VASODILATOR EFFECT OF VASOPRESSIN.

Publication of this thesis was made possible by financial support of
CABLON MEDICAL BV, Leusden, The Netherlands.

VASODILATOR EFFECT OF VASOPRESSIN.

Vaatverwijdende werking van vasopressine.

PROEFSCHRIFT

Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof.Dr. P.W.C. AKKERMANS M.A.
en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op
woensdag 9 november 1994 om 15.45 uur.

door

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geboren te Groesbeek

PROMOTIE COMMISSIE:

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Part of this thesis is based on the following publications:

Articles:

1 Derkx FHM, Brink HS, Merkus P, Smits J, Brommer EJP, Schalekamp MADH. Vasopressin V_2-receptor mediated hypotensive response in man. J.Hypertension 1987; 5 (suppl.5), S107-109.

2 Brommer EJP, Brink HS, Derkx FHM, Schalekamp MADH, Stibbe J. Normal homeostasis of fibrinolysis in nephrogenic diabetes insipidus in spite of defective V_2-receptor-mediated responses of tissue plasminogen activator release. Eur.J.Clin.Invest. 1990; 20, 72-78.

3 Brink HS, Derkx FHM, Boomsma F, Brommer EJP, Schalekamp MADH. 1-Desamino-8-D-arginine vasopressin (DDAVP) in patients with congenital nephrogenic diabetes insipidus. Neth.J. Med. 1993; 43, 5-12.

4 Brink HS, Derkx FHM, Boomsma F, Schalekamp MADH. The effects of 1-Desamino-8-D-arginine vasopressin (DDAVP) on renal hemodynamics and renin secretion. (in press)

5 Brink HS, Derkx FHM, Brommer EJP, Man in't Veld AJ, Boomsma F, Schalekamp MADH. Vascular effects of 1-Desamino-8-D-Arginine vasopressin (DDAVP) in subjects with essential hypertension or autonomic failure. (submitted)

Abstracts:

1 Brink H, Derkx F, Brommer E, Stibbe J, Kolstee H, Schalekamp M. The fibrinolytic, Factor VIII:C, von Willebrand factor, and hemodynamic responses to DDAVP in patients with hereditary nephrogenic diabetes insipidus. Thromb. Haemost. 1987; 58(1), 517.

2 Brink HS, Derkx FHM, Boomsma F, Schalekamp MADH. The effects of 1-Desamino-8-D-arginine vasopressin (DDAVP) on renal hemodynamics and renin secretion. Kidney Int. 1992; 42, 793.

3 Brink HS, Derkx FHM, Man in't Veld, Boomsma F, Schalekamp MADH. Vasodilator effects of 1-desamino-8-D-arginine vasopressin (DDAVP) in subjects with essential hypertension or autonomic failure. (in press)

Chapter one

INTRODUCTION AND AIM OF THE STUDY.

1.1.1 Historical overview.

Arginine-vasopressin (AVP) is one of the two hormones released from the posterior pituitary, which is known for being both the antidiuretic hormone and a potent vasopressor. The vasopressor action of AVP was first discovered in 1895 when Oliver and Schäfer found that a crude extract of the pituitary, caused an elevation in blood pressure in dogs[1], when administered intravenously.

Howell showed in 1898 that it was the posterior lobe of the gland that contained the vasopressor substance[2]. The antidiuretic activity of the posterior pituitary extract was discovered in 1913 [3]. This opened the possibility for treatment of patients with central diabetes insipidus. In 1928 the crude posterior pituitary extract was separated into two fractions, "oxytocin" and "vasopressin", with different biological activities[4]. "Oxytocin" was shown to have an effect on uterus contractions, whereas "vasopressin" harbored the potential to cause a rise in blood pressure, and the antidiuretic effect.

Chemical analysis of "vasopressin" from different species revealed that two types of "vasopressin" exist; being "lysine vasopressin" and "arginine vasopressin"[5]. The molecular structure of arginine-vasopressin was elucidated by Du Vigneaud[6,7]. Human "vasopressin" was proved to be arginine vasopressin, a nonapeptide with a disulfide bridge between the two cysteic acid residues (Fig.1)[8]. From this moment, the name "vasopressin" which first represented an extract of the posterior pituitary was used for its active compound.

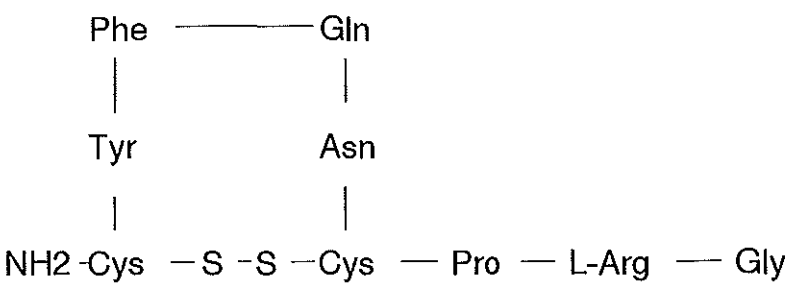

Figure 1. L-Arginine vasopressin.

1.1.2 Vasopressin metabolism.

The neurohormone AVP is stored in the posterior pituitary from where it is released into the circulation. It is synthesized in large cells in the supraoptic and paraventricular nuclei and transported along the axons in dense granules[9,10,11]. These dense granules are deposited in the nerve endings which are situated in close proximity to capillaries in the posterior pituitary[12]. On appropriate stimulation, the content of these granules is excreted into the blood stream by exocytosis[13].

Besides the AVP released from the pituitary into the circulation, it can also be found in several other places in the brain, and in the cerebro spinal fluid[14,15], where it most likely serves as a neurotransmitter and a neuromodulator[16,17,18,19]. The exact function is still obscure, but growing evidence points to a function of AVP in the preservation of memory and in the modulation of central cardiovascular regulation and the baroreflex[18,20,21,22,23,24,25,26].

Regulation of AVP excretion from the posterior pituitary depends on several influences. In normal physiology the major controlling factor is plasma osmolality[20,27,28]. A rise in plasma osmolality results in an increase in AVP excretion, which causes an increase in renal water reabsorption in the collecting ducts[29,30]. In this way AVP is the most important hormone involved in the regulation of renal water excretion[31]. AVP excretion can also be influenced by arterial blood pressure[32], total blood volume[33,34,35,36], plasma angiotensin II levels[37,38,39,40] and several other factors[41,42,43,44]. However, the physiologic importance of these factors is not clear[31,39,45,46,47,48].

Plasma AVP levels found in normal humans range from 0.5 to 10 pg/ml depending on the state of hydration. Under pathophysiological conditions like hypotensive hemorrhage and surgical procedures plasma AVP levels can increase to levels ranging from 50 to 600 pg/ml[32,49,50,51,52,53,54,55,56].

AVP is a short acting hormone with a plasma half-life of 17-35 minutes[57]. The major part of AVP is removed from the plasma through renal secretion and degradation. A minor part is cleared from plasma in the intestine and liver[58].

1.1.3 Vasopressin receptors.

Like most hormones AVP exerts its actions through stimulation of selective receptors[59,60]. From studies using different vasopressin analogues, two types of AVP receptors have been identified. According to the nomenclature proposed by Michell et al.[61],

they were called the V_1- and the V_2-receptor. Stimulation of the V_1-receptor induces phosphatidylinositol turnover and a rise in cytosolic calcium[61, 73,62,63,64]. V_2-receptor stimulation activates adenylate cyclase resulting in a rise in intracellular 3'-5'-adenosine monophosphate (c-AMP)[64,65,66].

Some examples of the variety of biologic effects of AVP that can be demonstrated in in vitro and in in vivo experiments are; vasoconstriction[67,68,69,70], induction of platelet aggregation[71], stimulation of glycogenolysis in hepatocytes[61, 73,64], inhibition of renin secretion from juxtaglomerular cells[72,73,74], the antidiuretic effect[3,66], stimulation of the release of Adrenocorticotrophic Hormone (ACTH) from the anterior pituitary[75,76,77] and elevation of plasma levels of clotting factor VIII (FVIII:C), von Willebrand factor (vWF:ag) and tissue-type plasminogen activator (t-PA:ag)[78,79]. From pharmacological experiments the type of receptor related to some of these effects is well defined. Vasoconstriction, platelet aggregation, glycogenolysis in hepatocytes, stimulation of prostaglandin production and inhibition of renin secretion by AVP, were demonstrated to be V_1-receptor related[56,61, 73,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96].

The antidiuretic effect is caused through stimulation of V_2-receptors located on the tubular cells of the renal collecting ducts[66,97]. The increase in intracellular c-AMP, following V_2-receptor stimulation, in its turn causes an increase in permeability for water of the apical membrane of these tubular cells resulting in the antidiuretic effect[29,66,98]. An exception to the sharp distinction between V_1- and V_2-receptors are the AVP-receptors involved in the corticotropin release hormone like effect of AVP in the anterior pituitary[77]. Based on work with several AVP-antagonists, Jard suggested these receptors to be called V_{1b}-receptors[99,100].

In a variety of tissues, the presence of AVP-receptors can been demonstrated by using pharmacological and autoradiography techniques (table 1). V_1-receptors were found on a great variety of cells, ranging from thrombocytes and vascular smooth muscle cells to adrenal cortical cells (table 1)[61, 73,82,84,86,101]. Up to now, V_2-receptors have only been found on tubular cells of renal collecting ducts and in several locations in the central nervous system[97,102,103].

Table 1. Biological effects of vasopressin.

Cell type	Effect	Receptor
vascular smooth muscle	vasocontriction	V_1
renal tubular cells	antidiuresis	V_2
blood platelets	aggregation	V_1
hepatocytes	glycogenolysis	V_1
juxtaglomerular cells	inhibition of renin secretion	V_1
renal mesangial cells	PGE_2-production	V_1
adrenal cortical cells	glucocorticoid production	V_1
anterior pituitary	ACTH-release	V_1
endothelium	FVIII:C release	?
	vWF:ag release	?
	t-PA release	?
brain	memory function	?

1.1.4 Cardiovascular effects of vasopressin.

AVP is one of the most potent vasoconstrictor hormones known. In in vitro experiments, it was demonstrated to be more potent than noradrenalin and angiotensin II on molecular base[67]. Yet, its function in blood pressure regulation and its possible role in the pathogenesis of hypertension remains subject of debate[56,104,105,106,107]

It was Howell[2] who described a fall in heart rate together with the increase in blood pressure, after the initial report of the effect on blood pressure of intravenous administration of crude pituitary extract in dogs. The following years it was observed from a variety of experiments that the hemodynamic effect of AVP infusion in animals is strongly affected by several factors, like the species used in the experiment and the preparation of the animal before AVP infusion. For example rats show a pressor response to much lower doses of AVP, on weight base, than dogs and the administration of anaesthetic or ganglion blocking drugs before AVP infusion can result in a much greater blood pressure response[5,56]. As AVP measurements, could only be performed by using bioassays, before the introduction of sensitive radioimmuno assays, the rat blood pressure assay, was the only officially recognized method for standardization of vasopressin preparations, and was

performed according to very strict protocols.

As mentioned above, the increase in blood pressure after AVP administration in intact animals, but also in man, is accompanied by a fall in heart rate and cardiac output[56,108,109,110,111,112,113]. As these effects on the heart rate and cardiac output, in animals, can be abolished by cutting the vagi surgically or chemically by either ganglion blocking agents or atropine, they are the result of baroreflex mediated counterregulation. When the baroreflex is blocked by either procedure, the effect of AVP on blood pressure becomes more pronounced[5,56,114,115,116]. The fall in heart rate is much greater in relation to the increase in blood pressure after AVP infusion than after administration of other vasopressor agents like angiotensin II or noradrenalin[117,118]. From these and other observations, can be concluded that AVP itself has an effect on the gain of the baroreflex[24,56,119].

Although the antidiuretic effect of posterior pituitary preparations in man was well established by many investigators after the initial report of Von den Velden in 1913[3], the pressor effect of AVP in humans was firstly demonstrated by Wagner and Braunwald in 1956[120]. In an experiment in which they administered "vasopressin" intravenously to three patients with primary autonomic insufficiency they observed a rise in arterial blood pressure together with an increase in peripheral resistance without any changes in cardiac output or heart rate. When Möhring et al[109] administered AVP to humans by continuous infusion, the same relation between a small increase in blood pressure and a marked fall in heart rate, as found in animal experiments, was observed at infusion rates resulting in plasma AVP levels of ±450 pmol/l. They also found in three patients with autonomic insufficiency, that infusion of small amounts of AVP resulting in plasma AVP levels slightly above the normal range, markedly increased blood pressure. Later, Simpson et al[121] demonstrated AVP to have marked effects on peripheral resistance and cardiac output, at plasma concentrations, that had no effect on blood pressure. From these experiments, can be concluded that also in humans, AVP is a potent vasoconstrictor, whose effects on blood pressure are largely counter balanced by the baroreflex.

In medical practice, i.v. administration of AVP is used in the treatment of gastro-intestinal bleeding[57,122]. In this situation however, AVP is not merely used for its effect on blood pressure, but rather for its effect on the portal venous pressure, which is reduced as a result of a diminished splanchnic arterial blood flow[123,124]. Unfortunately, the therapeutic use of AVP, in this setting, is frequently limited because of abdominal discomfort and angina pectoris, experienced by the patient. Another side effect frequently seen is a

pronounced vasoconstriction of the peripheral arteries, resulting in cold painful digits[110].

In several studies, marked differences in vasoconstrictor effect of AVP in different vascular beds were observed[125,126,127,128]. In dogs the blood flow reduction by AVP is most pronounced in the splanchnic bed, skeletal muscle, compact bone and skin[56,127,129]. Although AVP has long been thought to cause coronary vasoconstriction the reduction in coronary perfusion is most likely the result of the decrease in heart rate and left ventricular work[56,130,131]. Total renal perfusion, has been reported not to be affected by AVP[125,127,130]. However, other investigators found a shift in renal blood flow from outer cortex to inner cortex[132,133,134]. Not only regional differences in vasoconstrictor effect of AVP have been found, even an endothelium dependent vasodilator action has been demonstrated, in vitro, in the basilar artery of dogs[135].

Extensive research has been done to elucidate a possible role of AVP in the pathogenesis of hypertension. Up to now, only conflicting results have been obtained[56,104]. AVP was demonstrated to promote the development of hypertension in DOCA salt hypertensive rats[136,137,138,139], and AVP V_1-receptor antagonists were demonstrated to reduce blood pressure in spontaneously hypertensive rats[140]. In humans, plasma AVP levels were found not to be elevated in essential hypertension[56,141]. However, administration of a V_1-antagonist to hypertensive patients with end-stage renal insufficiency, has been demonstrated to cause a reduction in blood pressure[142]. In patients with malignant hypertension elevated plasma AVP levels can be found, but patients with inappropriate ADH secretion (SIADH), who have chronically elevated plasma AVP levels, are usually not hypertensive[143,144]. Although the precise role of AVP in blood pressure regulation in man is not clear, most evidence points at a function in short term blood pressure regulation[56,105].

1.2.1 1-Desamino-8-D-arginine vasopressin (DDAVP).

Du Vigneaud et al. were the first to show that alterations in the aminoacid composition of AVP, result in major changes in biological activity and specificity[145]. Since then, a large number of AVP analogs have been synthesized, with different agonist and antagonist actions on both V_1- and V_2-receptors [146,147,148,149,150,151,152,153,154,155,156].

1-Desamino-8-D-arginine vasopressin (DDAVP), first synthesized in 1965, is a vasopressin analog with specific antidiuretic properties and virtually no vasopressor effects[146,157]. The relative affinity of DDAVP for V_2-receptors is 1.6 times that of AVP, where

its relative affinity for V_1-receptors is only 0.009 times that of AVP[145]. The enhanced antidiuretic effect is most likely due to the deamination of hemicysteine in position 1. Substitution of the D-isomer of arginine for L-arginine at position 8 resulted in the loss of vasopressor action[157, 158,159]. Most likely this substitution also accounts for the prolongation of the plasma halflife of AVP as compared to AVP, as a result of impaired enzymatic cleavage of the molecule at the proline-arginine junction[145].

Nowadays, DDAVP is the drug of first choice in the treatment of central diabetes insipidus for several reasons. First of all the loss of vasopressor action considerably reduced the amount of side-effects as compared to treatment with AVP. Furthermore, its prolonged activity and the possibility of intra-nasal administration, made its use much more convenient to the patient[145,159,160,161,162,163].

1.2.2 DDAVP; effects on fibrinolysis and blood clotting.

Several vasoactive drugs, like AVP, adrenaline, and nicotinic acid, are known to increase plasma levels of tissue-type plasminogen activator (t-PA:ag) and clotting factor VIII (FVIII:C) in man[78,164]. A possible explanation of the phenomenon that such diverse drugs can elicit the same effect, is that the release of both FVIII:C and t-PA:ag are caused by their effect on vascular motility. Both Mannucci et al.[78] and Cash et al.[165], more or less independent of each other, evaluated this hypothesis in healthy volunteers, by infusion of DDAVP, which at that time was considered to have no vascular effects. In both experiments, intravenous administration of DDAVP resulted in an even greater rise of plasma levels of t-PA:ag than that observed after AVP infusion[78,165,166]. In Mannucci's experiment this phenomenon was also observed considering FVIII:C and vWF:ag[78]. Not only in healthy volunteers, but also in patients with mild to moderate hemophilia A and von Willebrand's disease, DDAVP can cause a rise in FVIII:C and vWF:ag[167,168,169,170]. As a result of this line of research, nowadays, DDAVP is widely used in the treatment of these patients, in order to stop and prevent hemorrhage, without the need for use of transfusions[171,172,173].

Not, only in patients with hemophilia or von Willebrand's disease but also in a variety of other clotting disorders, both inherited and acquired, DDAVP proved to be very effective in improving hemostasis[145,174,175,176,177,178,179,180,181,182]. DDAVP can even be used in patients without prior clotting disorders in order to reduce intraoperative blood loss during major surgery[183].

1.2.3 Hemodynamic effects of DDAVP

The initial reports on the effects of DDAVP on blood clotting and fibrinolysis claimed that no side effects of intravenous administration of DDAVP were observed[78,165,166]. However, shortly afterwards facial flushing and mild palpitations were reported as side-effects of the use of DDAVP[167,184]. Further evaluation of these side-effects revealed that intravenous administration of 0.3-0.4 g/kg DDAVP in healthy volunteers results in facial flushing, a lowering of diastolic blood pressure without any effect on systolic blood pressure, and a marked increase in heart rate[185,186,187]. These hemodynamic changes were accompanied by a marked rise in plasma renin and plasma noradrenalin levels[186, 186,187]. So it seems to be that DDAVP can cause vasodilatation.

In the original paper, in which these effects were described, it was suggested that DDAVP possibly acted as an antagonist of endogenous AVP at the V_1-receptor, as their hemodynamic effects are directly opposite of each other[186, 186]. However this would imply that the effects of DDAVP on FVIII:C, vWF:ag and t-PA:ag are based on another mechanism than the hemodynamic effects, as they are similar to the effects of AVP.

1.3 Aim of the thesis.

Our interest in DDAVP was raised after the reports on the side effects observed after intravenous administration of 0.3-0.4µg/kg DDAVP, used in order to increase plasma levels of FVIII:C and vWF:ag. Initially, the facial flushing and palpitations of which the patients complained were considered to be unpleasant but merely unimportant. In our view however, it opened a new approach to investigate the mechanism by which AVP is involved in the regulation of blood pressure in man.

As discussed in section 1.2.3, the first question that arose was whether it is V_1-receptor antagonism or V_2-receptor agonism that invoked the hemodynamic effects of DDAVP. Either answer to this question would open new possibilities in AVP research. If DDAVP would turn out to be a V_1-receptor antagonist of endogenous AVP, then it might be possible to use DDAVP in order to investigate the possible role of AVP in the pathogenesis of hypertension in man. When it is V_2-receptor stimulation that causes the hemodynamic effects of DDAVP, new questions about this receptor would rise. As V_2-receptors have only been demonstrated to be present in the kidney, the localization of the V_2-receptor that causes the hemodynamic changes after DDAVP infusion would need to be clarified. Furthermore, the question would arise whether V_2-receptor stimulation by AVP occurs

under physiologic and pathophysiologic conditions, and whether this is of importance to the hemodynamic effects of AVP in man.

Also the mechanism by which DDAVP invokes the changes in blood pressure and heart rate needs to be elucidated. Most likely the decrease in diastolic blood pressure and the increase in heart rate are the result of vasodilatation, up to now this is insufficiently substantiated in man.

With this in mind we studied both the hemodynamic effects and the effects on FVIII:C, vWF:ag and t-PA:ag of intravenous administration of DDAVP in man. Our objective was, to elucidate the mechanism by which DDAVP exerts these effects, and to evaluate at what plasma levels of DDAVP these effects can be demonstrated. This thesis contains the results of these studies.

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Chapter two

VASCULAR EFFECTS OF 1-DESAMINO-8-D-ARGININE VASOPRESSIN (DDAVP) IN HEALTHY VOLUNTEERS

2.1 Summary

Arginine vasopressin (AVP) causes antidiuresis via stimulation of AVP V_2-receptors on renal tubular cells, and vasoconstriction via extrarenal and intrarenal vascular V_1-receptors. There is evidence to suggest that AVP also stimulates vascular V_2-receptors. Data on vascular effects in normal subjects that are possibly mediated by V_2-receptors are scarce.

We studied the effects of the selective vasopressin V_2-receptor agonist 1-desamino-8-D-arginine vasopressin (DDAVP) on blood pressure, heart rate and the plasma levels of renin, clotting factor VIII (FVIII:C), von Willebrand factor (vWF:ag), tissue-type plasminogen activator activity (t-PA:ag), tissue-type plasminogen activator antigen (t-PA:ag), plasminogen activator inhibitor (PAI), and noradrenaline, adrenaline, and dopamine in healthy volunteers. Twelve subjects were randomly assigned to DDAVP (400 ng/kg i.v. in 10 minutes) or solvent. DDAVP caused a fall in diastolic blood pressure and a rise in heart rate while systolic pressure was unchanged. The rise in heart rate was associated with increments in plasma renin and noradrenaline. These responses are propably baroreflex-mediated. In view of the small drop in blood pressure however, the rise in heart rate after DDAVP was disproportionally high. Adrenaline and dopamine did not change. FVIII:C and vWF:ag rose as did t-PA:ag and t-PA:act. The rise of plasma t-PA:act was associated with the disappearance of PAI. It is unlikely that the infusion of 400 ng/kg DDAVP resulted in plasma levels high enough to cause an antagonistic effect at the V_1-receptor. We conclude that the responses described here are caused by V_2-receptor stimulation. The disproportionate rise in heart rate after DDAVP may be related to central V_2-receptor stimulation, as has been described in animals.

2.2 Introduction

Arginine-vasopressin (AVP) is a peptide hormone with a number of biological effects. These effects are brought about through stimulation of two different types of receptors[1]. Binding of AVP to the V_1-receptor causes a rise in cytosolic calcium and an increase in phosphatidylinositol turnover. Activation of vascular V_1-receptor leads, through this pathway, to vasoconstriction[2]. The antidiuretic effect of AVP is caused by stimulation of the V_2-receptor located on the tubular cells of the renal collecting ducts. Binding of AVP to this receptor is followed by activation of adenylate-cyclase, which causes an increase in

intracellular 3'-5'adenosine monophosphate (c-AMP)[3].

1-Desamino-8-D-arginine vasopressin (DDAVP) is a synthetic analog of AVP which is virtually devoid of vasopressor activity but has retained the antidiuretic properties of AVP[4,5]. For this reason DDAVP is widely used as a substitute for AVP in patients with central diabetes insipidus[6,7].

Mannucci et al.[8] and Cash et al.[9,10] reported that intravenous (i.v.) administration of a high dose of DDAVP in healthy volunteers, caused a rise in the plasma levels of tissue-type plasminogen activator (t-PA), clotting factor VIII (FVIII:C), and von Willebrand factor (vWF:ag). Because of its effects on FVIII:C and vWF:ag, DDAVP is used in patients with haemophilia A and von Willebrand's disease, to stop or prevent bleeding[11]. In this setting, i.v. administration of DDAVP caused facial flushing and palpitations as side-effects[12,13]. These effects are propably related to a vasodilator action of DDAVP[14,15].

The present study was carried out to quantify the effects of i.v. administration of DDAVP on blood pressure, heart rate, and the plasma levels of renin, FVIII:C, vWF:ag, t-PA, and noradrenaline, adrenaline, and dopamine in healthy volunteers in a double blind placebo-controlled study design.

2.3 Methods

2.3.1 Subjects

Twelve male healthy volunteers (aged 23-55 years) from the medical staff of our department participated in the study. They were randomly assigned to either DDAVP or placebo in a double blind fashion. They had not used any kind of medication for at least four weeks prior to the study. Informed consent was obtained from all participants. The study protocol was approved by the medical ethics committee of our hospital.

2.3.2 DDAVP-tests

At the beginning of the test, a Teflon cannula was inserted into the antecubital vein on each side. One cannula was used for infusion, the other to obtain blood samples.

After one hour of supine bedrest, DDAVP or placebo was infused in exactly 10 minutes. Before each test, DDAVP (Minrin, Ferring, Malmö, Sweden) or placebo (DDAVP solvent, provided by Ferring, Malmö, Sweden) were diluted in 100 ml saline. The final DDAVP solution contained 400 ng DDAVP per kg body weight.

Blood pressure and heart rate were recorded at 5 minute intervals, using an automatic oscillometric device (Accutor, Datascope Corp., Paramus, N.J.). Blood samples were drawn 10 minutes before the infusion and at 0, 10, 20, 30, and 60 minutes.

2.3.3. Assays

Blood samples were centrifuged for 10 minutes at 5000 rpm. The supernatant was decanted and snap frozen. Plasma was stored at -70°C until assay. The following substances were assayed: renin, noradrenaline, adrenaline, dopamine, FVIII:C, vWF:ag, tissue type plasminogen activator antigen (t-PA:ag), tissue-type plasminogen activator activity (t-PA:act), and plasminogen activator inhibitor (PAI).

Renin was measured by its ability to cleave angiotensin I from sheep renin substrate[16]. Noradrenaline was measured by high performance liquid chromatography with electrochemical detection[17]. Factor VIII:C was measured in a one stage assay[18], and vWF:ag was determined by rocket immunoelectrophoresis. T-PA:ag was measured with an enzyme-immunoassay, using a kit (Immulyse tPA) purchased from Biopool (Umeå, Sweden). T-PA:act was assayed in euglobulin fractions of plasma using a chromogenic substrate[19]. PAI was determined using a titration method which measures remaining free t-PA after the addition of t-PA to diluted plasma[20].

2.3.4.Statistical analysis

Baseline values of the data in both groups were compared using a Student-t test. The results of the infusion of placebo and DDAVP were compared in an analysis of variance for repeated measurements in the same subject. Plasma renin was analysed after logarithmic transformation.

2.4 Results

Baseline values of blood pressure, and heart rate, and the plasma levels of renin, noradrenaline, adrenaline, dopamine, FVIII:C, vWF:ag, t-PA:act, t-PA:ag, and PAI were not different between the DDAVP and placebo groups.

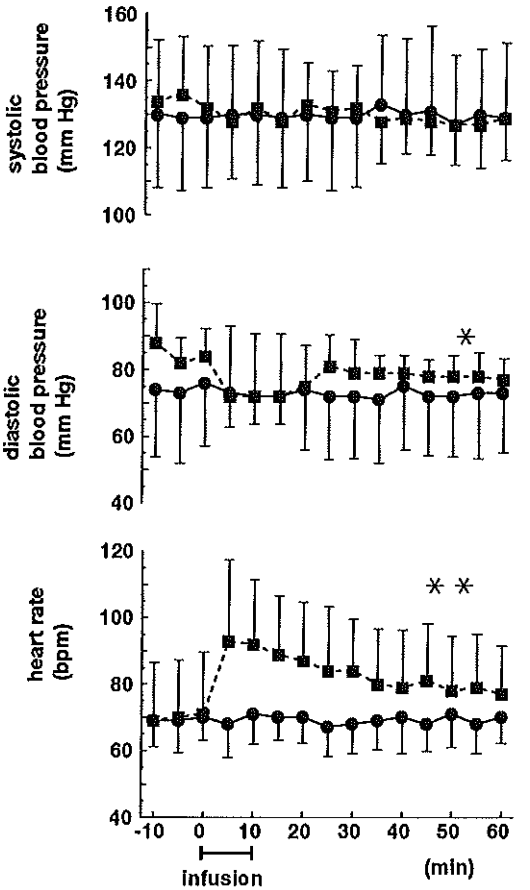

Figure 1. Effects of DDAVP (400 ng/kg i.v. in 10 minutes)(■- - -■) and placebo (●———●) on blood pressure and heart rate.
* $p < 0.05$, ** $p < 0.001$.

All volunteers receiving DDAVP experienced facial flushing 2 - 3 minutes after the beginning of the infusion. In none of the controls facial flushing was noted. In the placebo group heart rate and blood pressure did not change (Fig.1). The plasma levels of renin, noradrenaline, adrenaline, dopamine, FVIII:C, vWF:ag, t-PA:ag, t-PA:act, and PAI also did not change (Fig.2 - 5). DDAVP caused a 31% (18 - 44)(mean, 95% confidence interval, $p < 0.001$) increase in heart rate together with a 14% (2-20, $p < 0.05$) reduction in diastolic blood pressure (Fig.1). Systolic blood pressure did not change (Fig.1).

Plasma renin rose rapidly to 130% (61 - 200, $p < 0.001$) above baseline at the end

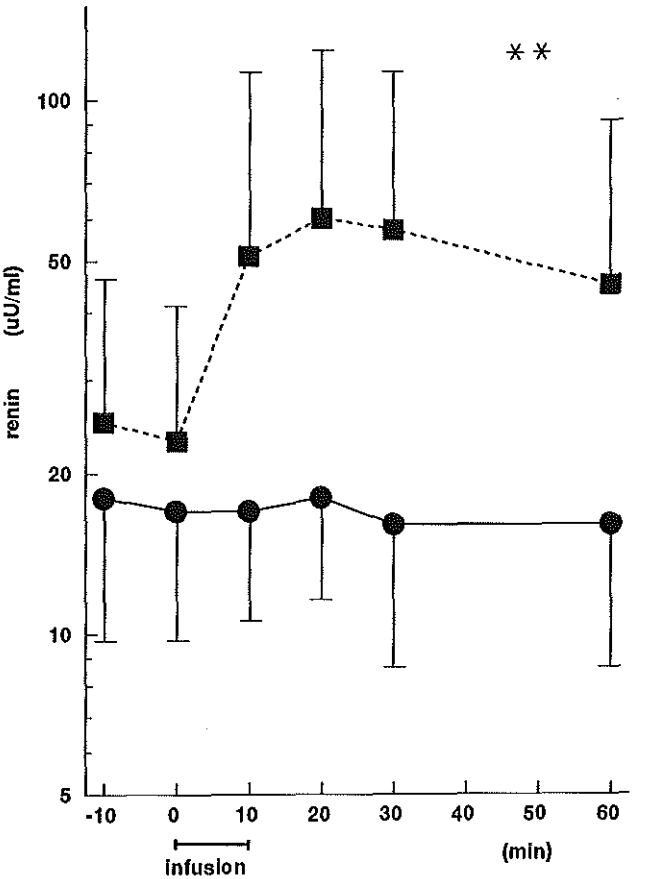

Figure 2. Effects of DDAVP (400 ng/kg i.v. in 10 minutes)(■- - -■) and placebo (●——●) on plasma renin. ** $p < 0.001$.

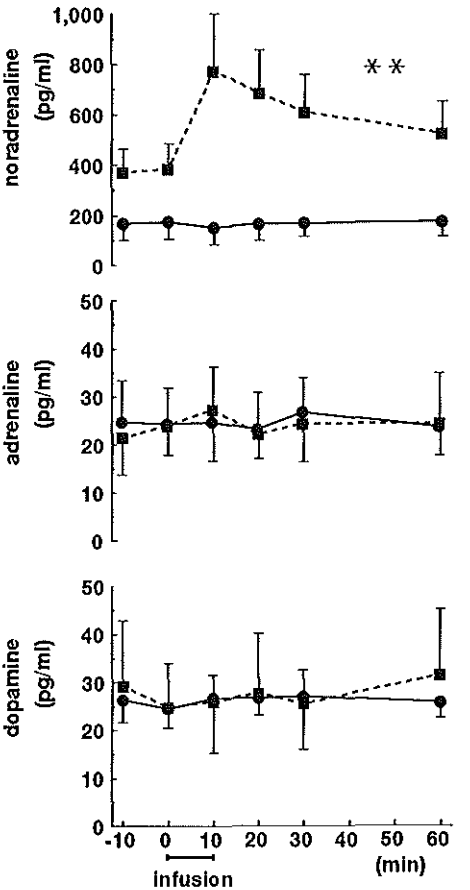

Figure 3. Effects of DDAVP (400 ng/kg i.v. in 10 minutes)(■- - -■) and placebo (●———●) on plasma noradrenaline, adrenaline, and dopamine. ** $p < 0.001$.

of the DDAVP infusion (10 minutes), and rose further to a maximum at 20 minutes (Fig.2). Plasma noradrenaline followed the same pattern (Fig.3). Adrenaline and dopamine did not change. FVIII:C and vWF:ag rose more gradually than renin and noradrenaline. vWF:ag rose to 668% (125 - 1212, $p<0.05$), and FVIII:C rose to 85% (7 - 163, $p<0.001$) above baseline at 60 minutes (Fig.4). T-PA:ag rose to 173% (1 - 348, $p < 0.001$) above baseline at 20 minutes (Fig.5).

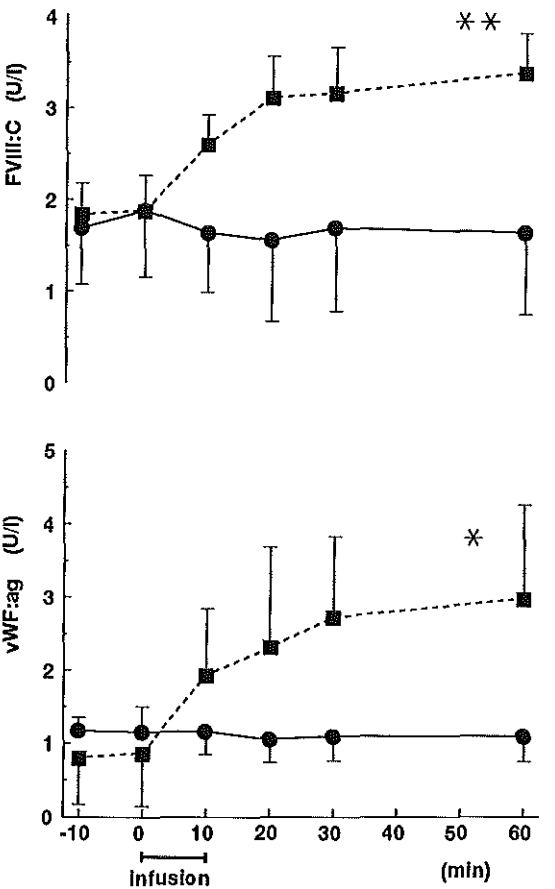

Figure 4. Effects of DDAVP (400 ng/kg i.v. in 10 minutes)(■- - -■) and placebo (●———●) on plasma FVIII:C and vWF:ag.
* $p < 0.05$, ** $p < 0.001$.

Prior to DDAVP infusion, t-PA:act was hardly detectable in plasma. In all subjects receiving DDAVP, t-PA:act rose, whereas at the same time PAI practically disappeared from plasma (Fig.5). At the end of the observation period (60 minutes) t-PA:ag and t-PA:act began to return to baseline and at the same time PAI reappeared.

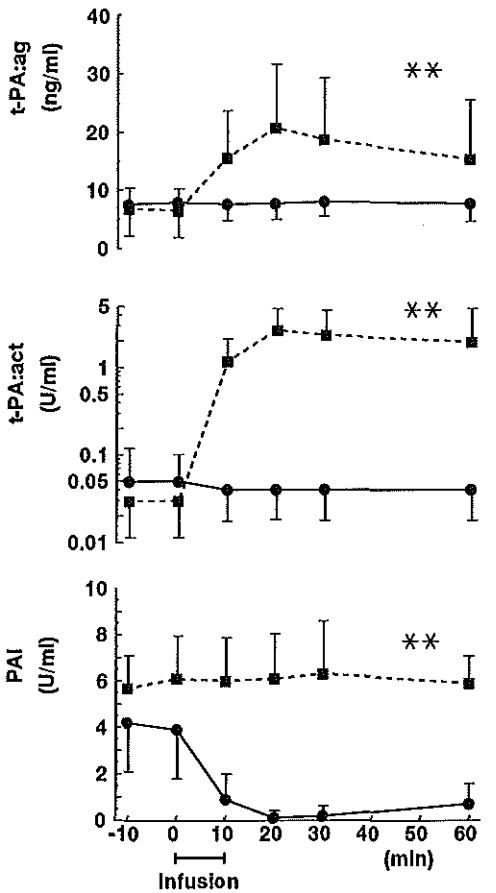

Figure 5. Effects of DDAVP (400 ng/kg i.v. in 10 minutes)(■- - -■) and placebo (●———●) on plasma t-PA:ag, t-PA:act, and PAI.
* $p < 0.05$, ** $p < 0.001$.

2.5 Discussion

In this study we confirmed the effects of DDAVP in healthy volunteers reported by others[15,21]. As compared to the studies of Mannucci et al.[22] and Williams et al.[15] this study provides more detailed data on the haemodynamic responses to DDAVP. These responses are likely to be caused by a vasodilator action of DDAVP. The increments in heart rate and in plasma renin and noradrenaline are propably caused by baroreflex stimulation. The increase in heart rate after DDAVP appears to be greater than with other vasodilators, considering the small drop in blood pressure. Increments in heart rate as big as we observed have also been reported with other vasodilators, but only when the changes in blood pressure were more marked than with DDAVP[22,23,24]. Considering the baroreflex as a closed loop negative feedback system, the disproportionate effect of DDAVP can be characterised as an increase in the gain of the baroreflex. A similar action on the baroreflex has been attributed to AVP[25,26,27,28,29]. Infusion of AVP, which acts as a potent vasoconstrictor, causes little rise in blood pressure, but, in spite of this small effect on pressure, the heart rate falls markedly. AVP and DDAVP may therefore affect the baroreflex through a similar mechanism. This possibility was addressed by Unger et al.[30] who found that intravenous infusion of AVP in rats caused an increase in the gain of the baroreflex through simulation of V_2-receptors in the brain.

The increase in plasma renin we observed, most likely reflects renal sympathetic stimulation[31]. Renal vasodilatation may also play a role. A direct V_2-receptor-mediated increase in renin secretion is unlikely since only V_1-receptors seem to be present on juxtaglomerular cells[3].

The changes in FVIII:C and vWF:ag we observed, are similar to those described by others[32,33]. The time course of these changes differed from the time course of renin and noradrenaline changes. This may indicate that the regulation of the endothelial release of FVIII:C and vWF:ag is essentially different from the regulation of renin and noradrenaline release. However differences in plasma half life can also contribute to the observed changes over time.

Since t-PA binds to PAI[20,34], the increase in t-PA:act in plasma underestimates the total amount of active t-PA released from endothelium. The decrease in t-PA:act at the end of the experiment appears to be due to a decrease in t-PA:ag as well as the reappearance of PAI.

Derkx et al.[14] found that DDAVP at concentrations in the order of micrograms per

ml was an antagonist AVP at the V_1-receptor of blood platelets and juxtaglomerular cells. Considering the distribution volume of DDAVP[35,36] the amount of DDAVP we used in this study it too small to give such high levels. It is therefore unlikely that antagonism of endogenous AVP at the V_1-receptor can account for the effects of DDAVP. Thus the effects of DDAVP in healthy volunteers we describe here appear to be caused by V_2-receptor stimulation.

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Chapter three

1-DESAMINO-8-D-ARGININE VASOPRESSIN (DDAVP) IN PATIENTS WITH CONGENITAL NEPHROGENIC DIABETES INSIPIDUS.

3.1 Summary

In healthy subjects, i.v infusion of the selective V_2-vasopressin receptor agonist 1-desamino-8-D-arginine vasopressin (DDAVP 400 ng/kg in 10 minutes) causes a marked increase in heart rate with a slight decrease in diastolic blood pressure. These haemodynamic responses are associated with increments in the plasma levels of renin, noradrenaline (NA), clotting factor VIII (FVIII:C), von Willebrand factor (vWF:ag), and tissue-type plasminogen activator (t-PA), and a fall in the plasma level of plasminogen activator inhibitor (PAI). None of these were observed in three patients with congenital nephrogenic diabetes insipidus (NDI), who had a genetic defect of the V_2-receptor. Plasma AVP levels in these patients were normal or slightly elevated, which makes it unlikely that the lack of DDAVP responsiveness was caused by down-regulation of vasopressin V_1-receptors. In one NDI patient, arginine vasopressin (AVP) was given in incremental doses, 62.5 - 4000 pg/kg/min. The heart rate and blood pressure responses to AVP were normal, indicating the absence of an V_1-receptor defect. The responses of vWF:ag and t-PA to venous occlusion in the patients with NDI were similar to the responses in five healthy volunteers, which indicates that in NDI the endothelial release of both vWF:ag and t-PA is normal. We conclude that DDAVP causes its effects on heart rate and blood pressure, and on the plasma levels of renin, noradrenaline, FVIII:C, vWF:ag, and t-PA through V_2-receptor stimulation.

3.2 Introduction

The antidiuretic hormone, arginine vasopressin (AVP), causes its effects through stimulation of two different types of receptors[1]. Michell et al.[2] postulated that the vasopressor action of AVP is mediated through stimulation of the V_1-receptor, which causes an increase in phosphatidylinositol breakdown and a rise in cytosolic calcium. The V_2-receptor is responsible for the antidiuretic action of AVP. Binding of AVP to the V_2-receptor, causes activation of adenylate cyclase which results in a rise in intracellular 3'-5'adenosine monophosphate (c-AMP).

1-Desamino-8-D-arginine vasopressin (DDAVP) is a synthetic analog of AVP, which has retained the antidiuretic, but not the vasopressor action of AVP[3]. Because of these properties, DDAVP is used in the treatment of central diabetes insipidus[4]. Intravenous (i.v.) administration of DDAVP causes an increase in plasma levels of clotting factor VIII[5] (FVIII:C), von Willebrand factor[6] (vWF:ag) and tissue-type plasminogen activator[5,7] (t-

PA:ag). As t-PA in plasma binds to plasminogen activator inhibitor (PAI)[8], an increase in the plasma level of t-PA will lead to a fall in plasma PAI[9]. Because of its effects on FVIII:C and vWF:ag, DDAVP can be used in patients with haemophilia A or von Willebrand's disease to stop or prevent bleeding[10]. Initially, only an increase in heart rate and mild facial flushing were reported to be side effects of DDAVP treatment[11].

DDAVP (400 ng/kg body weight i.v. in 10 minutes), which is a dose that is also used in patients with bleeding disorders, causes a marked increase in heart rate and a minor decrease in diastolic blood pressure together with a rise in the plasma levels of renin and noradrenaline[12,13]. These findings, and the results of in vitro experiments, made Derkx et al.[12] suggest that DDAVP may act as a V_1-receptor antagonist. However, in a pharmacokinetic study, we found that the doses of DDAVP we used resulted in plasma levels three orders of magnitude lower than the DDAVP concentrations required for V_1-receptor antagonism in vitro[9].

Congenital nephrogenic diabetes insipidus (NDI) is an X-linked disorder in which the renal collecting tubules are insensitive to stimulation by AVP leading to marked polyuria[14]. In contrast to central diabetes insipidus, patients with NDI do not respond to treatment with AVP or DDAVP. The molecular basis of this disease is a genetic defect of the vasopressin V_2-receptor or a defect at the post-receptor level. Kobrinsky et al.[15] reported that i.v. DDAVP in patients with NDI did not result in a rise in the plasma levels of FVIII:C and vWF:ag or facial flushing. In our previous work, we found that in NDI not only the plasma levels of FVIII:C and vWF:ag, but also blood pressure, heart rate and plasma levels of t-PA did not change after DDAVP i.v.[9]. These results were later confirmed by Bichet et al.[16].

From these studies it was concluded that the haemodynamic effects and the increases in FVIII:C, vWF:ag, and t-PA were caused through V_2-receptor stimulation[9,15,16]. However, there are several other possible explanations for the absence of the effects of DDAVP in NDI. First, patients with NDI might have chronically elevated plasma AVP levels leading to down-regulation of V_1-receptors. Second, it is possible that not only the V_2-receptor but also the V_1-receptor is not functioning properly in NDI. Third, in NDI the release of vWF:ag and t-PA from endothelium might be defective.

In this study, we investigated the haemodynamic effects of DDAVP, together with its effects on blood clotting and fibrinolysis in three patients with NDI. The functional integrity of the vascular V_1-receptor was tested in one of these patients. The release from endothelium of vWF:ag and t-PA, in response to venous occlusion, was studied in normal

volunteers and in the patients with NDI.

3.3 Patients and methods

3.3.1 Patients

Three patients with NDI participated in this study. All three had a lifelong history of polyuria and polydipsia. Patients 1 and 2, aged 28 and 30 years, were brothers who both suffered several episodes of severe dehydration in childhood resulting in mental retardation. The diagnosis of NDI was established when they were 4 and 6 years old. Patient 3, aged 32 years, had several male relatives, who had a daily urine output of 15 to 20 litres. In this patient the diagnosis of NDI was established at an early age, so adequate fluid replacement therapy prevented dehydration in early childhood.

In all three patients NDI was diagnosed during a water deprivation test, in which they showed a defective urine concentrating ability and an absent rise in urine osmolality after the intramuscular administration of AVP. On physical examination prior to the present study, the three patients with NDI did not show any sign of dehydration. The results of urine analysis and serum electrolyte, urea and creatinine concentrations were normal. All three patients gave informed consent prior to the study. Because patients 1 and 2 were mentally retarded, informed consent was also provided by their parents. The study protocol was approved by the Medical Ethics Review Committee of our hospital.

3.3.2 DDAVP-test

In both arms an i.v. cannula was inserted in the antecubital vein, of which one was used for DDAVP infusion and the other for the collection of blood, at the beginning of the experiment. After one hour of supine bedrest, 400 ng/kg body weight of DDAVP (Ferring, Malmö, Sweden) dissolved in 100 ml saline, was infused in 10 minutes. Blood samples were collected at -10, 0, 10, 20, 30, and 60 minutes from the beginning of the DDAVP infusion. Blood pressure and heart rate were measured at 5-minute intervals. In all blood samples osmolality, plasma renin, noradrenaline, FVIII:C, vWF:ag, t-PA antigen (t-PA:ag), and plasminogen activator inhibitor (PAI) were measured. AVP was measured in the blood samples collected prior to the DDAVP infusion. The data from the DDAVP-tests in the three patients with NDI were compared with the effects of DDAVP in normal volunteers which have been described previously[12,17].

3.3.3 AVP-infusion

In patient 3 the effects of AVP infusion on heart rate and blood pressure were studied. After one hour of bed rest, AVP (Ferring, Malmö, Sweden) was infused through an i.v. cannula. Every 30 minutes, the rate of infusion was doubled from 62.5 pg/kg/min AVP up to 4000 pg/kg/min AVP. Heart rate and blood pressure were measured at 5 minute intervals. All heart rate and blood pressure measurements, in both the DDAVP test and the AVP infusion, were performed using an automatic device (Accutor I, Datascope Corp., Paramus, NJ)[18].

3.3.4 Venous occlusion test

The three patients with NDI and five normal male volunteers (aged 25 - 41 years) were subjected to a venous occlusion test. After introduction of a teflon cannula into the antecubital vein, a manometer cuff was wrapped around the upper arm. After 30 minutes of resting, the cuff was inflated to 100 mm Hg for a duration of 15 minutes. Blood was drawn from the cannula just before inflation, and just before deflation of the cuff. The blood samples were assayed for vWF:ag, t-PA:ag, and PAI. Cannulation of the cubital vein of the arm subjected to venous occlusion did not give rise to the development of haematomas in any of the subjects.

3.3.5 Assays

Renin was measured by its ability to cleave angiotensin I from sheep renin substrate[19]. Noradrenaline was measured by high performance liquid chromatography[20]. Factor VIII:C was measured in a one stage assay[21] and vWF:ag was determined by rocket immunoelectrophoresis. T-PA:ag was measured with an enzyme-immunoassay, using a kit (Immulyse tPA) purchased from Biopool (Umeå, Sweden). PAI was determined using a titration method which measures remaining free t-PA after the addition of t-PA to diluted plasma[22]. AVP was measured by radioimmunoassay (Incstar, Stillwater, Minnesota, U.S.A.), after extraction from plasma using a Sep-Pak C-18 column. The lower limit of detection was 2 pg/ml. The recovery of AVP added to AVP free plasma was 95%.

3.4 Results

3.4.1 DDAVP-test

The effects of DDAVP in both the normal volunteers and the three patients with NDI, on blood pressure, heart rate and the plasma levels of renin, noradrenaline, FVIII:C, vWF:ag, t-PA:ag, and PAI are depicted in Figs. 1-4[12,17]. To compare the effects of DDAVP

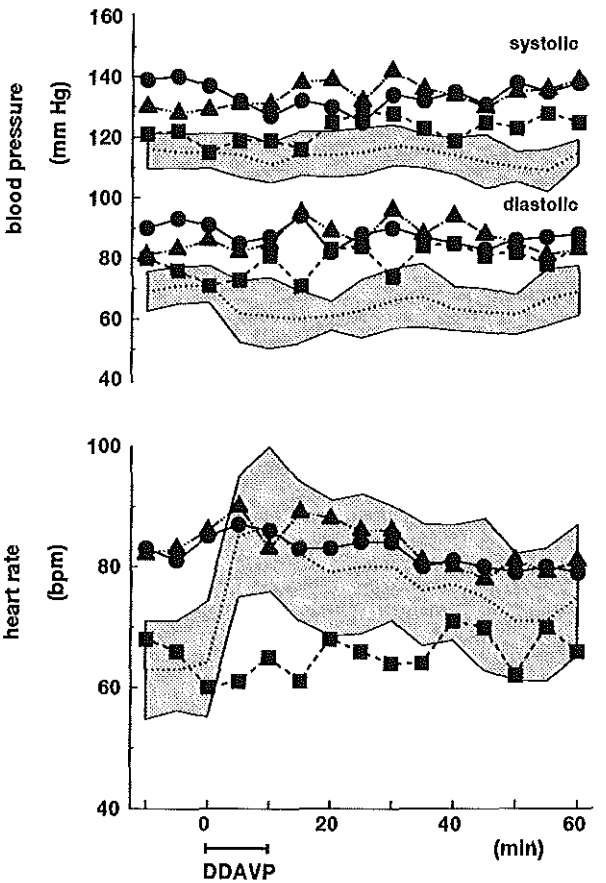

Figure 1. Effect of DDAVP (400 ng/kg i.v. in 10 minutes) on blood pressure and heart rate in patients 1 (●——●), 2 (■- - -■), and 3 (▲-·-·-·▲) with congenital nephrogenic diabetes insipidus, and 6 normal volunteers. The dotted line and the shaded area represent the geometric mean ± sd of the data from the volunteers[12].

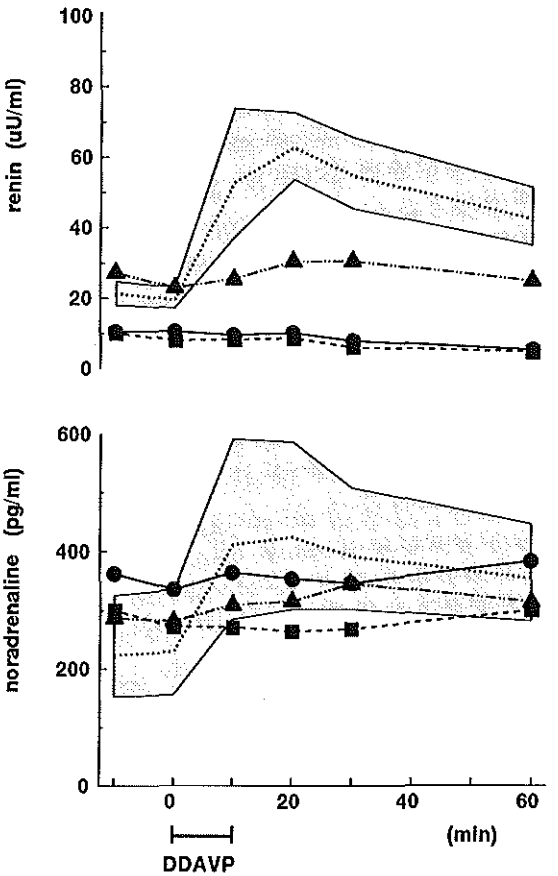

Figure 2. Effect of DDAVP (400 ng/kg i.v. in 10 minutes) on the plasma levels of renin and noradrenaline in patients 1 (●——●), 2 (■- - -■), and 3 (▲-·-·-·▲) with congenital nephrogenic diabetes insipidus, and 6 normal volunteers. The dotted line and the shaded area represent the geometric mean ± sd of the data from the volunteers[12].

in patients with NDI with those in the normal volunteers all data were expressed as their percentage of baseline values (t = 0 min). The geometric mean and 95 % confidence intervals of these effects in the normal volunteers are given in Tables 1 and 2, and are compared with the effects in the patients.

Before the administration of DDAVP, heart rate and blood pressure had stabilized in the three patients with NDI (Table 1), indicating that they had reached basal conditions.

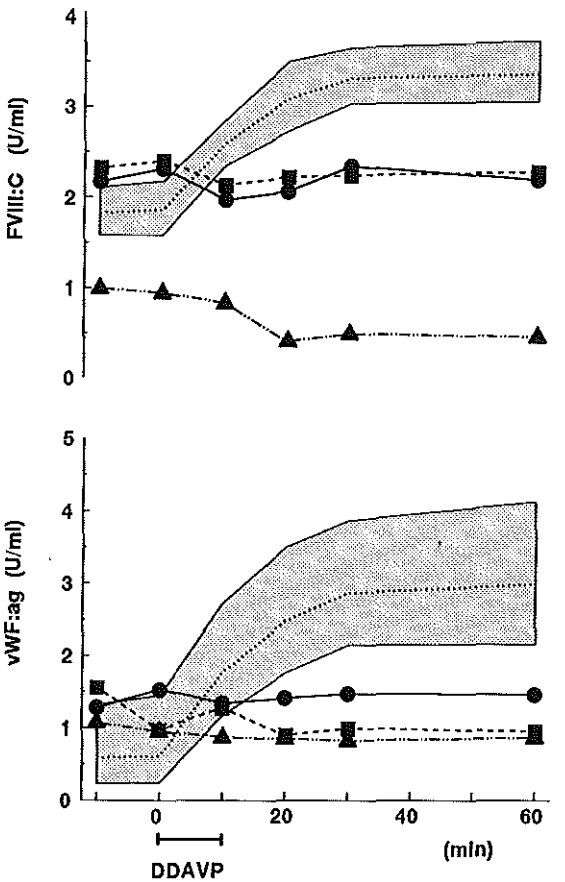

Figure 3. Effect of DDAVP (400 ng/kg i.v. in 10 minutes) on the plasma levels of clotting factor VIII (FVIII:C) and von Willebrand factor (vWF:ag) in patients 1 (●——●), 2 (■- - -■), and 3 (▲-·-·-·▲) with congenital nephrogenic diabetes insipidus, and 6 normal volunteers. The dotted line and the shaded area represent the geometric mean ± sd of the data from the volunteers[17].

DDAVP infusion had no effect on blood pressure in the NDI patients and caused a slight decrease in diastolic blood pressure in the healthy volunteers. The marked increase in heart rate in the normal volunteers was absent in the patients with NDI (Table 1). After DDAVP the plasma levels of renin, noradrenaline, FVIII:C, vWF:ag, and t-PA:ag showed a considerable increase in the normal volunteers, whereas PAI decreased. These effects were not observed in the patients with NDI (Tables 1 and 2).

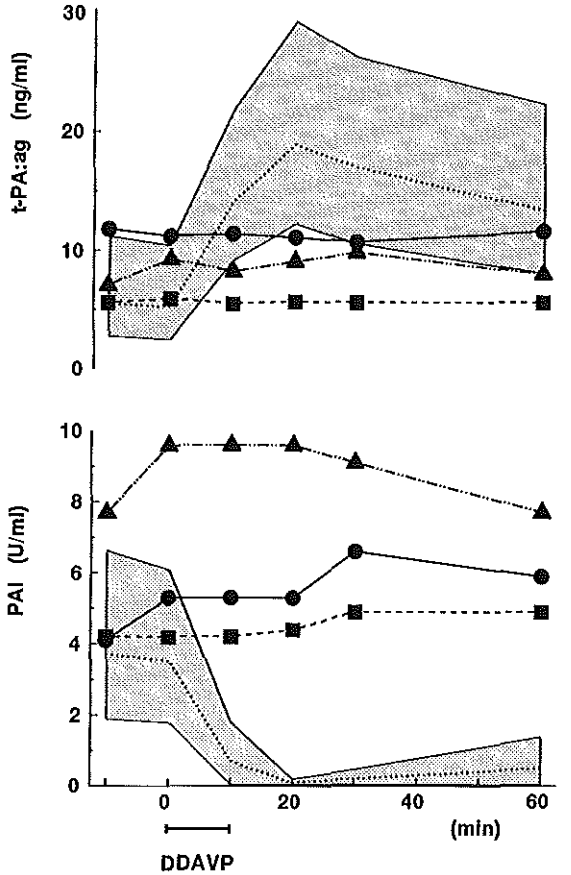

Figure 4. Effect of DDAVP (400 ng/kg i.v. in 10 minutes) on the plasma levels of tissue-type plasminogen activator antigen (t-PA:ag) and plasminogen activator inhibitor in patients 1 (●———●), 2 (■- - -■), and 3 (▲—·—·—·▲) with congenital nephrogenic diabetes insipidus, and 6 normal volunteers. The dotted line and the shaded area represent the geometric mean ± sd of the data from the volunteers[17].

3.4.2 AVP assay

Normal values of plasma AVP in our laboratory, in 16 normal subjects (aged 18-48 years), ranged from 2 - 6 pg/ml. The plasma levels of AVP in patients 1 (11 pg/ml) and 2 (14 pg/ml) were slightly above normal. Plasma AVP in patient 3 (3 pg/ml) was within the normal range.

Table 1. Effect of DDAVP in 6 normal volunteers and 3 patients with NDI.

time (min)	-10	0	10	20
Systolic blood pressure				
volunteers	100 (92-109)	100	97 (83-112)	99 (91-108)
patient 1	101	100	93	95
patient 2	105	100	103	109
patient 3	101	100	102	108
Diastolic blood pressure				
volunteers	102 (81-129)	100	86 (66-111)	86 (74-99)
patient 1	99	100	86	90
patient 2	113	100	114	117
patient 3	94	100	99	103
Heart rate				
volunteers	98 (81-118)	100	136 (113-163)	124 (102-150)
patient 1	98	100	101*	98*
patient 2	113	100	108*	113
patient 3	95	100	97*	102
Plasma renin				
volunteers	110 (99-122)	100	271 (153-480)	323 (248-420)
patient 1	100	100	90**	96***
patient 2	127	100	102*	106***
patient 3	119	100	110*	132***
Plasma noradrenaline				
volunteers	98 (84-113)	100	179 (147-218)	185 (127-269)
patient 1	108	100	109**	105*
patient 2	110	100	100**	97*
patient 3	102	100	110**	112*

Data presented as percentage of the baseline value (t= 0). The geometric mean and the 95% confidence interval are given for the normal volunteers. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2 Effects of DDAVP in 6 normal volunteers and 3 patients with NDI.

time (min)	-10	0	30	60
FVIII:C				
volunteers	98	100	178	181
	(90-107)		(129-247)	(120-273)
patient 1	94	100	101*	95*
patient 2	97	100	93**	95*
patient 3	105	100	52***	49***
vWF:ag				
volunteers	96	100	471	491
	(60-154)		(48-4632)	(104-2309)
patient 1	84	100	96	95*
patient 2	161	100	102	98*
patient 3	113	100	86	90*
t-PA:ag				
volunteers	107	100	327	258
	(91-126)		(148-726)	(131-508)
patient 1	105	100	96*	104*
patient 2	95	100	95*	95*
patient 3	76	100	105*	86*
PAI				
volunteers	96	100	26	34
	(80-114)		(0-61)	(0-105)
patient 1	81	100	121*	110
patient 2	100	100	113*	114
patient 3	82	100	95*	82*

Data presented as percentage of the baseline value (t= 0). Geometric mean and 95% confidence interval are given for the normal volunteers. FVIII:C; clotting factor VIII, vWF:ag; von Willebrand factor, t-PA:ag; tissue-type plasminogen activator antigen, PAI; plasminogen activator inhibitor, * $p< 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.4.3 AVP Infusion

The infusion of AVP in patient 3 resulted in an increase in blood pressure and a fall in heart rate, which was first noted at an infusion rate of 500 pg/kg/min (fig.5). At the highest infusion rate (4000 pg/kg/min) both the systolic and diastolic blood pressure showed an increase of maximally 10 mm Hg above baseline. This was accompanied by a fall in heart rate to approximately 15 bpm below baseline. At the same time the patient

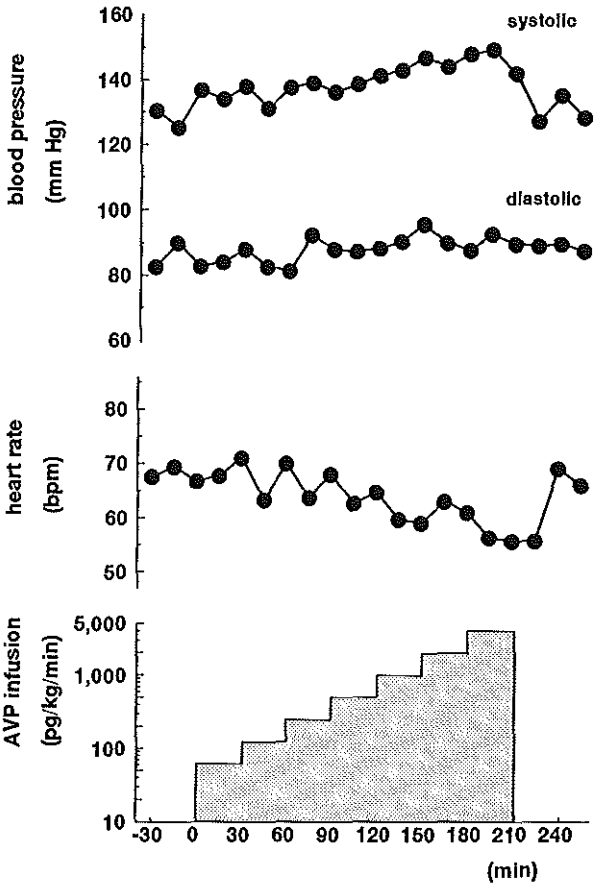

Figure 5. The effect of arginine vasopressin infusion (62.5 - 4000 pg/kg/min) on heart rate and blood pressure in patient 3 with congenital nephrogenic diabetes insipidus.

noted that his hands were getting cold. After discontinuation of the AVP infusion, both heart rate and blood pressure rapidly returned to baseline, and the patient reported that his hands were getting warm again. Throughout the whole experiment the patient did not experience nausea or precordial pain.

3.4.4 Venous occlusion test

The effects of venous occlusion on the plasma levels of vWF:ag, t-PA:ag and PAI, in the three patients with NDI and the normal volunteers, are presented in Table 3. In four volunteers and in patients 2 and 3, vWF:ag increased. In one volunteer and in patient 1, vWF:ag decreased. In all three patients, and in the volunteers t-PA:ag increased. In three normal volunteers, PAI dropped to unmeasurable levels. In two of them PAI showed only a minor decrease. In patient 1 PAI did not change whereas PAI increased in patients 2 and 3.

Table 3. Effect of venous occlusion in 5 normal volunteers and 3 patients with NDI.

		vWF:ag (U/ml)		t-PA:ag (ng/ml)		PAI (U/ml)	
		before	after	before	after	before	after
volunteers	1	0.90	1.25	4.6	11.2	2.63	0
	2	1.12	1.38	6.8	28.4	3.07	2.92
	3	0.63	1.09	3.3	13.3	2.01	0
	4	1.22	1.14	7.3	10.5	4.44	3.84
	5	1.02	1.43	5.5	21.7	5.13	0
patients	1	1.85	1.52	12.2	16.4	2.54	2.54
	2	1.35	1.86	3.3	11.4	1.34	2.45
	3	0.91	1.53	10.7	16.0	5.90	7.80

3.5 Discussion

NDI is the result of a defect of the V_2-receptor, either of the receptor itself or at the post-receptor level[14]. Thus the lack of any response to DDAVP we observed in NDI indicates that the haemodynamic effects of DDAVP in normal volunteers, and the effects on FVIII:C, vWF:ag, t-PA, and PAI are caused by V_2-receptor stimulation[9,16]. The possibility that the effects of DDAVP in our patients with NDI are absent as a result of down-regulation of V_1-receptors is unlikely since they showed no signs of chronic dehydration like renal function impairment or an elevation of serum osmolality. Furthermore, the plasma AVP levels in these patients were hardly elevated. Apparently the patients with NDI were capable to compensate for their urinary water losses by adequate drinking.

In order to exclude the possibility that the effects of high dose DDAVP are the result of a V_1-receptor antagonistic effect, the functional integrity of the V_1-receptor in NDI

must be considered. The patient we studied showed a vasoconstrictor response to the AVP infusion similar to those observed by Möhring et al.[23] in normal volunteers. Although AVP was infused in only one patient with NDI, the increase in blood pressure together with the fall in heart rate which rapidly returned to baseline after discontinuation of the infusion, can be considered as evidence to conclude that at least in this patient with NDI the V_1-receptor is functioning.

The disproportionate fall in heart rate, compared to the increase in blood pressure, during AVP infusion, as observed in normal subjects has been attributed to an increase in the gain of the baroreflex caused by AVP[24]. Unger et al.[25] found in rats that the effect of AVP on the baroreflex was caused by V_2-receptor stimulation. This may explain why our NDI patient showed no disproportionate fall in heart rate in response to the V_1-receptor-mediated increase in blood pressure during AVP infusion. It also explains why in normal individuals the rise in heart rate after DDAVP is disproportionate to the V_2-receptor-mediated vasodilatation.

The possibility that the endothelial release of vWF:ag and t-PA is impaired in NDI, has been addressed by Bichet et al.[26] who demonstrated that in NDI the plasma levels of both substances rise after adrenaline infusion. In the present study we examined the release from endothelium of vWF:ag and t-PA by venous occlusion. In the patients with NDI as well as in the normal volunteers, t-PA:ag increased with this manoeuvre. In two of the three patients with NDI and four of the five normal volunteers vWF:ag also increased, but in one of the patients and in one of the healthy subjects plasma levels of vWF:ag fell. Although the responses showed some degree of variability, these findings do not support that the lack of response of t-PA and vWF:ag to DDAVP in NDI is caused by a more general endothelial defect.

From the data presented in this study we conclude that the effects of i.v. administration of DDAVP in normal subjects on heart rate, blood pressure, and the plasma levels of renin, noradrenaline, FVIII:C, vWF:ag, and t-PA:ag are the result of V_2-receptor stimulation.

It is unlikely that these effects are caused by V_1-receptor antagonism because the dose of DDAVP is much to low to have such an antagonistic effect[9,12]. The localization of the V_2-receptor involved in these effects of DDAVP still has to be elucidated.
It is also unknown if, under certain pathophysiological conditions, endogenous AVP may cause V_2-receptor mediated vasodilatation, an effect that would counteract the vasoconstrictor action of AVP. The absence of an enhanced blood pressure response to

exogenous AVP in our patient with NDI does not support that such a V_2-receptor-mediated counteracting effect is of much physiologic importance. However this was an observation in a single patient, and more studies are needed to address this point.

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Chapter four:

VASCULAR EFFECTS OF 1-DESAMINO-8-D-ARGININE VASOPRESSIN (DDAVP) IN SUBJECTS WITH ESSENTIAL HYPERTENSION OR AUTONOMIC FAILURE.

4.1 Summary

The extrarenal effects of a single high dose of the V_2-receptor agonist 1-desamino-8-D-arginine vasopressin (DDAVP, Desmopressin) in subjects with essential hypertension and healthy subjects were compared. In addition, the extrarenal effects of lower doses were studied in subjects with essential hypertension. In eight healthy volunteers DDAVP (400 ng.kg i.v. in 10 minutes caused a slight fall (10, 5 - 15 mm Hg, mean, 95 % confidence interval, $p < 0.001$) in diastolic blood pressure without a change in systolic pressure. This was accompanied by marked increments in heart rate (26, 15 - 37 bpm, $p < 0.001$) and in the plasma levels of renin (220, 132 - 309 %, $p < 0.001$), noradrenaline (110, 77 -144 %, $p < 0.001$) and tissue-type plasminogen activator antigen (t-PA:ag)(253, 110 - 396 %, $p < 0.001$). These effects are compatible with a vasodilator action of DDAVP. In eight subjects with essential hypertension, DDAVP caused a fall in diastolic pressure (11, 4 - 17 mm Hg) and systolic pressure as well (13, 8 - 18 mm Hg, $p < 0.005$ with respect to healthy volunteers). The effects on heart rate, plasma renin, noradrenaline and t-PA:ag were not different from those in healthy volunteers. Plasma levels of DDAVP were measured in 6 subjects with essential hypertension. Peak levels after the single high dose of DDAVP were 2.85 (1.93-4.22) ng/ml.

Incremental infusions of lower doses of DDAVP were given to 10 subjects with essential hypertension. Cumulative doses were 12.5, 25, 50, 100, 200, and 400 ng/kg i.v. at 15 minute intervals. The first significant effect on blood pressure was observed after a cumulative DDAVP dose of 100 ng/kg. After this dose systolic blood pressure fell by 12 (5 - 20) mm Hg and diastolic pressure by 10 (5 - 15)mm Hg. Heart rate rose by 10 (6 -15) bpm with this dose. Renin rose by 31 (11 - 50) % and noradrenaline by 45 (27 - 62) %. The first significant rise in t-PA:ag was observed after a cumulative DDAVP dose of 200 ng/kg (134 , 71 - 197 %).

The same protocol was followed in three patients with orthostatic hypotension due to peripheral autonomic neuropathy. Already after a cumulative dose of 25 ng/kg, systolic blood pressure fell by 16 (15 - 18) mm Hg (median, range) and diastolic pressure with 8 (5 - 12) mm Hg. Heart rate and noradrenaline did not change, whereas renin rose in two patients after the highest cumulative dose of 400 ng/kg. The results in these patients support the view that the increase in renin after DDAVP in healthy subjects and in subjects with essential hypertension is caused by sympathetic stimulation in response to the vasodilator action of DDAVP.

The cardiovascular effects of DDAVP observed with the incremental infusions

suggests that under certain pathological conditions endogenous AVP may cause V_2-receptor-mediated vasodilatation.

4.2 Introduction

In humans arginine vasopressin (AVP) is the natural antidiuretic hormone. Apart from its antidiuretic effect, AVP has also a potent vasoconstrictor action. These two effects are mediated through two different types of receptors[1]. Binding of AVP to the vascular V_1-receptors results in vasoconstriction, through stimulation of the breakdown of phosphatidylinositol and an increase in cytosolic calcium. V_2-receptors, located on renal tubular cells, are responsible for the antidiuretic effect. Binding of AVP to this receptor causes stimulation of adenylate cyclase and increased production of 1'5'adenosine monophosphate (c-AMP)[2].

1-Desamino-8-D-arginine vasopressin (desmopressin, DDAVP) is a selective V_2-receptor agonist[3]. In healthy volunteers intravenous administration of a single high dose of DDAVP (400 ng/kg i.v.) causes a slight fall in blood pressure and marked increases in heart rate and in the plasma levels of renin and noradrenaline[4,5]. These effects suggest that DDAVP has a vasodilator action. In addition to its hemodynamic effects, DDAVP also causes the release of clotting factor VIII (FVIII:C), von Willebrand factor (vWF:ag), and tissue-type plasminogen activator (t-PA) from endothelium[6,7]. All these effects of DDAVP are mediated by V_2-receptors[8,9,10].

In this study we compared the extrarenal effects of a single high dose of DDAVP (400 ng/kg i.v.) in subjects with essential hypertension and healthy volunteers, and the plasma levels of DDAVP were measured. In order to evaluate whether V_2-receptor mediated vasodilatation occurs at plasma DDAVP levels that approximate the levels of AVP that can be found under certain pathological conditions with stimulated AVP release[11,12], we also studied the hemodynamic effects of lower doses of DDAVP (12.5 ng/kg to 400 ng/kg i.v.) in subjects with essential hypertension and in patients with orthostatic hypotension due to autonomic neuropathy. The latter group of patients was included in the study because it was expected that in the absence of autonomic counterregulatory mechanisms the vasodilator effect of DDAVP would be more manifest.

4.3 Methods

4.3.1 Subjects

Eight male apparently healthy volunteers (aged 24-51 years) and eight male subjects with essential hypertension (aged 34 - 60) received a single high dose of DDAVP (400 ng/kg i.v.). Ten additional male subjects with essential hypertension (aged 32 - 60) participated in a study in which the extrarenal effects of lower doses of DDAVP (12.5 - 400 ng/kg i.v.) were investigated. Urine analysis and serum electrolytes, urea and creatinine in the hypertensive subjects were normal. Urinary excretion of vanyllilmandelic acid was also normal. Ultrasound imaging and renography using ^{99}Te-DTPA disclosed no evidence for the existence of renovascular hypertension.

The extrarenal effects of low doses of DDAVP were also investigated in three patients with autonomic failure. All three had orthostatic hypotension due to peripheral autonomic neuropathy. One patient had primary hereditary amyloidosis, one had amyloidosis accompanying multiple myeloma, and one patient had idiopathic peripheral autonomic neuropathy[13].

All participants in this study had not taken any kind of medication for at least two weeks prior to the study. The study protocol was approved by the Medical Ethics Committee of our hospital. All subjects gave their informed consent.

4.3.2 Single high dose DDAVP

One hour before the infusion of DDAVP a teflon cannula was inserted into the antecubital vein of the left arm, which was used as a vascular access both for the infusion of DDAVP and for blood sampling. After 1 hour of supine bed rest 400 ng/kg DDAVP (Minrin®, Ferring, Malmö, Sweden) dissolved in 100 ml 0.9 % NaCl was infused in 10 minutes. After the infusion the cannula was flushed with 10 ml 0.9 % NaCl.

Blood pressure and heart rate were recorded at 5 minute intervals, using an automatic oscillometric device (Accutorr®, Datascope Corp, Paramus, NY). Blood samples were drawn at -10, 0, 10, 20, 30, and 60 minutes.

4.3.3 Incremental low doses of DDAVP

In 10 subjects with essential hypertension and in 3 patients with autonomic failure DDAVP was administered in incremental doses at 15 minute intervals up to a cumulative dose of 400 ng/kg i.v. The doses of DDAVP were 12.5, 12.5, 25, 50, 100 and 200 ng/kg. A teflon cannula which was inserted into the left antecubital vein at the beginning of the

experiment, was used both for the administration of DDAVP and for blood sampling. The first dose was administered after 1 hour of supine bedrest and each dose was infused over a period of 1 minute. In the subjects with essential hypertension blood pressure and heart rate, which were measured with the automatic oscillometric device, were recorded at 5 minute intervals from 60 minutes before the first dose, up to 60 minutes after the final dose of DDAVP. In the patients with autonomic failure, blood pressure was continuously measured in a radial artery via a catheter. The heart rate was derived from the continuously recorded electrocardiogram. Blood samples were drawn 15 minutes before and immediately prior to each dose as well as 15 and 60 minutes after the last dose.

4.3.4 Assays

Plasma renin was measured by its ability to cleave sheep renin substrate[14]. Plasma noradrenaline was measured by high performance liquid chromatography with electrochemical detection[15]. t-PA:ag was measured by radioimmunoassay[16]. DDAVP was measured in plasma by radioimmunoassay, using ^{131}I labelled DDAVP and a specific antibody against DDAVP, both provided by Ferring[17]. The radioimmunoassay of DDAVP had a lower limit of detection of 1.5 pg/ml, and a cross-reactivity with AVP of 100%.

4.3.5 Statistical analysis

The effects of a single dose of DDAVP in normal volunteers and patients with essential hypertension and the effects of incremental doses of DDAVP in the hypertensive subjects and the patients with autonomic failure were analyzed by using an analysis of variance for repeated measures in the same subjects. To identify the lowest dose of DDAVP that caused a statistically significant change from baseline a Student Newman Keuls test was used. Plasma renin levels were analyzed after logarithmic transformation of data.

4.4 RESULTS

4.4.1 Single high dose DDAVP

Both the normal volunteers and the subjects with essential hypertension experienced facial flushing 2 - 3 minutes after the beginning of the DDAVP infusion. The effects on blood pressure and heart rate are depicted in Fig.1. In the healthy volunteers, DDAVP caused a fall in diastolic blood pressure of 10 (5 - 15) mm Hg from baseline

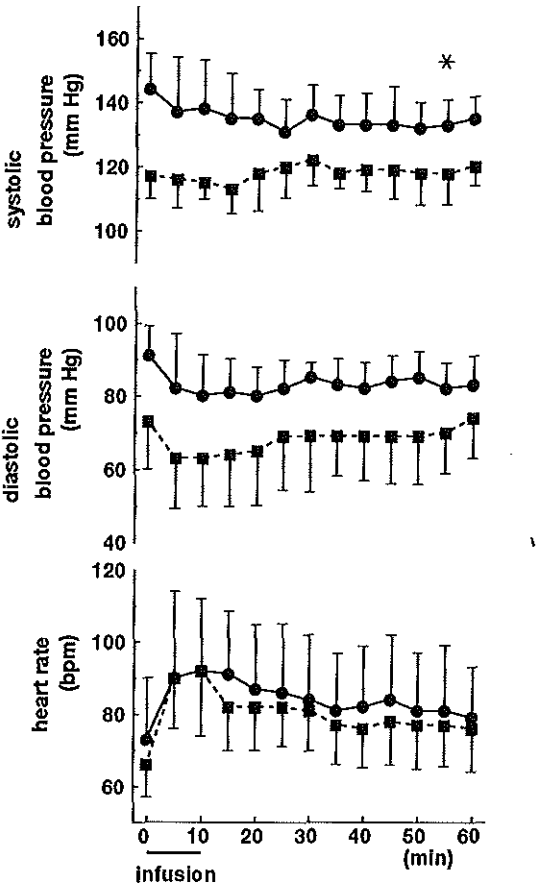

Figure 1. Effect of DDAVP (400 ng/kg i.v. in 10 minutes) on blood pressure and heart rate in 8 healthy volunteers (■- - -■), and 8 subjects with essential hypertension (●——●). Data presented as mean ± sd. * $p < 0.05$ for difference between groups.

(mean, 95% confidence interval, $p < 0.001$), without any significant change in systolic pressure. Heart rate rose by 26 (15 - 37) bpm ($p < 0.001$). Renin increased by 220 (132 - 309) % ($p < 0.001$), and noradrenaline by 110 (77 - 144) % ($p < 0.001$)(Fig.2). Plasma t-PA:ag rose by 253 (110 - 396) % ($p < 0.001$).

In the subjects with essential hypertension the diastolic blood pressure fell by 11 (4 - 17) mm Hg ($p < 0.05$)(Fig. 1). Systolic blood pressure fell by 13 (8 - 18) mm Hg

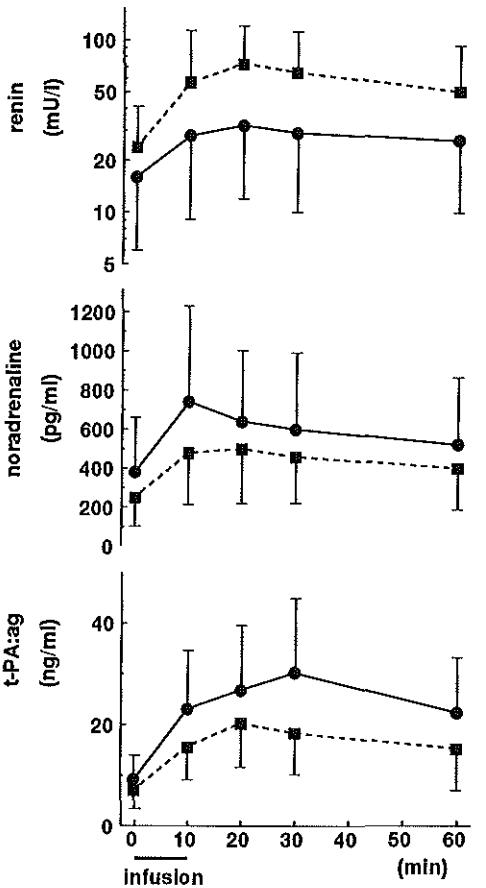

Figure 2. Effect of DDAVP (400 ng/kg i.v. in 10 minutes) on plasma renin, noradrenaline, and t-PA:ag in 8 healthy volunteers (■—■), and 8 subjects with essential hypertension (●——●). Data presented as mean ± sd.

($p < 0.01$). Heart rate rose by 19 (15 - 23) bpm ($p < 0.001$). Renin rose by 120 (43 - 198) % ($p < 0.001$) , and noradrenaline by 90 (48 - 131) % ($p < 0.001$), and t-PA:ag by 240 (202 - 277) % ($p < 0.001$) (Fig. 2).

As expected, the baseline blood pressure readings in the hypertensive subjects were higher than in the healthy subjects ($p < 0.01$). Baseline heart rate, renin, noradrenaline and t-PA:ag were not different between the two groups. The effects of DDAVP on diastolic pressure and heart rate, and on renin, noradrenaline and t-PA:ag were

also not different between the two groups. The hypertensives however showed a fall in systolic pressure, which was not observed in the normal volunteers.

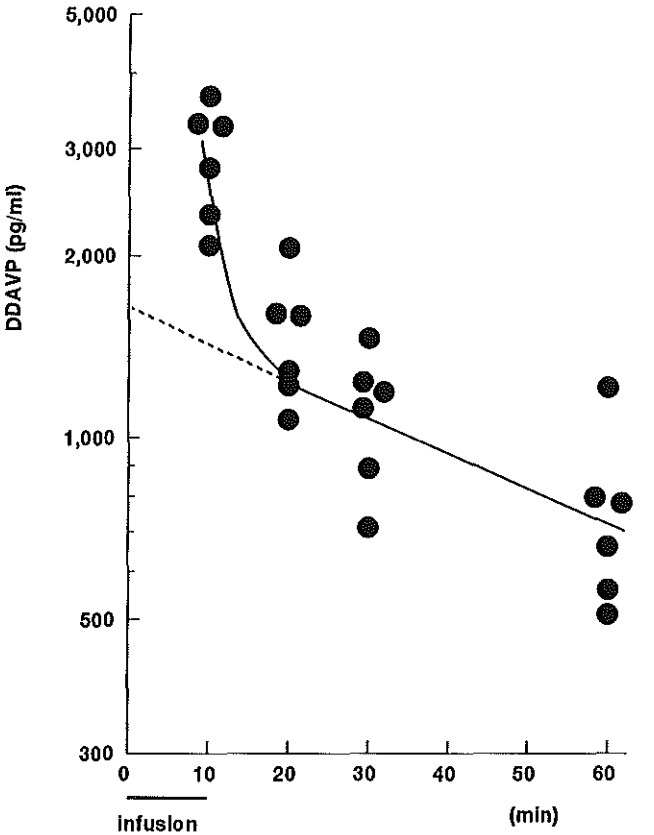

Figure 3. Plasma DDAVP levels in 6 subjects with essential hypertension after the infusion of DDAVP (400 ng/kg i.v. in 10 minutes).

DDAVP was measured in the plasma samples from six of the hypertensive subjects. Peak plasma levels at the end of the infusion were 2.85 (1.93 - 4.22) ng/ml (Fig. 3). DDAVP disappeared from plasma monoexponentially with an elimination half life of 48 (27 - 86) minutes. The distribution volume of DDAVP was calculated to be 0.29 (0.22 - 0.36) l/kg.

4.4.2 Incremental low doses of DDAVP

The subjects with essential hypertension receiving incremental doses of DDAVP experienced facial flushing. One subject reported facial flushing after the second dose of DDAVP (cumulative dose 25 ng/kg). Four subjects experienced facial flushing after the 50 ng/kg dose, and five after the 100 ng/kg dose. The effects of DDAVP on blood pressure and heart rate are depicted in Fig.4. Both systolic and diastolic blood pressure showed a

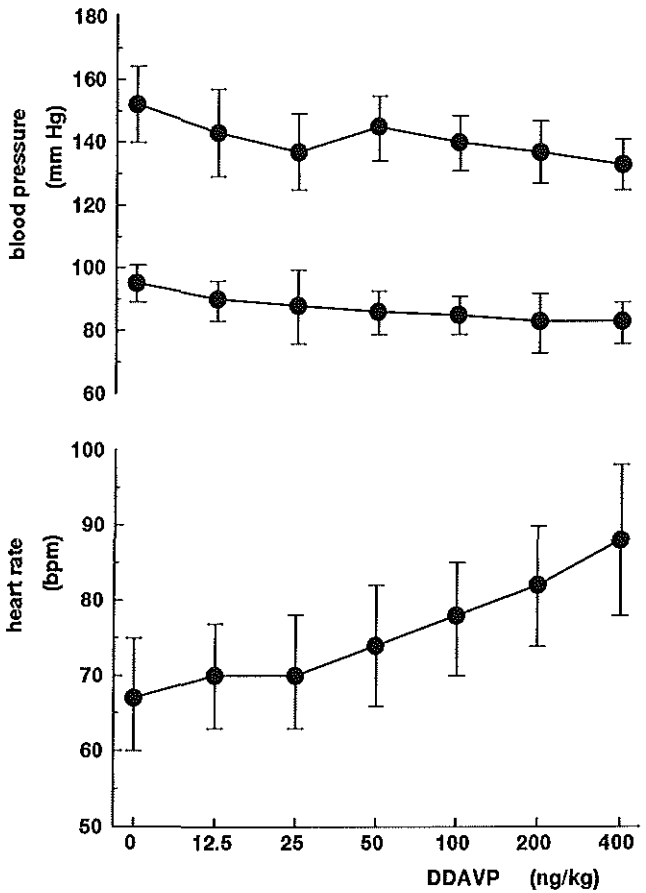

Figure 4. Effect of incremental doses of DDAVP (cumulative dose 12.5 to 400 ng/kg at 15 minute intervals) on blood pressure and heart rate in 10 subjects with essential hypertension. Data presented as mean ± sd. Systolic and diastolic pressure were significantly different from baseline ($p < 0.05$) at 100 to 400 ng/kg doses. Heart rate was significantly different from baseline ($p < 0.05$) at 50 to 400 ng/kg doses.

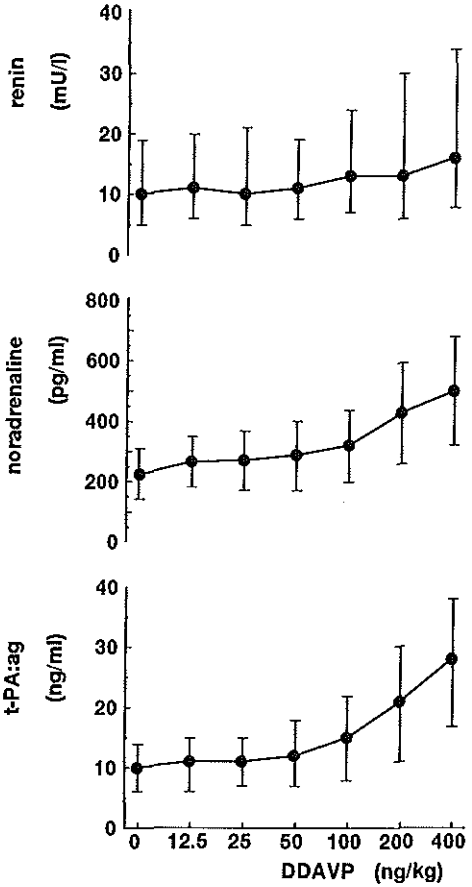

Figure 5. Effect of incremental doses of DDAVP (cumulative dose 12.5 to 400 ng/kg at 15 minute intervals) on plasma renin, noradrenaline, and t-PA:ag in 10 subjects with essential hypertension. Data presented as mean ± sd. Renin and noradrenaline were significantly different from baseline at ($p < 0.05$) 100 to 400 ng/kg doses. t-PA:ag was significantly different from baseline at 200 and 400 ng/kg doses ($p < 0.05$).

dose-dependent decrease. Systolic pressure fell to 19 (11 - 28) mm Hg below baseline ($p < 0.001$) after the highest cumulative dose (400 ng/kg). Diastolic pressure fell to 13 (6 - 19) mm Hg below baseline ($p < 0.001$). Heart rate, renin, noradrenaline and t-PA:ag all showed a dose dependent increase (Fig.4 and 5). Heart rate increased with 21 (16 - 25) bpm ($p < 0.001$). Renin rose with 68 (45 - 92) % ($p < 0.001$). Noradrenaline showed a 129

(91 - 166) % ($p < 0.001$) increase, and t-PA:ag rose by 233 (110 - 355) % ($p < 0.001$).

Analysis of the effects of the individual doses of DDAVP revealed that the increase in heart rate of 7 (4 - 10) bpm we observed was statistically significant ($p < 0.05$) after the 50 ng/kg dose. The first statistically significant changes in blood pressure were observed after the 100 ng/kg dose. With this dose systolic pressure fell by 12 (5 - 20) mm Hg ($p < 0.05$), and diastolic pressure by 10 (5 - 15) mm Hg ($p < 0.05$) from baseline. Both renin

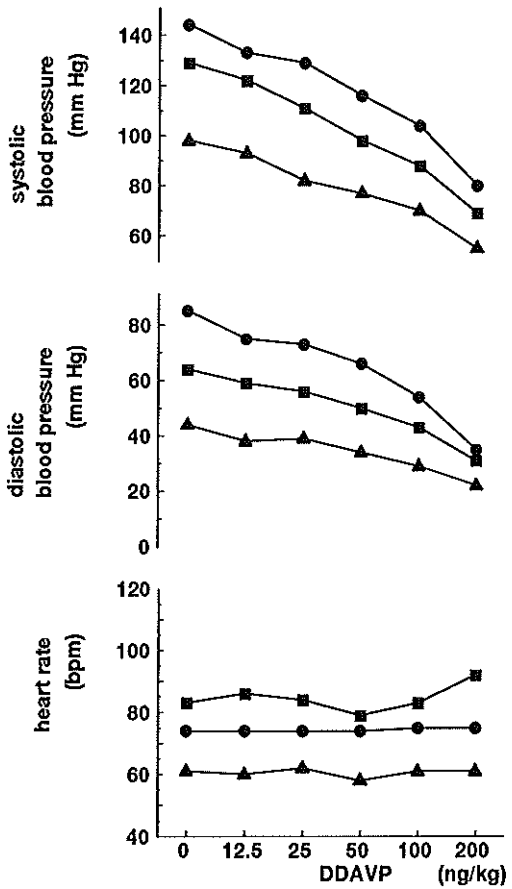

Figure 6. Effect of incremental doses of DDAVP (cumulative dose 12.5 to 200 ng/kg at 15 minute intervals) on blood pressure and heart rate in 3 patients with autonomic failure. Systolic and diastolic pressure were significantly different from baseline ($p < 0.05$) at 25 to 200 ng.kg doses.

and noradrenaline showed a statistically significant change from baseline after the 100 ng/kg dose. Renin increased by 31 (11 - 50) % ($p < 0.05$), and noradrenaline by 45 (27 - 62) % ($p < 0.05$). A significant increase in t-PA:ag of 134 (71 - 197) % ($p < 0.05$) was observed after the 200 ng/kg dose.

In the patients with autonomic failure DDAVP caused a dose dependent fall in blood pressure (Fig.6). Already the second dose of DDAVP (cumulative dose 25 ng/kg) caused a statistically significant fall in systolic blood pressure of 16 (15 - 18) mm Hg

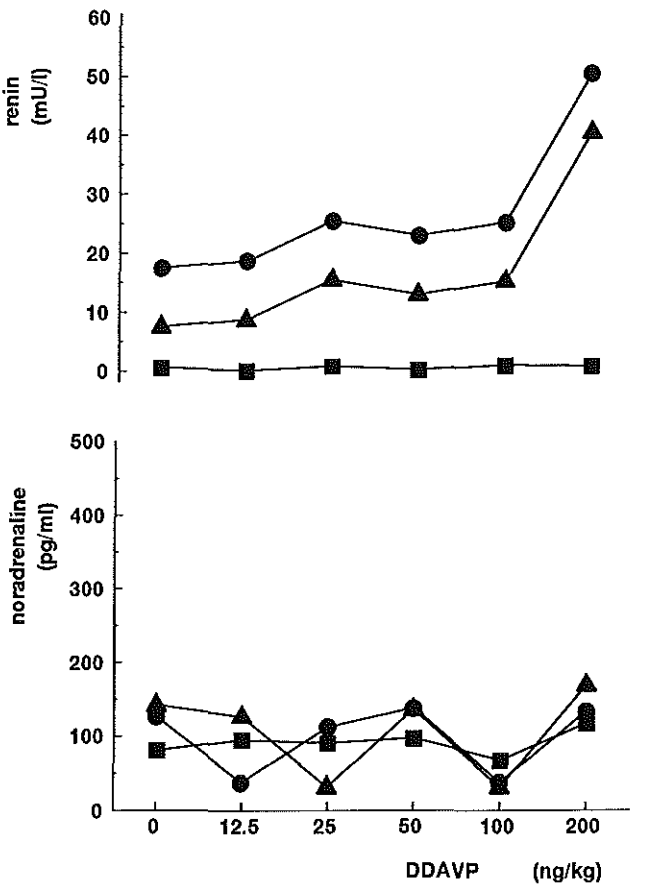

Figure 7. Effect of incremental doses of DDAVP (cumulative dose 12.5 to 200 ng/kg at 15 minute intervals) on plasma renin and noradrenaline in 3 patients with autonomic failure.

(median, range) ($p < 0.05$) and diastolic pressure of 8 (5 - 12) mm Hg ($p < 0.05$). No changes in heart rate were observed in these patients. In two out of the three patients renin increased after the highest dose of DDAVP (Fig.7). Noradrenaline did not show any significant change.

4.5 Discussion

The effects of a high dose of DDAVP (400 ng/kg i.v. in 10 minutes) on blood pressure, heart rate, and the plasma levels of renin, noradrenaline and t-PA:ag, in the eight healthy volunteers who participated in this study were not different from those described previously[4,5]. These effects are compatible with the view that in normal subjects DDAVP acts as a vasodilator. Our results indicate that this is also true for subjects with essential hypertension. From Fig.1 it appears that the effect of DDAVP on heart rate may be somewhat blunted in the subjects with essential hypertension as compared to the healthy volunteers. This might be related to the well known decrease in baroreflex sensitivity in hypertension. However, the difference in heart rate response was statistically not significant. In the healthy volunteers DDAVP caused a fall in diastolic blood pressure without a change in systolic pressure. In the hypertensives both diastolic and systolic blood pressure fell. This also might be related to a diminished baroreflex response.

The dose-response study in the hypertensives showed significant effects on blood pressure, heart rate, and the plasma levels of renin and noradrenaline at doses 2 to 4 times lower than given during the single high-dose infusion.

The patients with autonomic failure showed marked hypotensive responses to DDAVP at doses that were even 8 times lower than the single high dose. As expected, no changes in heart rate were observed in the autonomic failure patients and the lack of sympathetic counterregulation might well explain the increased response of blood pressure to DDAVP.

The rise in plasma noradrenaline after DDAVP is probably a sign of sympathetic stimulation in response to vasodilatation. The rise in t-PA:ag might also be a response to vasodilatation. It is also possible that the rise in t-PA:ag is a direct consequence of V_2-receptor stimulation.

In the patients with autonomic failure we found a blunted renin response to DDAVP. This supports the view that the rise in renin after DDAVP in normal volunteers and in subjects with essential hypertension is secondary to sympathetic stimulation and not a direct result of V_2-receptor stimulation. The rise in renin after the highest dose of DDAVP in

two patients with autonomic failure is possibly caused by a fall in renal perfusion pressure related to the fall in blood pressure[18,19].

The distribution volume and plasma half life of DDAVP we found are in accordance with other studies[20,21]. Our dose-response studies showed significant effects on blood pressure and heart rate at doses that were 4 to 8 times lower than the high dose that was used in the single dose infusions. The plasma DDAVP levels we found after single high-dose DDAVP infusion were much higher than the natural AVP levels that can occur under physiological and certain pathological conditions[11,12]. From the distribution volume and plasma half life we estimate that in the patients with autonomic failure after the lowest hypotensive dose (cumulative dose 50 ng/kg) plasma DDAVP levels were 150 - 200 pg/ml. We estimate that in the hypertensive subjects the lowest hypotensive dose (cumulative dose 100 ng/kg) resulted in plasma levels of 300 - 400 ng/kg. These plasma levels are comparable to the levels of AVP that are found under pathological conditions with stimulated AVP secretion.

We conclude that under certain pathological conditions endogenous AVP may cause V_2-receptor-mediated vasodilatation. In these conditions this mechanism possibly serves to attenuate the V_1-receptor-mediated vasoconstrictor effect of AVP.

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Chapter five

EFFECTS OF 1-DESAMINO-8-D-ARGININE VASOPRESSIN (DDAVP) ON RENAL HEMODYNAMICS AND RENIN SECRETION IN SUBJECTS WITH ESSENTIAL HYPERTENSION.

5.1 Summary

Intravenous (i.v.) infusion of the selective vasopressin (V_2) agonist 1-desamino-8-D-arginine vasopressin (DDAVP) in humans causes a fall in blood pressure, an increase in heart rate, and a rise in plasma renin and noradrenaline. The present study was designed to demonstrate the vasodilatory properties of DDAVP in the renal circulation.

Seven male hypertensive subjects (31-63 years), received an i.v. infusion of DDAVP (400 ng/kg in 10 minutes) at the time of renal vein catheterization and renal angiography as part of the diagnostic work-up of their hypertension. ^{131}I-Hippurate clearance was used to measure effective renal plasma flow (ERPF). True renal plasma flow was calculated as ERPF divided by the extraction ratio of ^{131}I-hippurate (E_{hip}).

^{125}I-Thalamate clearance was used to measure glomerular filtration rate (GFR). Measurements were made before and 15-20 minutes after administration of DDAVP. Angiography was performed after these measurements were made. In all patients the renal arteries were normal and E_{hip} and the extraction of ^{125}I-thalamate (E_{thal}) were not different for the left and right kidney.

After DDAVP systolic blood pressure fell from 163 (114-211) mm Hg (mean, 95% confidence interval) to 146 (113-179) mm Hg ($p < 0.05$). Diastolic blood pressure fell from 103 (70-137) mm Hg to 87 (58-117) mm Hg ($p < 0.01$). Heart rate rose from 79 (48-110) bpm to 102 (61-143) bpm ($p < 0.001$). The plasma level of ^{131}I-hippurate did not change, and E_{hip} of the left kidney fell from 0.77 (0.68-0.87) to 0.72 (0.57-0.87)($p < 0.05$), and of the right kidney from 0.80 (0.75-0.84) to 0.71 (0.61-0.82)($p < 0.005$). E_{thal} of the left kidney fell from 0.18 (0.13-0.24) to 0.16 (0.08-0.24)($p < 0.05$), and of the right kidney from 0.22 (0.18-0.26) to 0.17 (0.10-0.25)($p < 0.005$).

These data indicate that the true renal plasma flow was increased after DDAVP and filtration fration was decreased. Peripheral plasma renin rose by 175 (55-564) percent ($p < 0.001$), and the renal vein-to-artery renin ratio of the left kidney rose from 1.44 (0.83-2.05) to 1.70 (0.97-2.43)($p < 0.05$), and of the right kidney from 1.43 (1.05-1.81) to 1.85 (0.98-2.72)($p < 0.05$).

We conclude that stimulation of renal V_2-receptors by DDAVP leads to renal vasodilation and increased renin recretion. The data provide evidence for the existence of functional renal V_2-receptors at sites other than the renal tubular cells.

5.2 Introduction

The nonapeptide arginine vasopressin (AVP) has both potent vasoconstrictor and antidiuretic effects[1,2]. The vasoconstrictor action of AVP is mediated by vascular V_1-receptors, while the antidiuretic effect is mediated by V_2-receptors on the tubular cells of the renal collecting ducts[3].

1-Desamino-8-D-arginine vasopressin (DDAVP) is a synthetic analog of AVP with selective V_2-receptor agonistic properties[4,5]. Because of its potent antidiuretic effect and its lack of vasoconstrictor action, DDAVP is used in the treatment of patients with central diabetes insipidus[6]. High doses of DDAVP induce an increase of the plasma levels of clotting factor VIII, von Willebrand factor and tissue type plasminogen activator[7,8]. Because of the effects on clotting factor VIII and von Willebrand factor, high doses of DDAVP are used in various haemorrhagic disorders[9]. High doses of DDAVP also cause facial flushing, a fall in blood pressure, a marked increase in heart rate, and a rise of plasma noradrenaline, all compatible with systemic vasodilation[10].

The present study addresses the possible vasodilatory effects of DDAVP in the renal circulation.

5.3 Patients and methods

Seven male patients, aged 31-63 years, were studied after they had given their informed consent. They were admitted to our hospital for a diagnostic work-up, because their hypertension was difficult to control. Urine analysis and serum electrolytes, urea and creatinine were normal. Urinary excretion of vanillylmandelic acid was also normal. All medication was stopped for at least two weeks prior to the study.

Renal function and perfusion were measured using constant infusion clearance techniques without urine collection[11,12,13]. A solution containing 0.5 µCi/ml of ^{131}I-sodium iodohippurate (^{131}I-hip) (50,000 c.p.m./ml) and 2 µCi/ml ^{125}I-iothalamate (^{125}I-thal)(50,000 c.p.m./ml) was infused at a rate of 0.1 ml/min after a bolus of 0.20 ml/kg of this solution had been given. Approximately 90 minutes after the beginning of the infusion of ^{131}I-hip and ^{125}I-thal, angiography catheters were introduced, by using the Seldinger technique, in a femoral artery and the ipsilateral femoral vein. Approximately 35 minutes later, blood samples were taken simultaneously from the renal vein and

abdominal aorta, first at one side, and immediately thereafter on the other[12] (Fig.1). DDAVP (Minrin, Ferring, Malmö, Sweden), 400 ng/kg dissolved in 100 ml saline, was infused in a peripheral vein in exactly 10 minutes (Fig.1). Renal vein sampling was repeated 20 minutes after the infusion of DDAVP (Fig.1). A renal aniogram was made after completion of the study.

Peripheral venous blood samples were taken at regular time intervals as indicated in Fig.1.

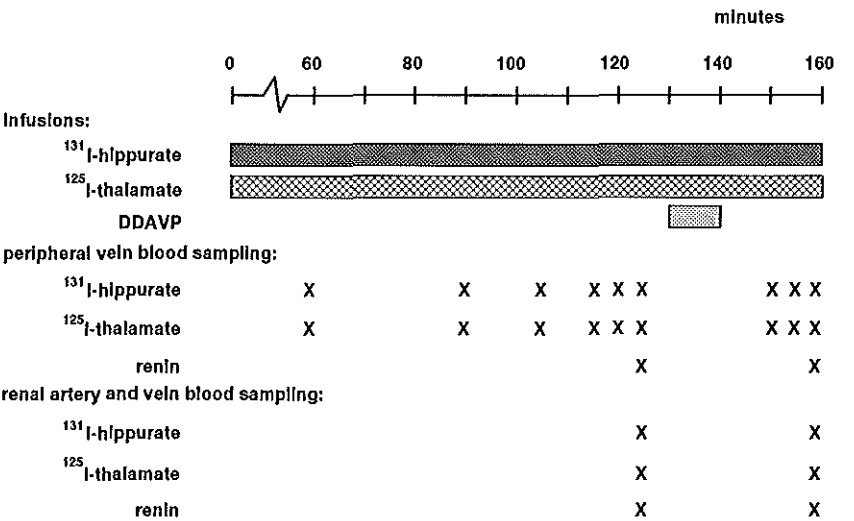

Figure 1. Diagram of the timing of the infusion of DDAVP, and ^{131}I-hippurate, and ^{125}I-thalamate, and the acquisition of blood samples.

All blood samples were immediately centrifuged, ^{125}I and ^{131}I radioactivity levels in plasma were measures seperately. The single kidney extraction ratios of ^{131}I-sodium hippurate (E_{hip}) and ^{125}I-iothalamate (E_{thal}) were calculated as (A-V)/A, where A = radioactivity in plasma from the abdominal aorta, and V = radioactivity in plasma from the renal vein. The total extraction efficiency (both kidneys) was calculated as the mean of the extraction ratios on each side. In all blood samples plasma renin was measured with an enzyme kinetic assay, in which the in vitro generated angiotensin I

was measured by radioimmunoassay[14].

Blood pressure was measured intra-arterially from the catheter in the abdominal aorta. Heart rate was determined from a simultaneously recorded ECG signal.

The data were analyzed using a one way analysis of variance for repeated measures in the same subjects. Data on plasma renin were analysed after log transformation.

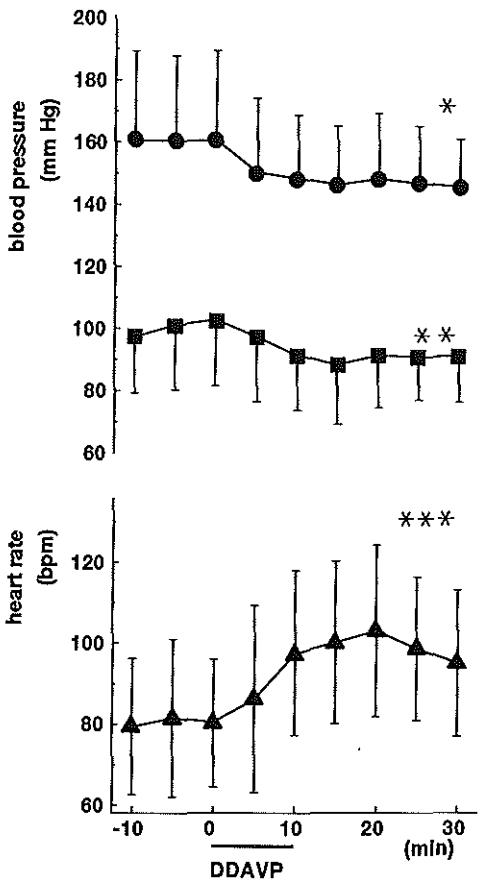

Figure 2. Effect of DDAVP (400 ng/kg i.v. in 10 minutes) on systolic blood pressure (●——●), diastolic blood pressure (■——■), and heart rate (▲——▲) (mean ± sd). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

5.4 Results

Renal angiograms were normal in all seven patients. As shown in Fig.2, DDAVP infusion caused a fall in systolic blood pressure by 9.5 (0.8-19.9) percent (mean, 95% confidence interval)($p < 0.05$) and in diastolic pressure by 15.2 (0.2-30.1) percent ($p < 0.01$). This effect was accompanied by a 21.7 (4.0-39.4) percent ($p < 0.001$) increase in heart rate.

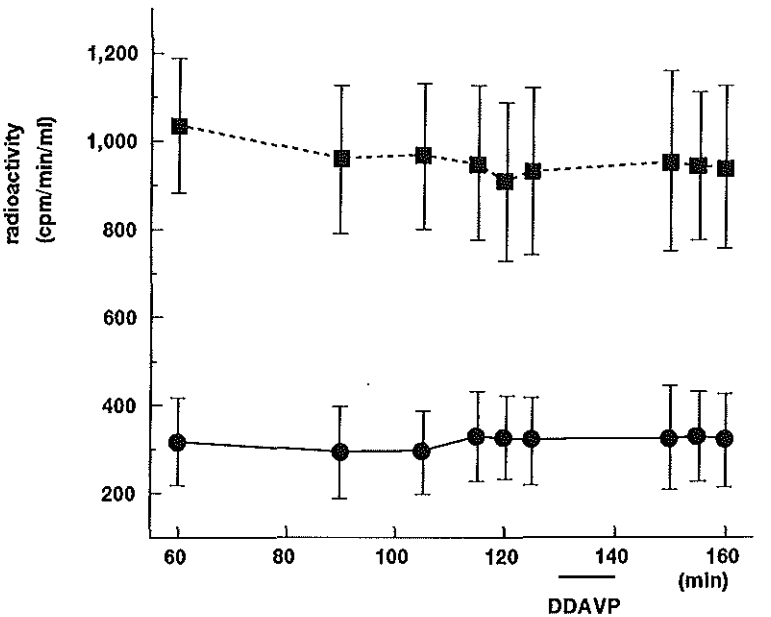

Figure 3. Radioactivity from ^{125}I-thalamate (■- - -■) and ^{131}I-hippurate (●———●) in plasma measured before and after DDAVP (400 ng/kg i.v. in 10 minutes) (mean ± sd).

After 90 minutes of ^{131}I-hip and ^{125}I-thal infusion the plasma levels of both compounds were stable (Fig.3). The infusion of DDAVP did not significantly alter the plasma levels of ^{131}I-hip and ^{125}I-thal (Fig.3). However, E_{hip} and E_{thal} were reduced by DDAVP; E_{hip} of the left kidney fell from 0.77 (0.68-0.87) to 0.72 (0.57-0.87) ($p < 0.05$) and of the right kidney from 0.80 (0.75-0.84) to 0.71 (0.61-0.82) ($p < 0.005$), and E_{thal} of the left kifney fell from 0.18 (0.13-0.24) to 0.16 (0.08-0.24) ($p < 0.05$) and of the right kidney from 0.22 (0.18-0.26) to 0.17 (0.10-0.25) ($p < 0.005$)(Fig.4).

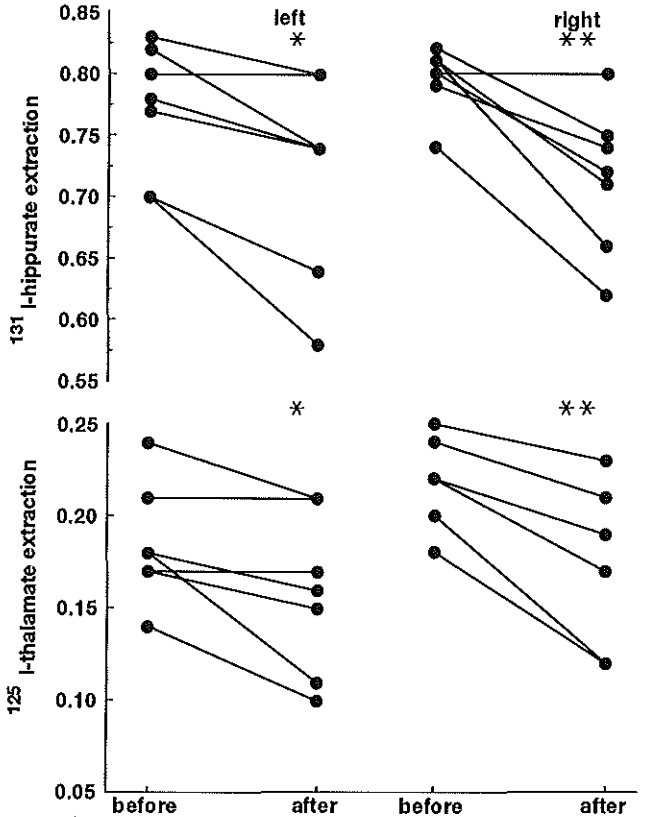

Figure 4. Single kidney extraction of ^{131}I-hippurate and ^{125}-thalamate of the left kidney and the right kidney measured before and after DDAVP (400 ng/kg i.v. in 10 minutes). * $p < 0.05$; ** $p < 0.005$.

Peripheral venous plasma renin rose by 175 (55-564) percent ($p < 0.001$), and renal vein-to-artery renin ratio of the left kidney rose 1.44 (0.83-2.05) to 1.70 (0.97-2.43) ($p < 0.05$), and of the right kidney from 1.43 (1.05-1.81) to 1.85 (0.98-2.72) ($p < 0.05$) (Fig.5).

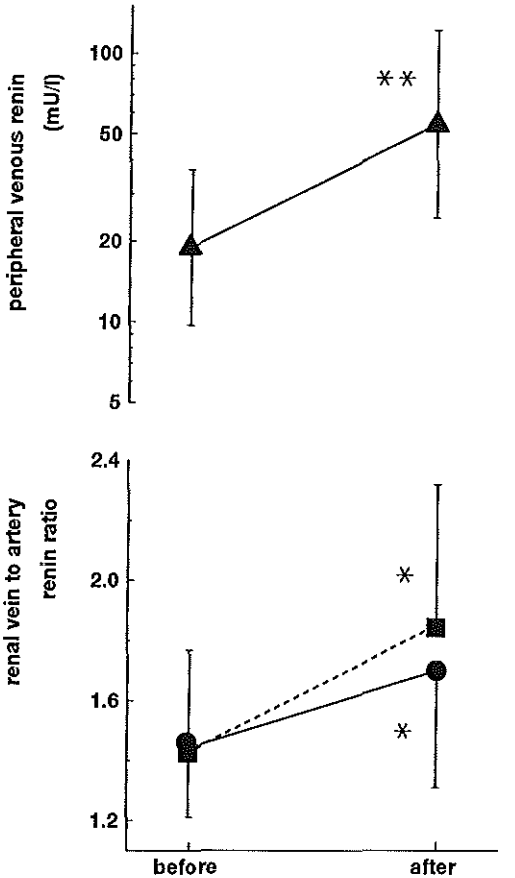

Figure 5. Peripheral venous renin (▲——▲) and single kidney vein-to-artery renin ratio of the left kidney (●——●) and right kidney (■- - -■) measured before and after DDAVP (mean ± sd).
* $p < 0.05$; ** $p < 0.001$.

5.5 Discussion

The hemodynamic effects of an i.v. infusion of 400 ng/kg DDAVP in healthy volunteers are a slight fall in diastolic blood pressure without a change in systolic blood pressure[9,15]. Together with these changes in blood pressure marked increments in heart rate and in the plasma levels of renin and noradrenaline are observed. In previous work we demonstrated these effects to be caused by extrarenal V_2-receptor stimulation[16]. The patients with essential hypertension that participated in the present

study showed not only a fall in diastolic blood pressure but also in systolic blood pressure. Possibly the blood pressure in hypertensive subjects is somewhat more responsive to the vasodilator action of DDAVP.

In the present study we assessed the effects of DDAVP on renal hemodynamics by measurements of the peripheral and renal venous plasma levels of ^{131}I-hip and ^{125}I-thal during constant systemic infusion of these compounds[10,11]. As shown in Fig.3, the plasma levels of both ^{131}I-hip and ^{125}I-thal had reached a stable plateau prior to the infusion of DDAVP. Thus it was possible to calculate ERPF and GFR as the clearances of ^{131}I-hip and ^{125}I-thal respectively by dividing the infusion rates by the steady state plasma levels. Such calculations were not possible after DDAVP infusion, because the time interval between the infusion of DDAVP and the last measurements of ^{131}I-hip and ^{125}I-thal was to short (30 minutes) to reach a new steady state. For ERPF a semiquantitative estimate of the effect of DDAVP could be made, because in patients with a normal renal function the plasma half-life of ^{131}I-hip is about 20 minutes. For GFR such a semiquantitative estimate connot be made because of the longer plasma half-life of ^{125}I-thal.

Thus, since plasma ^{131}I-hip did not change after DDAVP, we may conclude that there was little or no change in ERPF. This, together with the observed fall in E_{hip} is an indication that the true renal plasma flow was increased by DDAVP. This increase in RPF occurred while blood pressure fell. Thus DDAVP had caused renal vasodilatation. As with other drugs causing renal vasodilation the renal vasodilatory effect of DDAVP was associated with a fall in E_{hip}, which may be explained by a shortened plasma transit time through the kidney[12,17].

E_{thal} is equal to single kidney filtration fraction. The fall in E_{thal} we observed therefore demonstrates a fall in filtration fraction.

DDAVP caused a marked rise in peripheral plasma renin. Our study demonstrates that this was due to increased renal secretion, since both renal plasma flow and renal vein-to-artery renin ratio were increased after DDAVP. Infusion of AVP, in contrast to DDAVP, causes inhibition of renin secretion through stimulation of V_1-receptors on juxtaglomerular cells[9,18,19,20]. At extremely high concentrations, DDAVP can act as an antagonist of the AVP-induced inhibition of renin secretion from kidney slices[9]. DDAVP itself, on the other hand, did not stimulate renin secretion[9]. Thus it is unlikely that the increase in renal renin secretion after DDAVP we observed, is a direct result of V_2-receptor stimulation. It rather is a secondary response to renal

vasodilatation. Possibly, sympathetic stimulation may also contribute to the increased release of renin after DDAVP[34,21].

In previous work it was demonstrated that DDAVP causes its vasodilatory effect through V_2-receptor stimulation[15,22]. V_2-receptors have been localized in the brain, and it has been suggested that DDAVP exerts its vasodilator action through the central nervous system[23]. One would expect such effect to be accompanied by a reduction in sympathetic tone. DDAVP however is known to cause an increase in the activity of the sympathetic nervous system as is reflected in the increase in plasma noradrenaline[9,16]. Furthermore, in dogs with a destroyed central nervous system, the selective V_2-receptor agonist 4-valine-8-D-arginine vasopressin (VDAVP) has been shown to cause vasodilation through a peripheral action[24]. A more recent study in humans disclosed that even AVP can cause vasodilation in the forearm through stimulation of peripheral V_2-receptors[25]. It may therefore be concluded that, although until recently the AVP receptors in the vascular wall were considered to be of the V_1-type[26], and V_2-receptors were considered to be located exclusively on renal tubular cells[27], it is unlikely that DDAVP causes its vasodilator action in man via the central nervous system.

It is possible that DDAVP causes its vasodilator action by stimulated production of vasodilatory prostaglandins like PGE_2 and PGI_2. AVP is known to stimulate renal PGE_2 production in rats via V_1-receptors on glomerular mesangial cells[28,29,30,31], whereas DDAVP had not such effect[26]. It has been reported that both AVP and DDAVP stimulate PGE_2 production in patients with central or nephrogenic diabetes insipidus[32]. However the response to DDAVP was much weaker than to AVP. PGI_2 synthesis by vascular smooth muscle cells, from rat mesenteric arteries and aorta, can be stimulated by AVP but not by DDAVP[24,25]. In humans, DDAVP has been reported to cause an increase in the production of PGI_2[33]. However, in more recent studies this could not be confirmed[34,35]. Furthermore, blockade of prostaglandin synthesis by high dose aspirin did not inhibit the effects on blood pressure and heart rate of DDAVP[36]. It seems therefore unlikely that increased PGI_2 production can account for the vasodilator action of DDAVP.

We conclude that DDAVP causes renal vasodilatation via vascular V_2-receptors, despite the fact that up to now peripheral V_2-receptors have only been demonstrated in renal medullary tubules and collecting ducts. Since the amounts of DDAVP we infused result in plasma levels far exceeding the AVP levels that occur

under physiological and in abnormal conditions[37,38], like dehydration or hemorrhagic shock, it remains to be seen if V_2-receptor-mediated vasodilation is a physiologically important effect contributing to the overall hemodynamic actions of endogenous AVP.

5.6 References

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Chapter six
SUMMARY AND CONCLUSIONS

6.1 Introduction

In humans arginine vasopressin (AVP) is the natural antidiuretic hormone. Apart from its antidiuretic action, AVP can cause a variety of other effects like vasoconstriction, platelet aggregation, glycogen breakdown in the liver, the release of clotting factor VIII (FVIII:C), von Willebrand factor (vWF:ag), and tissue-type plasminogen activator (t-PA) from endothelium, and inhibition of renin release from juxtaglomerular cells. In the central nervous system AVP acts as a neurotransmitter.

AVP causes its effects by stimulation of two different types of receptors[1]. The vasoconstrictor effect is mediated by stimulation of vascular V_1-receptors. Stimulation of this receptor causes an increase in phosphatidylinositol breakdown and a rise in cytosolic calcium. Platelet aggregation, glycogen breakdown and inhibition of renin release are also mediated by V_1-receptors. The antidiuretic action is mediated by stimulation of V_2-receptors located on tubular cells of the renal collecting ducts. Binding of AVP to this receptor causes activation of adenylate cyclase, which leads to a rise in intracellular 3'5'adenosine monophosphate (c-AMP).

1-Desamino-8-D-arginine vasopressin is a synthetic analog of AVP which has virtually lost the vasopressor action has but retained the antidiuretic properties of AVP[2]. Because of its selective V_2-receptor agonist effect, DDAVP is used as a treatment in patients with central diabetes insipidus.

Both AVP and also intravenous administration of a high dose of DDAVP (200 - 400 ng/kg) have been shown to cause an increase in the plasma levels of FVIII:C, vWF:ag, and t-PA[3]. At these doses facial flushing and palpitations are reported as side effects of DDAVP, suggesting that its infusion causes hemodynamic changes.

6.2 Aim of the thesis

This thesis contains the results of the studies that were undertaken to investigate the hemodynamic effects, as well as the effects on FVIII:C, vWF:ag, and t-PA of intravenous administration of DDAVP in man. Our objective was to elucidate the mechanism by which DDAVP exerts these effects, and to evaluate the possibility that this mechanism can also be activated by endogenous AVP.

6.3 DDAVP in healthy volunteers

In twelve apparently healthy volunteers we investigated the hemodynamic responses and the effects on FVIII:C, vWF:ag, and t-PA of DDAVP, using a double blinded placebo controlled study design. The participants in this study were randomly assigned to either DDAVP (400 ng/kg i.v. in 10 minutes) or a comparable amount of solvent. Placebo had no effect on any of the parameters measured. DDAVP caused a minor fall in diastolic blood pressure without a change in systolic pressure. This was accompanied by a striking increase in heart rate and a rise in plasma renin and noradrenaline. These findings support the view that at these doses DDAVP causes vasodilatation and baroreflex-mediated sympathetic stimulation. DDAVP caused also a rise in FVIII:C, vWF:ag, and both t-PA-antigen (t-PA:ag) and t-PA-activity (t-PA:act). The increase in t-PA:act was accompanied by a fall in plasminogen activator inhibitor (PAI). From this study we concluded that DDAVP itself and not another constituent of the DDAVP solution causes vasodilatation and a rise in plasma FVIII:C, vWF:ag, and t-PA:ag and t-PA:act.

6.4 DDAVP in patients with congenital nephrogenic diabetes insipidus

Derkx et al.[4] found that high concentrations of DDAVP can inhibit V_1-receptor mediated effects of AVP. They concluded that the vasodilator effect of DDAVP might be caused by antagonism of the V_1-receptor mediated vasoconstrictor effect of endogenous AVP. However, it is unlikely that the infusion of 400 ng/kg of DDAVP can result in plasma levels as high as the DDAVP concentrations that were shown to cause a V_1-receptor antagonistic effect in vitro.

Since DDAVP is a selective V_2-receptor agonist, it is likely that its vasodilator action is caused by V_2-receptor stimulation. To test this hypothesis we infused DDAVP (400 ng/kg i.v. in 10 minutes) into three patients with congenital nephrogenic diabetes insipidus (NDI). Patients with NDI suffer from polyuria and polydipsia due to an X-linked recessive genetic defect of the V_2-receptor or a defect at the post-receptor level. This defect is propably not restricted to the renal tubules. Patients with congenital NDI may therefore expected to be unresponsive to DDAVP. Indeed single high dose of DDAVP did not change blood pressure, heart rate and the plasma levels of renin, noradrenaline, FVIII:C, vWF:ag, t-PA:ag, t-PA:act, and PAI. The absence of these extrarenal effects of DDAVP in these patients suggests that in healthy volunteers these effects are mediated via V_2-receptors.

It is unlikely that the patients with NDI not only have a V_2-receptor defect but also a diminished V_1-receptor function, because plasma AVP levels were normal, which argues

against down-regulation of V_1-receptors. Moreover infusion of AVP in one af the patients caused a normal pressor response.

The lack of vWF:ag and t-PA responses in hte NDI patients was not caused by generalized endothelial dysfunction. The responses to venous occlusion in the patients with NDI were similar to those in five normal volunteers.

We therefore conclude that in normal volunteers, DDAVP causes vasodilatation and the release from endothelium of FVIII:C, vWF:ag, t-PA:ag and t-PA:act through V_2-receptor stimulation.

6.5 DDAVP in subjects with essential hypertension

Eight subjects with essential hypertension and eight normal volunteers received a single high dose of DDAVP (400 ng/kg i.v. in 10 minutes). The vasodilator effect and the effects on t-PA:ag of DDAVP were compared between the two groups. In contrast to the normal volunteers DDAVP in the hypertensives caused not only a fall in diastolic blood pressure but also a fall in systolic pressure. The rise in heart rate in the hypertensives appeared to be somewhat blunted as compared to the normal volunteers. Both the fall in systolic pressure and the somewhat blunted rise in heart rate might be related to the well known diminished baroreflex sensitivity in hypertension. The responses of renin, noradrenaline and t-PA:ag were the same in the hypertensives and the normal volunteers. We conclude that the effects of DDAVP in the subjects with hypertension are not essentially different from those in healthy volunteers.

6.6 DDAVP plasma levels after a single high dose

In six of the subjects with essential hypertension we measured DDAVP plasma levels after the infusion of a single high dose (400 ng/kg i.v. in 10 minutes). These plasma levels were much higher than the natural AVP levels that can be found under physiological conditions in man. They are also higher than the AVP levels under pathological conditions with stimulated AVP release. It remains therefore uncertain whether the observed vasodilator effect of V_2-receptor stimulation by DDAVP can also be elicited by endogenous AVP.

6.7 Incremental low doses of DDAVP

We investigated the hemodynamic effects of incremental low doses of DDAVP in ten subjects with essential hypertension and in three patients with orthostatic hypotension due to peripheral autonomic neuropathy. We expected blood pressure of patients with loss of sympathetic function to be hyperresponsive to the vasodilator action of DDAVP. In the hypertensives DDAVP had significant effects on blood pressure and heart rate at doses 4 to 8 times lower than the single high dose infusion. The patients with orthostatic hypotension showed a significant fall in blood pressure at even lower doses without any change in heart rate. We estimate that the lowest hypotensive dose of DDAVP in the hypertensives and in the patients with autonomic failure resulted in plasma levels that are comparable to the levels of AVP that can be found under pathological conditions with stimulated AVP secretion. We conclude therefore that under certain pathological conditions endogenous AVP may cause V_2-receptor mediated vasodilatation.

As expected, DDAVP did not cause a rise in noradrenaline in the patients with autonomic failure. These patients also showed a diminished responsiveness of renin to DDAVP. This supports the view that the rise in renin after DDAVP in both the normal volunteers and the hypertensives is not a direct result of V_2-receptor stimulation but is secondary to sympathetic stimulation .

6.8 Effects of DDAVP on the renal circulation and on renin release

The possible vasodilator action of DDAVP in the renal circulation and the effect on renal renin secretion were investigated in seven patients with essential hypertension. The clearance of ^{131}I-hippurate (^{131}I-hip) as a measure of effective renal plasma flow (ERPF), and the clearance of ^{125}I-thalamate (^{125}I-thal) as a measure of glomerular filtration rate (GFR) were measured before and after the infusion of a single high dose of DDAVP (400 ng/kg i.v. in 10 minutes). The renal clearances were calculated from the peripheral venous plasma levels and the infusion rate of both compounds during a constant infusion. Single kidney extraction ratios of ^{131}I-hip (E_{hip}) and ^{125}I-thal (E_{thal}) and the renal vein-to-artery renin ratio's were measured by renal vein sampling before and after DDAVP. Plasma ^{131}I-hip did not change after DDAVP, which indicated that there was little or no change in ERPF. Thus, the significant fall in E_{hip} we observed, is propably caused by an increase in true renal plasma flow (RPF). This increase in RPF occurred while blood pressure fell. We conclude therefore that DDAVP in the dose we used causes renal vasodilatation.

The renal vein-to-artery renin ratios showed a significant rise, despite the increase in

RPF. This demonstrates that the release of renin by the kidney is increased, and this has led to the rise in peripheral vein renin.

6.9 Conclusions

From the studies described in this thesis it can be concluded that a high dose of DDAVP causes systemic and renal vasodilation through V_2-receptor stimulation. The observed effects of lower doses of DDAVP indicate that V_2-receptor mediated vasodilatation may also occur in certain pathological conditions with elevated plasma levels of endogenous AVP. Such high plasma AVP levels are found when circulating blood volume is severely reduced. Under these circumstances stimulation of V_2-receptors on renal tubular cells reduces urine production, and helps to preserve circulating volume. V_1-receptor-mediated vasoconstriction causes an increase in peripheral vascular resistance, and this helps to maintain blood pressure. It is possible that V_2-receptor mediated vasodilatation serves as a mechanism to protect vital organs from the vasoconstrictor effect of V_1-receptor stimulation.

6.10 References

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Hoofdstuk zeven
SAMENVATTING EN CONCLUSIES

7.1 Inleiding

Arginine-vasopressine (AVP) is bij de mens het natuurlijke antidiuretisch hormoon. Naast de antidiuretische werking, kan AVP diverse andere effecten veroorzaken zoals vasoconstrictie, plaatjes-aggregatie, glycogeen-afbraak in de lever, en de afgifte van stollingsfactor VIII (FVIII:C), von Willebrand factor (vWF:ag) en weefsel-plasminogeen activator (t-PA) door endotheel, en inhibitie van renine-secretie uit juxtaglomerulaire cellen. In het centrale zenuwstelsel heeft AVP een neurotransmitter-functie.

De effecten van AVP worden veroorzaakt door stimulatie van twee verschillende receptoren[1]. Het vaatvernauwende effect wordt veroorzaakt door stimulatie van V_1-receptoren in de vaatwand. Stimulatie van deze receptor leidt tot een toename van de phosphatidylinositol omzetting en een stijging van het calcium in de cytosol. Plaatjes-aggregatie, glycogeen-afbraak en inhibitie van renine-secretie worden eveneens veroorzaakt via V_1-receptoren. De antidiuretische werking van AVP verloopt via activatie van V_2-receptoren op de tubuluscellen van de verzamelbuisjes in de nier. Binding van AVP met deze receptoren activeert adenylaatcyclase, dit leidt vervolgens tot een stijging van het intracellulair 3'5'adenosine monofosfaat (c-AMP).

1-Desamino-8-D-arginine vasopressine (DDAVP) is een synthetisch AVP-derivaat[2], dat de vaatvernauwende werking verloren heeft, maar de antidiuretische werking heeft behouden. Vanwege deze selectieve V_2-receptor-agonistische werking wordt DDAVP gebruikt voor de behandeling van patiënten met centrale diabetes insipidus.

Van zowel AVP als van intraveneuze toediening van hoge doses DDAVP (200 - 400 ng/kg) is gebleken dat dit een stijging van FVIII:C, vWF:ag en t-PA in plasma kan veroorzaken[3]. Bij deze doseringen zijn roodheid en warmtegevoel in het gezicht en hartkloppingen gemeld als bijwerkingen van DDAVP. Deze effecten wijzen erop dat DDAVP hemodynamische veranderingen veroorzaakt.

7.2 Doel van het onderzoek

Dit proefschrift bevat de resultaten van de studies die werden gedaan om de hemodynamische effecten alsmede de effecten op FVIII:C, vWF:ag en t-PA van intraveneuze toediening van DDAVP bij de mens te onderzoeken. Het doel van het onderzoek was om op te helderen via welke mechanisme DDAVP deze effecten veroorzaakt, en om de mogelijkheid te evalueren dat dit mechanisme ook kan worden geactiveerd door endogeen AVP.

7.3 DDAVP bij gezonde vrijwilligers

In een dubbel-blinde placebo-gecontroleerde studie werd bij twaalf gezonde vrijwilligers de hemodynamische effecten van DDAVP, en de effecten op FVIII:C, vWF:ag en t-PA onderzocht. De deelnemers kregen na randomisatie of DDAVP (400 ng/kg i.v. in 10 minuten) of een vergelijkbare hoeveelheid bewaarvloeistof. Placebo had geen effect op de parameters die werden gemeten. DDAVP veroorzaakte en geringe daling van de diastolische bloeddruk, zonder een daling van de systolische bloeddruk. Dit ging gepaard met een opvallende toename van de hartfrequentie en een stijging van renine en noradrenaline in plasma. Deze waarnemingen ondersteunen de hypothese dat deze dosis DDAVP vaatverwijding veroorzaakt, en via de baroreflex tevens sympathicus stimulatie geeft. DDAVP veroorzaakte ook een stijging van FVIII:C, vWF:ag en van zowel t-PA-antigeen (t-PA:ag) als t-PA-activiteit (t-PA:act). De stijging in t-PA:act ging gepaard met een daling van plasminogeenactivator-inhibitor (PAI). Uit de resultaten van deze studie concluderen wij dat DDAVP zelf, en niet een ander bestanddeel van de DDAVP oplossing, vaatverwijding en de toename in plasma van FVIII:C, vWF:ag, t-PA:ag en t-PA:act, veroorzaakt.

7.4 DDAVP bij patiënten met congenitale nefrogene diabetes insipidus

Derkx et al.[4] vonden dat hoge concentraties DDAVP aan V_1-receptor-stimulatie gerelateerde effecten van AVP kan remmen. Hieruit werd geconcludeerd dat het vaatverwijdende effect van DDAVP zou kunnen berusten op antagonisme van de V_1-receptor gemediëerde vasoconstrictieve werking van AVP. Het is echter onwaarschijnlijk dat infusie van 400 ng/kg DDAVP resulteert in plasma concentraties in dezelfde orde van grootte als de concentratie die een V_1-receptor antagonistisch effect bleek te hebben in vitro.

DDAVP is een selectieve V_2-receptor-agonist. Daarom is het waarschijnlijk dat de vaatverwijdende werking wordt veroorzaakt door stimulatie van V_2-receptoren. Teneinde deze hypothese te testen werd DDAVP (400 ng/kg i.v. in 10 minuten) toegediend aan drie patiënten met congenitale nefrogene diabetes insipidus (NDI). Patienten met congenitale NDI lijden aan polyurie en polydipsie als gevolg van een geslachtsgebonden recessief genetisch defect van de V_2-receptor dan wel een defect op post-receptor niveau. Dit defect is waarschijnlijk niet beperkt tot de verzamelbuisjes in de nier. Van patiënten met congenitale NDI mag dus verwacht worden dat zij niet reageren op DDAVP. Conform deze verwachting, veroorzaakte een enkelvoudige hoge dosis DDAVP geen enkele

veranderingen in bloeddruk, hartfrequentie, renine, noradrenaline, FVIII:C, vWF:ag, t-PA:ag, t-PA:act en PAI. De afwezigheid van deze extra-renale effecten bij deze patiënten wijst erop, dat bij gezonde vrijwilligers en essentiële hypertonici de extra-renale effecten van DDAVP worden veroorzaakt door V_2-receptor stimulatie.

Het is onwaarschijnlijk dat de patiënten met congenitale NDI naast een V_2-receptor defect tevens een verminderde V_1-receptor-werking hebben, want de plasma AVP spiegels waren normaal, hetgeen pleit tegen down-regulatie van V_1-receptoren. Tevens bleek, bij één van de patiënten, dat infusie van AVP een normale pressor-reactie veroorzaakte. De afwezige reactie van vWF:ag en t-PA bij de patienten met congenitale NDI werd niet veroorzaakt door een gegeneralizeerde endotheel-dysfunktie. De reacties op veneuze occlusie bij de patiënten met congenitale NDI waren gelijk aan die bij een vijftal gezonde vrijwilligers. Wij concluderen dat bij gezonde vrijwilligers, DDAVP, door stimulatie van V_2-receptoren, vaatverwijding en vrijmaking uit endotheel van FVIII:C, vWF:ag, t-PA:ag en t-PA:act veroorzaakt.

7.5 DDAVP bij essentiële hypertensie

Acht personen met essentiële hypertensie en acht gezonde vrijwilligers kregen een enkelvoudige hoge dosis DDAVP (400 ng/kg i.v. in 10 minuten) toegediend. De vaatverwijdende werking, en het effect op t-PA:ag secretie van DDAVP werden vergeleken tussen de twee groepen. In tegenstelling tot de normale vrijwilligers, veroorzaakte DDAVP bij de essentiële hypertonici niet alleen een daling van de diastolische bloeddruk, maar tevens een daling van de systolische bloeddruk. De toename van de hartfrequentie bij de hypertonici verliep iets vlakker dan bij de gezonde vrijwilligers. Zowel de daling van de systolische bloeddruk als het vlakker verloop van de hartfrequentie-stijging kunnen worden verklaard uit een verminderde baroreflex-gevoeligheid bij hypertonici. De reactie van renine, noradrenaline en t-PA:ag op DDAVP, waren niet verschillend in beide groepen. We concluderen dat de effecten van DDAVP bij hypertonici niet essentieel verschillen van de effecten bij normale vrijwilligers.

7.6 DDAVP plasma spiegels

Bij zes van de personen met essentiële hypertensie werden de DDAVP-concentraties in plasma gemeten na infusie van een enkelvoudige hoge dosis (400 ng/kg i.v. in 10 minuten). Deze concentraties bleken aanzienlijk hoger te zijn dan de concentratie van

endogeen AVP in plasma, zoals die onder fysiologische omstandigheden kunnen voorkomen bij de mens. De DDAVP-concentraties waren ook hoger dan AVP-concentraties in plasma onder pathologische omstandigheden die gepaard gaan met gestimuleerde AVP secretie. Het blijft dus onzeker of het vaatverwijdend effect van V_2-receptor stimulatie door DDAVP, ook kan worden opgewekt door endogeen AVP.

7.7 Oplopende lage doseringen DDAVP

We onderzochten de hemodynamische effecten van oplopende lagere doses DDAVP bij tien personen met essentiële hypertensie en drie patiënten met orthostatische hypotensie door perifere autonome neuropathie. We verwachtten dat de bloeddruk bij de patiënten met verminderde sympathicus-funktie een versterkte reactie op de vaatverwijdende werking van DDAVP zouden vertonen. Bij de hypertonici had DDAVP significante effecten op bloeddruk en hartfrequentie bij doseringen 4 tot 8 maal lager dan de enkelvoudige hoge dosering. De patiënten met orthostatische hypotensie vertoonden een significante daling van de bloeddruk zonder enige verandering in de hartfrequentie bij een nog lagere dosis. We schatten, dat de laagste dosis DDAVP, die een daling van de bloeddruk gaf bij de hypertonici en bij de patiënten met autonome dysfunktie, resulteerde in DDAVP-concentraties in plasma van dezelfde orde van grootte als AVP-concentraties zoals die kunnen worden gevonden onder pathologische omstandigheden die gepaard gaan met een verhoogde AVP-secretie. Wij concluderen hieruit dat onder bepaalde pathologische omstandigheden endogeen AVP vaatverwijding zou kunnen veroorzaken via V_2-receptor stimulatie.

Zoals verwacht veroorzaakte DDAVP bij de patiënten met orthostatische hypotensie geen stijging van het noradrenaline. Tevens vertoonden deze patiënten een verminderde reactie van renine op DDAVP. Dit steunt de hypothese dat de renine stijging na DDAVP zowel bij gezonde vrijwilligers als bij essentiële hypertonici, geen direct gevolg is van V_2-receptor stimulatie, maar een secundair gevolg is van sympathicus activatie.

7.8 Effecten van DDAVP op de renale circulatie en op renine- secretie

De mogelijke vaatverwijdende werking van DDAVP op de renale circulatie en het effect op de renine-secretie, werden onderzocht bij zeven patiënten met essentiële hypertensie. De ^{131}I-hippuran (^{131}I-hip) klaring, als maat voor de effectieve renale plasma flow (ERPF), en de ^{125}I-thalamate (^{125}I-thal) klaring als maat voor de glomerulaire

filtratiesnelheid (GFR), werden gemeten voor en na een enkelvoudige hoge dosis DDAVP (400 ng/kg i.v. in 10 minuten).De renale klaringen werden berekend uit de perifeer-veneuze plasma spiegels en de infusie-snelheid van beide stoffen tijdens een continue infuus. De renale extractie-ratio's van ^{131}I-hip (E_{hip}) en ^{125}I-thal (E_{thal}) en de renale veno-arteriele renine ratio's van beide nieren werden afzonderlijk gemeten middels niervene-sampling voor en na DDAVP. De ^{131}I-hip spiegel veranderde niet na DDAVP hetgeen aangeeft dat de ERPF niet of nauwelijks was veranderd. Dus de significante daling van de E_{hip} die we vonden is waarschijnlijk veroorzaakt door een toename van de renale plasma flow (RPF). Deze RPF-stijging vond plaats terwijl de bloeddruk daalde. We concluderen daarom dat DDAVP, in de dosering die wij gebruikten, vaatverwijding in de nier veroorzaakte.

De renale veno-arteriele renine-ratio's vertoonden een significante stijging. Dit toont aan dat de secretie van renine door de nier is toegenomen, en dat hierdoor het perifere renine steeg.

7.9 Conclusies

Uit de studies, beschreven in dit proefschrift, kan worden geconcludeerd dat een hoge dosis DDAVP systemische en renale vaatverwijding veroorzaakt via V_2-receptor stimulatie. De waargenomen effecten van lagere doseringen DDAVP geven aan dat V_2-receptor-gemediëerde vaatverwijding ook kan optreden onder bepaalde pathologische omstandigheden met verhoogde plasma-spiegels van endogeen AVP. Dergelijke sterk verhoogde AVP-spiegels worden gevonden wanneer het circulerend bloedvolume ernstig is verlaagd. Onder deze omstandigheden neemt de urine-productie af door stimulatie van V_2-receptoren op de renale tubuluscellen, hetgeen helpt om het circulerend volume te bewaren. Vasoconstrictie door stimulatie van V_1-receptoren veroorzaakt een stijging van de perifere vaatweerstand hetgeen helpt om de bloeddruk op peil te houden. Het is echter mogelijk dat V_2-receptor-gemediëerde vaatverwijding dient als een mechanisme om vitale organen te beschermen tegen het vaatvernauwend effect van V_1-receptor stimulatie.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 9 mei 1958 te Groesbeek. In aansluiting op het behalen van het atheneum B diploma werd in 1976 begonnen met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam. Tijdens deze studie was hij vanaf 1979 gedurende twee jaar student-assistent op de afdeling Pathologische Anatomie II (Prof. M. de Vries) en vanaf 1980 gedurende drie jaar student-assistent op de afdeling Anatomie III (Prof. P. Krediet).

Na het arts-examen in 1983 vervulde hij zijn dienstplicht bij de Koninklijke Luchtmacht op vliegbasis Volkel als dienstplichtig arts. Van 1984 tot 1989 volgde hij de opleiding interne geneeskunde op de afdeling Inwendige Geneeskunde I van het Dijkzigt Ziekenhuis Rotterdam (opleider: Prof. Dr. M.A.D.H. Schalekamp). Op 1 november 1989 werd hij geregistreerd als internist.

Vanaf april 1990 werd hij opgeleid tot nefroloog in het Academisch Ziekenhuis Groningen. De registratie als nefroloog vond op 1 april 1992 plaats. Aansluitend was hij gedurende ruim twee jaar werkzaam als nefroloog op zowel de dialyse afdeling van Beatrixoord (Haren, Gn), de Stichting Thuisdialyse Noord-Nederland, als in het Academisch Ziekenhuis Groningen.

Vanaf 1 mei 1994 is hij werkzaam op de afdeling Intensive Care van het Academisch Ziekenhuis Nijmegen.

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NAWOORD

Dit proefschrift is voortgekomen uit klinisch wetenschappelijk onderzoek verricht op de afdeling Inwendige Geneeskunde I van het Academisch Ziekenhuis Dijkzigt te Rotterdam. Uitsluitend door de belangeloze vrijwillige deelname aan de diverse experimenten van een aantal gezonde proefpersonen en patiënten, en de inspanningen van een aantal medewerkers van de afdeling was het mogelijk dit onderzoek te voltooien. Verder werd er een belangrijke bijdrage geleverd vanuit het instituut TNO-IVVO te Leiden alwaar een deel van de laboratorium-bepalingen werden verricht. Al die mensen die direct of indirect bij de totstandkoming van dit proefschrift betrokken zijn geweest wil ik in dit nawoord bedanken. Zonder iemand tekort te willen doen wil ik een aantal personen met name bedanken.

Als eerste mijn promotor Prof. Dr. M.A.D.H. Schalekamp. Maarten, ik wil je bedanken voor de energie en tijd die je in mij hebt geïnvesteerd zowel tijdens als na mijn opleiding tot internist. Je hebt me op weg geholpen op het glibberige pad van het klinisch-wetenschappelijk onderzoek. Het pad dat ik op jouw advies insloeg lag in een andere richting dan de hoofdweg van het door jou geleidde onderzoek. Mede hierdoor was het voor ons beiden niet eenvoudig de juiste route te vinden, echter dankzij jouw inspanningen als gids is uiteindelijk dit proefschrift tot stand gekomen.

Frans Derkx, door de jaren heen heb je, ondanks jouw ernstige preoccupatie met het renine-angiotensine-aldosteron systeem, steeds tijd weten te vinden om mij te helpen met de voortgang van het onderzoek.

Rene de Bruin, de wijze waarop jij de gegevens van de diverse laboratorium-bepalingen beheert was van onschatbare waarde voor het verzamelen van de data waarop dit proefschrift is gebaseerd.

Remco Hooijer, een mengsel van kalk en azijn vormt de basis van een goede vriendschap. Zonder jouw hulp met zowel hardware als software zou het schrijven van dit boekje aanzienlijk langer geduurd hebben.

Pa, vaak is gebleken dat jij mij beter kende dan ik zelf wilde toegeven. Jouw niet aflatende belangstelling heeft mij gestimuleerd om dit proefschrift af te maken.

Elly, mijn proefschrift kon worden geschreven dankzij het feit dat jij achter me stond. Jouw onbeperkt vertrouwen in het eindresultaat was voor mij de basis om door te gaan ook bij tegenslag. Aan jou en onze kinderen wil ik dit proefschrift opdragen.