney, and that stimulation of these receptors elicits such marked renal effects, makes it attractive to suppose an important physiological role for this third naturally occurring catecholamine in the kidney.

This thesis describes the renal effects of some dopamine and alpha-adrenergic receptor antagonists before and during infusion of dopamine in various doses in normal man and in patients with renal disease. An attempt is made to draw some conclusions on the physiological and possibly pathophysiological role of endogenous dopamine.

Chapter 1 provides an extensive review of the renal effects of dopamine. In the historical section the importance of adequate dose-finding studies is highlighted. After a description of the various receptors which may be stimulated by dopamine, the renal effects of exogenous and of endogenous dopamine are discussed separately. Several mechanisms may be responsible for dopamine-induced natriuresis. The renal vasodilation per se has often been considered as the main factor; however, recent evidence supports the assumption that direct tubular effects of dopamine are at least complementary. The inhibition of aldosterone release by dopamine may also contribute to the natriuretic effect of dopamine. A pathophysiological role of defective dopamine generation in essential hypertension and some oedematous disorders like congestive heart failure has been suggested but the available evidence does not allow firm conclusions in this respect. This does not exclude a possibly valuable role in the treatment of such diseases for selective and orally active dopamine agonists which have recently become available for clinical use.

Chapter 2 states the purpose of the studies which were performed in normal volunteers and in patients with renal disease and moderately impaired renal function. A general description of the study population, the study protocols and the methods is given.

Chapter 3 describes the renal effects of dopamine dose-response curves in normal volunteers and patients with renal disease. In earlier studies of Beukhof et al in patients

with IgA-glomerulopathy, and of ter Wee et al in a larger group of patients with various renal diseases, an impaired response of ERPF and GFR to infusion of a fixed dose of 1.5-2.0 μg/kg/min dopamine compared to normal volunteers was found. Below a base-line GFR of 73 ml/min/1.73 m^2 dopamine did not change ERPF or GFR; above this level the dopamine-induced rise in ERPF and GFR was larger with increasing base-line GFR. Even when the base-line GFR of a patient with renal disease was within the normal range his response to the fixed dose of dopamine was impaired compared to a healthy control subject. It was concluded that in patients with renal disease, nephron loss could be compensated for by a progressive utilization of the so called reserve filtration capacity. The possibility was proposed that an increase in endogenous renal dopamine was involved in this recruitment of reserve filtration capacity: renal vasodilation resulting from stimulated renal dopamine generation compensates for a fall in renal blood flow after nephron loss in a patient with renal disease. If such a hypothesis is true, the validity of a fixed-dose dopamine infusion for testing reserve filtration capacity can even be questioned: an impaired renal haemodynamic response cannot be assumed to represent only a fall in recruitable renal vasodilatory potential but may also reflect the competition of exogenous dopamine and stimulated endogenous dopamine for binding to dopamine receptors which induce renal vasodilation.

We decided to test the hypothesis of enhanced renal dopamine generation in patients with renal disease both by studying the effects of dopamine antagonists and by performing dose-response studies with exogenous dopamine. If renal disease would be associated with an increased renal dopamine generation, an enhanced sensitivity to dopamine antagonists, revealed by a renal vasoconstrictory response and possibly a fall in sodium excretion, should be found. A flattened dose-response curve, not only absolutely but also percentually, for exogenous dopamine would form another, albeit less persuasive argument for enhanced renal dopamine generation.

The results in chapter 3 show that for the renal haemodynamic parameters in patients with renal disease compared to healthy volunteers, an impaired percentual response was indeed found. In the normal volunteers a marked dose-dependent renal vasodilatory response was established which was already evident at a dose of 0.25 μg/kg/min, and reached its maximum at a dose of 4 μg/kg/min. The increase in GFR was modest. The reduction in the renal vasodilatory response in the patients with renal disease was found for the complete dose-range of dopamine. However, the natriuretic response to dopamine did not differ between the patients and the healthy volunteers. This casts some doubt on the hypothesis of an enhanced renal dopamine generation, although a local vascular increase in dopamine generation cannot be excluded. The fact that patients with renal disease had an impaired renal vasodilatory but a conserved natriuretic response to dopamine, formed our first argument for the assumption that the dopamine-induced natriuresis does not depend on renal vasodilation. As discussed above, direct tubular effects or an aldosterone-inhibiting action of dopamine might be other factors involved in the increase in sodium excretion. Additional arguments for a direct tubular action were supplied by the observed changes in the excretions of calcium, γ-glutamyltransferase and β-2-microglobulin, and in the tubular resorption of phosphate.

Further arguments to refute the hypothesis that enhanced endogenous dopamine generation in patients with renal disease is responsible for their impaired renal vasodilatory response to exogenous dopamine were provided by the study described in chapter 4. We examined the effect of the dopamine antagonist metoclopramide on base-line values and dopamine dose-response curves for renal haemodynamics and sodium excretion in healthy volunteers and in patients with renal disease.

Metoclopramide shifted the dopamine dose-response curve for renal vasodilation in the healthy volunteers and may therefore be assumed to act as a dopamine antagonist in the human kidney. Neither in the healthy volunteers nor in the patients with renal disease was any effect on base-line values of ERPF or FF found, thereby undermining the assumption that the impaired renal vasodilatory response in the patients with renal disease is due to enhanced endogenous dopamine generation. However, metoclopramide induced a fall in sodium excretion and a shift of the dopamine dose-response curve for natriuresis. This might represent the contribution of endogenous dopamine to sodium excretion on the one hand and is a second argument for a natriuretic action of dopamine which is independent of its renal vasodilatory effect on the other hand. One might presume that, while the renal effects of exogenous dopamine comprise both renal vasodilation and natriuresis, endogenous dopamine has no influence whatsoever on renal vessels and its only role is in modulating sodium excretion. However the demonstration of dopamine receptors in several renal vessel types and the observation that very high doses of metoclopramide may decrease renal plasma flow argue against such an assumption. The observed changes in the fractional excretions of γ-glutamyltransferase and β-2-microglobulin again support a direct (proximal) tubular effect of dopamine. The observed fall in aldosterone concentration during dopamine infusion and its rise during metoclopramide draw attention to the contributory role of aldosterone in the natriuretic effect of dopamine.

In chapter 5 the effects of another dopamine antagonist, sulpiride, have been examined. For metoclopramide other human studies had suggested dopamine antagonist activity at the renal level; for sulpiride comparable evidence from studies in man was scant despite the fact that in animal studies this drug is a very potent antagonist of dopamine-induced renal vasodilation. Therefore, and also because sulpiride in contrast to many other potent or selective dopamine antagonists is clinically available, we started a study of its effect on dopamine-induced renal vasodilation in healthy volunteers. To our surprise no effect whatsoever on the dopamine dose-response curves for ERPF or FF could be found. A fall in sodium excretion at base-line and its impaired response to dopamine infusion was assumed by us to represent a dopamine antagonist action of sulpiride on the natriuretic effects of endogenous and exogenous dopamine, respectively, which made it less likely that the dose of sulpiride had been too low to detect antagonist activity on dopamine-induced renal vasodilation. In the animal studies investigating the effects of sulpiride on dopamine-induced renal vasodilation, pretreatment with alpha-adrenergic antagonists had been used to block the alpha-adrenergic effects of dopamine. As sulpiride is known to possess some alpha-antagonist activity, we discussed the possibility that this might have obscured the

dopamine antagonist activity in our studies. We decided to repeat our sulpiride studies after pretreatment with alpha-blockers. Pending the results of alpha-blocker experiments, we discontinued the sulpiride studies in patients with renal disease of whom three had been investigated sofar. The results of these patients are also mentioned in chapter 5. Meanwhile the contrast between the antinatriuretic effect of sulpiride and the lack of effect on dopamine-induced renal vasodilation formed another argument for a dissociation between dopamine-induced renal vasodilation and natriuresis.

Chapter 6 is devoted to the relation of alpha-adrenergic and dopaminergic renal effects. In the first two parts of this chapter dopamine dose-response curves without and with sulpiride are described after pretreatment with the selective alpha-1-blocker prazosin and the aselective alpha-adrenoceptor antagonist phentolamine, respectively. We conclude that sulpiride does not show any activity as an antagonist of dopamine-induced renal vasodilation in man during pretreatment with either prazosin or phentolamine. The earlier observed fall in sodium excretion is confirmed and again supports a role for endogenous dopamine in maintaining sodium excretion. We cannot explain why this potent antagonist of dopamine-induced renal vasodilation in various animal studies fails to show any such effect in our human experiments. During both forms of pretreatment with an alpha-blocker dopamine lost its natriuretic effect. Therefore we performed a separate study comparing dopamine dose-response curves with and without prazosin pretreatment in which prazosin was found to impair not only the natriuretic but also the renal vasodilatory action of dopamine, although base-line values were not altered by prazosin. A comparable study in patients with renal disease gave somewhat different results: while base-line sodium excretion fell and the patients also exhibited an abolished natriuretic response to dopamine during prazosin pretreatment, base-line values of ERPF, GFR or FF nor their renal vasodilatory response to dopamine were affected by prazosin. Several possibilities to explain this unexpected reduction in the natriuretic response to dopamine are discussed. One of the proposed theories suggests that alpha-blockade results in enhanced endogenous renal dopamine generation. Although this theory covers most of the observations in this study, no other clinical or experimental studies or data on dopamine levels are available to support such a theory which, therefore, remains speculative. Another theory involves the selective effects of the DA_1 dopamine and the alpha-1 adrenoceptor on phospholipase-C and its relation to sodium excretion. Stimulation of alpha-1 adrenoceptors is a prerequisite for phospholipase activation and might explain why alpha-blockade in our studies abolished a phospholipase-C-mediated natriuretic response to dopamine. In this theory we also propose the blunted response of the ERPF to dopamine infusion during prazosin pretreatment to be a consequence (and not a cause) of the abolished natriuretic response. In fact, a synergistic effect of alpha-1 adrenoceptor and DA_1 dopamine receptor stimulation on phospholipase-C-mediated sodium excretion might explain the disproportionate rise in sodium excretion during infusion of dopamine in doses at which ERPF starts to fall due to the vasoconstrictory effect of alpha-adrenergic stimulation. The present studies also examined for the first time the acute (within one day of starting the drug) effects of prazosin on renal haemodynamics and sodium

excretion. The lack of effect on these parameters agrees with the results from studies on the renal effects of chronic prazosin treatment.

Finally, in chapter 7 we investigated plasma and urine free dopamine levels under the study conditions which had been used in the previous chapters. No change in these levels could be detected under our base-line study conditions which included moderate hydration in volunteers which were in a supine position (except for voiding), and repeated venapuncture. No evidence for a possible circadian rhythm during our study period was found. Infusion of dopamine at doses of 0.25 and 0.5 μg/kg/min resulted in huge increases in plasma dopamine levels while urine dopamine rose to levels slightly above the physiological range. At higher infusion rates which were associated with observable renal effects in our earlier studies, a sharp increase in urine dopamine was observed while that of plasma dopamine became less outspoken. We concluded that the observation made under physiological circumstances that there is a far better correlation between the assumed renal effects of endogenous dopamine and urine dopamine than plasma dopamine, is also valid for the renal effects of exogenous dopamine. Phentolamine but not prazosin administration was associated with a small rise in plasma dopamine. Urine dopamine was changed by neither of them, a finding which undermines the theory of an increase in endogenous renal dopamine during alpha-blockade which had been proposed in chapter 6. When our preliminary data on dopamine levels in patients with renal disease are confirmed, including the low urine dopamine excretion (even when corrected for a normal GFR), this will form another objection to our earlier formulated hypothesis of an enhanced endogenous renal dopamine generation in patients with renal disease.

In conclusion our pharmacological studies have confirmed an impaired renal vasodilatory response in patients with renal disease. However, their natriuretic response to dopamine is conserved. No supportive evidence was found for the assumption that the impaired renal vasodilatory response to dopamine in the patients with renal disease is due to enhanced endogenous dopamine generation. Both in normal volunteers and patients with renal disease, endogenous dopamine seems to have a role in sodium excretion. The dopamine-induced natriuresis does not depend on renal vasodilation but is probably due to direct proximal tubular effects although inhibition of aldosterone secretion may contribute to the natriuretic effect of dopamine. Metoclopramide but not sulpiride was shown to act as an antagonist of dopamine-induced renal vasodilation; both drugs antagonized dopamine-induced natriuresis. Alpha-blockade, both selective alpha-1-adrenergic using prazosin and aselective using phentolamine, abolished dopamine-induced natriuresis by an as yet unknown mechanism.

Studies using selective agonists and antagonists for DA_1 and DA_2 dopamine receptors may allow a better definition of the contribution of various receptors to the renal effects of endogenous and exogenous dopamine.

SAMENVATTING EN CONCLUSIES.

Dopamine geldt als een waardevol geneesmiddel in de medische praktijk. De uitdrukking "renale dosis" dopamine is elke internist bekend. Dit toont niet alleen de betekenis die toegekend wordt aan de renale effecten van dit middel, maar ook het besef dat deze effecten gebonden zijn aan bepaalde doses. Infusie van dopamine leidt tot een toename van renale doorbloeding, glomerulaire filtratie en zoutuitscheiding. Dit is het gevolg van stimulatie van specifieke dopamine receptoren. Bij infusie van grotere hoeveelheden dopamine dalen de renale doorbloeding en glomerulaire filtratie echter weer. Dit berust waarschijnlijk op stimulatie van adrenerge receptoren, die renale vasoconstrictie teweegbrengt. Het leggen van relaties tussen de effecten van dopamine en de stimulatie van specifieke receptoren is gebaseerd geweest op farmacologisch onderzoek met agonisten en antagonisten van de dopamine en adrenerge receptoren.

Dat dopamine in de nier aanwezig is en in nog veel grotere hoeveelheden in de urine, dat specifieke dopamine receptoren in de nier bestaan, en dat stimulatie van deze receptoren opvallende veranderingen in de nierfunctie sorteert zijn argumenten om een fysiologische rol voor dit derde natuurlijk voorkomende catecholamine te veronderstellen.

Dit proefschrift beschrijft de renale effecten van een aantal dopamine antagonisten en alfa-blokkers voor en tijdens infusies van dopamine in verschillende doses bij de gezonde mens en bij patienten met nierziekten. Er is gepoogd een aantal conclusies te trekken over de fysiologische en de mogelijke pathofysiologische rol van endogeen dopamine.

Hoofdstuk 1 is een uitgebreid overzicht van de literatuur over de renale effecten van dopamine. In het gedeelte over de geschiedenis van het dopamine onderzoek wordt het belang geillustreerd van "dose-finding" onderzoek. Na een beschrijving van de verschillende receptoren die door dopamine gestimuleerd kunnen worden, komen de renale effecten van exogeen en endogeen dopamine afzonderlijk aan de orde. De door dopamine teweeggebrachte toename in de zoutuitscheiding lijkt langs verschillende wegen tot stand te kunnen komen. Renale vasodilatatie is hierbij vaak als de belangrijkste factor beschouwd. Recent onderzoek steunt echter de veronderstelling dat directe tubulaire effecten van dopamine een rol spelen in de door dopamine teweeggebrachte natriurese. Ook remming van aldosteronafgifte door dopamine kan hieraan bijdragen. Er wordt wel gesuggereerd dat een tekort schieten van de vorming van dopamine van betekenis is in de pathofysiologie van essentiele hypertensie en aandoeningen als decompensatio cordis maar de nu beschikbare en in hoofdstuk 1 besproken gegevens zijn niet eenduidig. Dit neemt niet weg dat een aantal recent voor klinisch gebruik ter beschikking gekomen selectieve en oraal werkzame dopamine agonisten van waarde kunnen zijn in de behandeling van deze aandoeningen.

In hoofdstuk 2 wordt aangegeven wat het doel was van de onderzoeken beschreven in dit proefschrift. Deze werden uitgevoerd bij gezonde vrijwilligers en bij patienten met nierziekten met een matig gestoorde nierfunctie. Een globale beschrijving wordt

gegeven van de onderzochte groep vrijwilligers en patienten, van de onderzoeksprotocollen en van de gebruikte methoden.

Hoofdstuk 3 beschrijft de renale effecten van dopamine dosis-respons curves bij gezonde vrijwilligers en bij patienten met nierziekten. In voorgaande onderzoeken van Beukhof et al bij patienten met IgA-nefropathie, en van ter Wee et al in een grote groep patienten met verschillende nierziekten, was een verminderde respons van ERPF en GFR gevonden in vergelijking met gezonde proefpersonen in reactie op infusie van een vaste dosis dopamine van 1.5-2 µg/kg/min. Beneden een uitgangs-GFR van 73 ml/min/1.73 m^2 beinvloedde dopamine de ERPF of GFR niet meer. Boven deze waarde was de toename in ERPF en GFR groter naarmate de uitgangs-GFR hoger lag. Zelfs als de uitgangs-GFR van een patient met een nierziekte zich binnen de voor gezonden geldende grenzen bevond bleek de reactie op de vaste dosis dopamine verminderd in vergelijking met een gezonde proefpersoon. De conclusie was dat bij patienten met nierziekten het opgetreden verlies aan nefronen gecompenseerd wordt door een inschakeling van de zogenaamde renale reserve filtratie capaciteit. De veronderstelling was dat een toename in endogene renale dopamine vorming betrokken zou kunnen zijn bij deze recrutering van de renale reserve filtratie capaciteit: renale vasodilatatie als gevolg van een toegenomen renale dopamine vorming compenseert de afname in nierdoorbloeding na verlies van nefronen bij een patient met een nierziekte. Als een dergelijke hypothese klopt is het zelfs de vraag of het gebruik van een vaste dosis dopamine voor het onderzoek van de renale reserve filtratiecapaciteit juist is: een afgenomen renaal hemodynamische respons hoeft niet beslist een verminderd vermogen tot renale vasodilatatie te betekenen maar kan ook een gevolg zijn van competitie van grotere hoeveelheden endogeen renaal dopamine met het exogeen toegediende dopamine voor binding aan dopamine receptoren.

We besloten de hypothese van een toegenomen endogene renale dopamine vorming bij patienten met nierziekten te onderzoeken door de effecten van dopamine antagonisten te beoordelen en door dopamine dosis-respons-curves voor exogeen toegediend dopamine te maken. Indien nierziekte inderdaad gepaard gaat met met een toename in de vorming van endogeen renaal dopamine zou dit moeten leiden tot een verhoogde gevoeligheid voor dopamine antagonisten, tot uiting komend in renale vasoconstrictie en mogelijk ook een afname in de zoutuitscheiding. Een afgevlakte dosis-respons-curve, niet alleen absoluut maar ook percentueel, voor exogeen dopamine zou een volgend, zij het minder belangrijk argument zijn ten gunste van een toegenomen endogene renale dopamine vorming.

De resultaten uit hoofdstuk 3 tonen dat bij patienten met nierziekten er in vergelijking met gezonde vrijwilligers inderdaad sprake was van een verminderde percentuele respons in de gemeten parameters voor de renale hemodynamiek. Bij gezonden bleek een opvallende renale vasodilatatie op te treden die al duidelijk werd bij een dosis van 0.25 µg/kg/min en maximaal was bij een dosis van 4 µg/kg/min. De GFR steeg slechts in bescheiden mate. Bij patienten met nierziekten bleek sprake van een verminderde renale vasodilatatie voor alle onderzochte doses. Anderzijds was er bij gezonden en bij patienten met nierziekten een even sterke toename in natriurese onder

invloed van dopamine. Dit deed enige twijfel rijzen aan de eerder geformuleerde hypothese van een toegenomen renale dopamine vorming bij patienten met een nierziekte, hoewel er sprake zou kunnen zijn van een slechts locaal toegenomen vorming van dopamine in de vaatwand. De bevinding dat patienten met een nierziekte een verminderde renale vasodilatatie vertoonden onder dopamine terwijl de toename in zoutuitscheiding niet verschilde van die bij gezonden, vormde een eerste argument voor de veronderstelling dat de door dopamine teweeggebrachte natriurese niet afhankelijk is van renale vasodilatatie. Zoals eerder besproken zouden directe tubulaire effecten of een remming van aldosteron door dopamine ook betrokken kunnen zijn bij de toename van de zoutuitscheiding. Ondersteuning voor een direct tubulair effect van dopamine werd geleverd door de waargenomen veranderingen in de excretie van calcium, γ-glutamyltransferase en β-2-microglobuline en de tubulaire resorptie van fosfaat.

De hypothese dat een toegenomen endogene dopamine vorming verantwoordelijk is voor het verminderde vermogen tot renale vasodilatatie bij patienten met nierziekte werd verder ondermijnd door het onderzoek beschreven in hoofdstuk 4. We onderzochten de effecten van de dopamine antagonist metoclopramide op uitgangswaarden en dopamine dosis-respons curves voor ERPF, GFR, FF en zoutuitscheiding bij gezonde vrijwilligers en bij patienten met nierziekten. Metoclopramide verschoof de dosis-respons curve voor ERPF en FF bij gezonde vrijwilligers en kan daarom beschouwd worden als een dopamine antagonist in de menselijke nier. Metoclopramide had geen enkel effect op de uitgangswaarden van ERPF of FF bij gezonde vrijwilligers maar evenmin bij patienten met nierziekten. Op basis van deze laatste bevinding bleek de veronderstelling dat het afgenomen vermogen tot renale vasodilatatie bij de patienten met nierziekten berust op een toename in endogene dopamine vorming niet houdbaar. Anderzijds bracht metoclopramide een opvallende daling van de zoutuitscheiding teweeg en verschoof de dosis-respons curve voor de natriurese onder invloed van dopamine. Dit zou een afspiegeling kunnen zijn van de bijdrage van endogeen dopamine aan de zoutuitscheiding. Ook vormt het een nieuw argument voor een natriuretische werking van dopamine die los staat van de renale vasodilatatie. Misschien is het zo dat, waar de renale effecten van exogeen dopamine zowel renale vasodilatatie als een toename in zoutuitscheiding omvatten, endogeen dopamine geen enkele invloed op niervaten heeft en alleen van betekenis is voor het moduleren van de zoutexcretie. Het aantonen van dopamine receptoren in verschillende types niervaten en de bevinding dat zeer hoge doses metoclopramide wel een toename van de renale doorbloeding teweeg kunnen brengen pleiten tegen een dergelijke veronderstelling. De waargenomen veranderingen in de excretie van γ-glutamyltransferase en β-2-microglobuline bieden weer steun aan een direct (proximaal) tubulair effect van dopamine. De daling in plasma aldosteron tijdens dopamine infusie en de toename ervan tijdens metoclopramide attenderen op de mogelijke bijdrage van een geremde aldosteron afgifte aan het natriuretische effect van dopamine.

In hoofdstuk 5 zijn de renale effecten van een tweede dopamine antagonist, sulpiride, onderzocht. In het geval van metoclopramide hadden andere humane onderzoeken reeds dopamine antagonistische activiteit in de nier gesuggereerd. In het geval

van sulpiride waren gegevens van humaan onderzoek dienaangaande schaars, ook al was sulpiride in dieronderzoek een zeer krachtige antagonist van door dopamine geinduceerde renale vasodilatatie gebleken. Om deze reden en vanwege het feit dat sulpiride in tegenstelling tot vele andere krachtige of selectieve dopamine antagonisten wel voor humaan gebruik beschikbaar is, onderzochten wij het effect van sulpiride op door dopamine tweeggebrachte renale vasodilatatie bij gezonde vrijwilligers. Tot onze verrassing ontbrak elk effect op de dopamine dosis-respons curves voor de ERPF en FF. Wel trad er tijdens sulpiride enige daling op van de zoutuitscheiding ten opzichte van de uitgangssituatie en was de natriuretische respons op dopamine verminderd. Wij veronderstelden dat dit de dopamine antagonistische werking van sulpiride op de natriuretische effecten van respectievelijk endogeen en exogeen dopamine vertegenwoordigt. Het door ons waargenomen effect van sulpiride op de zoutuitscheiding pleit ook tegen de veronderstelling dat de dosis sulpiride te laag was om antagonistische activiteit op de door dopamine teweeggebrachte vasodilatatie aan te tonen. In de dieronderzoeken waarin het effect van sulpiride op door dopamine geinduceerde renale vasodilatatie onderzocht werd, was behandeling vooraf met alfa-blokkers toegepast om de alfa-adrenerge effecten van dopamine te antagoneren. Omdat van sulpiride bekend is dat het ook alfa-blokkerende eigenschappen bezit, overwogen we of deze de dopamine antagonistische activiteit in ons onderzoek gemaskeerd zouden kunnen hebben. We besloten ons onderzoek met sulpiride te herhalen na voor-behandeling met alfa-blokkers. In afwachting van deze resultaten met alfa-blokkers, onderbraken we het onderzoek met sulpiride bij patienten met nierziekten, van wie we op dat moment drie onderzocht hadden. De resultaten van deze patienten worden in hoofdstuk 5 vermeld. Het contrast tussen de remming van de zoutuitscheiding door sulpiride en het ontbreken van elk effect op de door dopamine teweeggebrachte renale vasodilatatie verschafte verdere steun aan de veronderstelling dat de door dopamine geinduceerde vasodilatatie en natriurese gedissocieerd zijn.

Hoofdstuk 6 is gewijd aan de relatie tussen alfa-adrenerge en dopaminerge renale effecten. De eerste twee onderdelen van dit hoofdstuk leiden tot de conclusie dat ook na behandeling vooraf met ofwel de selectieve alfa-blokker prazosin ofwel de aselectieve alfa-adrenerge antagonist fentolamine sulpiride niet werkt als een antagonist van door dopamine teweeggebrachte renale vasodilatatie bij de mens. Wel wordt ook nu een afname in zoutuitscheiding waargenomen, die opnieuw de veronderstelling steunt dat endogeen dopamine een rol speelt bij het handhaven van de zoutuitscheiding. Het blijft onverklaard waarom sulpiride de door dopamine teweeggebrachte renale vasodilatatie wel krachtig remt in verschillende dieronderzoeken, maar niet bij de mens.

Zowel tijdens de behandeling met prazosin als die met fentolamine bracht dopamine geen toename van de zoutuitsheiding meer teweeg. We deden hiernaar verder onderzoek door dopamine dosis-respons curves zonder en met behandeling vooraf met prazosin te vergelijken. Hierbij bleek dat prazosin, zonder dat de uitgangswaarden beinvloed werden, zowel de natriurese als de renale vasodilatatie onder invloed van dopamine remde. Een vergelijkbaar onderzoek bij patienten met nierziekten gaf andere

resultaten: enerzijds nam na behandeling vooraf met prazosin de zoutuitscheiding af zowel voor als tijdens de dopamine dosis-respons curves. Anderzijds werden de uitgangswaarden van ERPF, GFR en FF noch hun respons op dopamine beinvloed door prazosin. Verschillende mogelijkheden om deze onverwachte afname in de natriurese onder invloed van dopamine tijdens alfa-blokkade te verklaren worden bediscussieerd. Een van de geopperde theorieen suggereert dat alfa-blokkade resulteert in een toegenomen endogene renale dopamine vorming. Hoewel de meeste waarnemingen in dit onderzoek hiermee verklaard worden, ontbreken andere klinische of experimentele onderzoeken of gegevens over dopamine spiegels ter ondersteuning van een dergelijke theorie, die daarmee speculatief blijft. Een volgende hypothese gaat uit van de selectieve effecten van de DA_1 dopamine en de alfa 1-adrenoceptor op fosfolipase-C en de relatie hiervan met de zoutuitscheiding. Stimulatie van alfa-1-adrenoreceptoren is vereist voor activatie van fosfolipase en zou kunnen verklaren waarom alfa-blokkade in ons onderzoek een door fosfolipase-C gemedieerde toename in de zoutuitscheiding onder invloed van dopamine blokkeert. De afgenomen respons van de ERPF op dopamine tijdens behandeling met prazosin wordt binnen deze theorie beschouwd als een gevolg en niet als een oorzaak van de geblokkeerde natriuretische respons. Een synergistisch effect van alfa-1-adrenoceptor en DA_1 dopamine receptor stimulatie op een door fosfolipase-C gemedieerde natriurese zou de sterke toename in zoutuitscheiding kunnen verklaren tijdens infusie van dopamine in doses waarbij de ERPF juist begint te dalen onder invloed van het vasoconstrictoire effect van alfa-adrenerge stimulatie. In ons onderzoek wordt voor het eerst het acute effect (binnen een dag na de eerste dosis) van prazosin op renale hemodynamiek en zoutuitscheiding onderzocht. Het ontbreken van effect hierop is in overeenstemming met de resultaten van onderzoeken naar de renale effecten van chronische prazosin behandeling.

Tenslotte onderzochten we in hoofdstuk 7 plasma en urine spiegels van vrij dopamine onder de omstandigheden zoals die ook in de voorgaande hoofdstukken bestonden. De spiegels veranderden niet tijdens handhaving van de basale omstandigheden, die uitgingen van een matige hydratie bij vrijwilligers in een liggende houding (mictie uitgezonderd) met herhaalde venapuncties. We vonden geen aanwijzingen voor een mogelijk circadiaan ritme tijdens de uren van ons onderzoek. Infusie van dopamine in doses van 0.25 en 0.5 µg/kg/min leidde tot een zeer sterke toename van de plasma dopamine spiegels terwijl de urine dopamine spiegels tot even boven fysiologische waarden stegen. Bij hogere infusiesnelheden, in doses die gepaard gingen met waarneembare renale effecten in onze eerdere onderzoeken werd een scherpe stijging van urine dopamine spiegels gevonden terwijl de toename in plasma dopamine spiegels veel minder uitgesproken werd. Wij concludeerden dat de bevinding onder fysiologische omstandigheden dat er een veel betere correlatie is van de veronderstelde renale effecten van endogeen dopamine met de urine dopamine spiegels dan met de plasma dopamine spiegels, ook geldig is voor de renale effecten van exogeen dopamine. Fentolamine toediening gaf een geringe toename van plasma dopamine, dit was niet het geval met prazosin. De urine dopamine spiegels veranderden niet na toediening van fentolamine of prazosin, wat pleit tegen de eerder genoemde hypothese van een

toename in endogene renaal dopamine tijdens alfa-blokkade. Bevestiging van onze voorlopige gegevens over dopamine spiegels bij patienten met nierziekten, m.n van de lage dopamine excretie in de urine (ook indien gecorrigeerd voor GFR), zou een volgend argument zijn tegen onze eerder geformuleerde hypothese over een toegenomen endogene renale dopamine vorming bij patienten met nierziekten.

Concluderend hebben onze farmacologische onderzoeken bevestigd dat er sprake is van een verminderd vermogen tot renale vasodilatatie onder invloed van dopamine. De natriuretische respons op dopamine is echter intact. We vonden geen ondersteuning voor de veronderstelling dat het afgenomen vermogen tot renale vasodilatatie onder invloed van dopamine bij patienten met nierziekten berust op toegenomen vorming van endogeen renaal dopamine. Endogeen dopamine lijkt zowel bij gezonde vrijwilligers als bij patienten met nierziekten wel een rol te spelen bij de zoutuitscheiding. De door dopamine teweeggebrachte toename in zoutuitscheiding hangt niet af van het optreden van renale vasodilatatie maar is waarschijnlijk te danken aan directe effecten van dopamine op de proximale tubulus, hoewel ook remming van aldosteron afgifte een rol kan spelen. Metoclopramide bleek als een antagonist van de door dopamine teweeggebrachte vasodilatatie te fungeren. Dit gold niet voor sulpiride. Beide middelen antagoneerden de onder invloed van dopamine optredende natriurese. Toediening van alfa-adrenerge antagonisten, zowel selectief met prazosin, als aselectief met fentolamine, blokkeerde de toename in zoutuitscheiding onder invloed van dopamine via een vooralsnog onduidelijk mechanisme.

Onderzoek met selectieve agonisten en antagonisten voor DA_1 en DA_2 dopamine receptoren zal wellicht een betere afgrenzing mogelijk maken van de bijdrage van de verschillende receptoren aan de renale effecten van endogeen en exogeen dopamine.