The interaction between vasopressin and modulators of the cardiovascular system

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CHAPTER 1

INTRODUCTION

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1 Historical background

Arginine Vasopressin (vasopressin, AVP), or the antidiuretic hormone (ADH), is secreted by the posterior lobe of the pituitary gland. Oliver and Schaefer in 1895 were the first to describe its vasoconstrictor activity. They demonstrated that hypophyseal extracts display vasoconstrictive properties. In 1913, the antidiuretic effect of injections of extracts of the pituitary gland was first observed in patients with diabetes insipidus. The synthesis and isolation of vasopressin in the 1950s confirmed that one and the same hormone of the pituitary gland is responsible for the vasoconstrictive and the antidiuretic effects. Subsequently, several analogues of vasopressin were synthesized, such as desmopressin, a vasopressin agonist with selective antidiuretic properties, which is used in the treatment of diabetes insipidus. An important subsequent step was the development of the peptide vasopressin-antagonists in the nineteen sixties and onwards. This development of analogues and antagonists enabled scientists to evaluate the role of vasopressin in human physiology and pathophysiology. This progress was further stimulated by the discovery of nonpeptide vasopressin antagonists, which display sufficient bioavailability for oral use.

2.1 Biosynthesis of vasopressin

The oxytocin-vasopressin superfamily is found both in vertebrates and invertebrates, and characterised by a peptide structure of 9 amino acids. The preserved nonapeptide pattern throughout the evolution indicates that both the precursor structures and the processing enzymatic machinery were largely conserved to ensure the generation of a specific conformation. Virtually all vertebrate species possess an oxytocin- and a vasopressin-like peptide, respectively, thus allowing the tracing of two evolutionary lineages. The ancestral gene encoding the precursor protein antedates the divergence between the two groups, about 700 million years ago. Vasopressin is a peptide with a disulfide bond between the two cysteine-amino acids in the positions 1 and 6. All mammal vasopressins have the same amino acid sequence with an arginine in position 8, except the porcine vasopressin, which has a lysine in position 8 (Figure 1).
Vasopressin is synthesized predominantly in the neurosecretory cells of the supraoptic- and secondarily the paraventricular-nucleus, both located in the hypothalamus. The gene is located on chromosome 20p13 and consists of 3 exons. These exons form the preprohormone of 168-amino-acids. The first 23 amino acids form the signal peptide, which is responsible for the connection to the ribosomes. During the protein synthesis this signalling peptide is removed, after which the prohormone is transported via the endoplasmic reticulum (ER) and the Golgi apparatus into secretory granules. In these granules the prohormone disintegrates, under the influence of the enzymes monoxygenase, exopeptidase, endopeptidase and lyase, to yield three proteins. These proteins are vasopressin (9 amino acids), vasopressin-neurophysin (92 amino acids) and vasopressin-glycopeptide (39 amino acids), respectively. Vasopressin-neurophysin is responsible for correct intracellular transport and processing. The function of the vasopressin-glycopeptide is unknown. After internalisation of vasopressin into the granules, it is transported from the paraventricular- and the supraoptic-nuclei, via axons located in the internal zone of the median eminence, towards the nerve endings at capillaries in the posterior pituitary.  

At present more than 35 mutations have been related to hereditary forms of central diabetes insipidus. Most of the mutations are found in the signalling peptide or in the vasopressin-neurophysin part, and only two are found in the primary structure of vasopressin itself. This finding emphasizes the importance of the correct configuration of the (pre) prohormone necessary for correct intracellular processing. Most known forms of familial central diabetes insipidus have an autosomal dominant inheritance pattern, which becomes
clinically manifest in the period several months after birth until early adolescence. The reason why this autosomal dominant disease displays a delayed clinical onset so far remains unknown. Several authors suggest that the large accumulation of misfolded precursors is ultimately toxic towards the secretory cells that produce them. This hypothesis is supported by in vitro experiments that demonstrated the occurrence of cellular toxicity due to the accumulation of misfolded precursors in the ER, resulting in morphological derangements. Another possibility is the formation of heterodimers by the normal allele and the mutant (pre) prohormone resulting in an impaired expression of the initial normal function of the non-mutant gene.

It has already been suggested two decades ago that vasopressin can be generated in peripheral tissues. Recently two independent publications demonstrated a local generation of vasopressin outside the central nervous system. Guillon et al. demonstrated by means of PCR and perfusion experiments that the rat and human adrenal medulla may express and release vasopressin under basal conditions and also as a result of stimulation by acetylcholine. Activation of the cortical V1 receptor (see paragraph 2.1) results in both steroid secretion and cortical growth. In the medulla, both V1 and V3 receptor subtypes are expressed. V3 receptors have been shown to stimulate catecholamine secretion. The role of the V1 receptor in the medulla remains unclear. Hupf et al. described the occurrence of low basal levels of vasopressin and the corresponding mRNA in the rat heart. After applying ventricular wall stress the vasopressin levels as well as vasopressin mRNA levels appeared to be increased. Systemic effects of this local vasopressin production remain uncertain. However, the combination of wall stress and coronary infusion of a V1-antagonist resulted in a decreased coronary perfusion pressure. This finding implicates that besides the centrally regulated release of vasopressin with its systemic effects, vasopressin may serve as a local tissue hormone.

### 2.2 Regulation of vasopressin secretion

The central secretion of vasopressin is regulated by several stimuli. The most important one is a change in plasma osmolality (P_{osm}). Changes in P_{osm} are perceived by specialized so called ‘osmoreceptor-neurons’ localized in the
hypothalamus. Plasma vasopressin levels (P_{AVP}) start to increase after an increase of P_{osm} above a threshold level of about 280-290 mOsm/kg. The rise of the P_{AVP}, in the lower range of (0-10 pg/ml), causes after this threshold level an increase by 1 pg/ml, whereas the P_{osm} increases by only 1 %. An increase of P_{AVP} by 1 pg/ml significantly increases osmolality of the urine. \(^{20}\) Maximal osmotic stress will increase the P_{AVP} to a level of 15-20 pg/ml.

P_{AVP} is also affected by changes in blood volume and blood pressure, detected by mechano-receptors of the vagal nerve endings in the aorta, the cardiac atria and pulmonary veins. Furthermore, endings of the glossopharyngeal nerve from the carotid sinus stimulate the release of vasopressin. \(^{21}\) Small decreases by about 8% of the extracellular fluid volume do not result in an increase of P_{AVP}. Beyond that point P_{AVP} increases steeply up to levels of 50-100 pg/ml. These levels result in a maximal antidiuresis and an increased peripheral resistance to prevent cardiovascular collapse. Besides these two important regulatory systems, several other factors are known to increase P_{AVP} such as hypoglycaemia, \(^{22}\) hypoxia, \(^{23}\) acidosis, \(^{24}\) pharyngeal stimuli, \(^{25}\) pain, \(^{26}\) and emetic stimuli. \(^{27}\) Vasopressin release can also be directly stimulated by hormones and mediators, such as acetylcholine, histamine, nicotine, dopamine, prostaglandins and angiotensin II, and inhibited by others such as opioids and the atrial natriuretic peptide. \(^{28}\)

### 3 Vasopressin-receptors and their subtypes

#### 3.1 The V\(_1\)-receptor (or V\(_{1\alpha}\)-receptor)

Michell et al. subdivided vasopressin-receptors into two major subtypes, that is the V\(_1\)- and the V\(_2\)-receptor, respectively. \(^{29}\) The V\(_1\)-receptors (Fig. 2) are primarily members of the G\(_{q/11}\) family, but the G\(_i\) family can also be involved under certain conditions. \(^{30,31}\) In analogy with \(\alpha_1\) and the H\(_1\)-receptors, the V\(_1\)-receptor activates, via a G\(_{q/11}\)-protein, phospholipase C-\(\beta\) which cleaves phosphatidylinositol-4,5-bisphosphate, thus yielding inositol-1,4,5-triphosphate (IP\(_3\)) and diacylglycerol (DAG). IP\(_3\) induces the release of calcium ions from intracellular stores. \(^{32}\) Furthermore the activation of the V\(_1\)-receptor leads to an enhanced influx of extracellular Ca\(^{2+}\) ions, via L-type Ca\(^{2+}\)-
channels. The mechanism underlying channel activation so far remains unknown. The V₁-receptor also activates phospholipase D, resulting in the hydrolysis of other phospholipids to produce phosphatidic acid, which is further metabolised to yield DAG. DAG in its turn activates protein kinases C and this results in the phosphorylation of key proteins further downstream. Finally, the V₁-receptor mediates the activation of phospholipase A₂, and it activates the cyclooxygenase pathway via the mobilisation of arachidonic acid from phospholipids. All these effects result in either immediate or late responses. Direct responses cause an activation of the contractile elements of the vascular smooth muscle cells, stimulation of the glycogenolysis in the

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**Figure 2:** Signal transduction mechanism mediated by the V₁-receptor. Abbreviations: PLA₂, phospholipase A₂; PLC-β, phospholipase C-β; PLD, phospholipase D; PIP₂, L-3-phosphatidyl-D-myoinositol-4,5-bisphosphate; IP₃, inositol-(1,4,5)-trisphosphate; DAG, 1,2-diacylglycerol; PA, phosphatidic acid; AA, arachidonic acid; PS’s, prostaglandins; LTE’s, leucotriens; TXA, Thromboxanes; PKC, Protein Kinase C; Ras GRF, Ras guanine-nucleotide releasing factor; PYK2, proline-rich tyrosine kinase 2; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MKK, MAPK kinase; M KK K, MAPK kinase kinase.
hepatocytes, and increased platelet aggregation. The late responses involve the V₁-receptor-mediated effects on cellular hyperplasia and hypertrophy. These effects seem to involve an increased expression of the proto-oncogenes c-fos and c-jun, which are both active in transcription regulation of many other genes necessary for cellular growth. Also the activation of MAP kinases seems to be partly regulated by vasopressin. The V₁-receptor as Gq-coupled receptor can activate the MAPKerkl/2 pathway via the upstream stimulation of MAPK Kinase Kinase Raf 1 (MKKKRaf 1). MKKKRaf 1 in this setting can be stimulated through protein kinase C and via an increase in intracellular Ca²⁺ concentration.

Until the nineteen seventies research was focused on the antidiuretic effects of vasopressin, because physiological and pathophysiological levels of vasopressin were thought not to play a role in the regulation of vascular tone. However, it is now assumed that vasopressin at normal or increased plasma levels does indeed play a role in the regulation of blood pressure. Several important findings support this view. Vasopressin has been demonstrated to be a potent vasoconstrictor in vitro. This vasopressin-induced vasoconstriction is most prominent in the splanchnic, muscular and cutaneous vascular beds. In vivo, the vasoconstrictor effects of vasopressin are compromised by a decrease in activity of the sympathetic nervous system. Under physiological conditions, even large elevations of plasma vasopressin levels do not influence blood pressure, as a result of a counteracting activity of the baroreflex system. Conversely, it has been demonstrated that ganglionic blockade enhanced vasopressin-induced pressor activity. The comparison of vasopressin dose-response relationships in conscious normal and sinoaortic denervated dogs, revealed an enhancement after denervation of the pressor sensitivity to vasopressin that was far stronger than that observed for either angiotensin II or norepinephrine. Lumbers et al. demonstrated that there were no peripheral vasopressin-mediated interactions with baroreceptor discharge, when measured by electrical activity in dissected single fibres from the carotid sinus nerve of anaesthetized dogs. This finding suggests that the vasopressin-induced augmentation of the baroreflex system is centrally located and not dependent on alterations in the sensitivity of carotid sinus baroreceptors. Several authors evaluated the effect of an increased vasopressin-plasma concentration enhancing the inhibitory action of the baroreceptors. The inhibitory action stimulated by vasopressin was
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prevented by local destruction of the area postrema and this effect appeared to be mediated by the $V_1$-receptor. This observation confirms the central localisation of vasopressin-induced sensitisation of the baroreflex. Interestingly, in contrast with this feedback mechanism of vasopressin-mediated vasoconstriction, several recent studies show that vasopressin may exert direct potentiating effects on the perivascular nerve fibers of the sympathetic nervous system. 53-55 This peripheral facilitation of the sympathetic nervous system has been demonstrated only in single vessel preparations and it results in an increase of vascular tone. This effect is probably $V_1$-receptor mediated. Conflicting results are presented regarding their pre- or post-synaptic site of action (Figure 3).

![Diagram of sympathetic nerve terminal](image)

**Figure 3**: Potentiating actions of vasopressin on the sympathetic nerve varicosity and the vascular smooth muscle cell. Abbreviations: NA, noradrenaline; $\alpha_1$ and $\alpha_2$, $\alpha_1$- and $\alpha_2$- adrenoceptors.

Although vasopressin-induced peripheral vasoconstrictor effects are considered to be the main hemodynamic function of the $V_1$-receptor, this
receptor may also mediate direct cardiac effects. It has been suggested that the strong reduction in cardiac output induced by circulating vasopressin is not caused by a sensitised baroreflex system, but rather provoked by direct cardiac actions of this peptide. 56 Graf et al. demonstrated, that the V₁-receptor is involved in the regulation of cardiac function and perfusion. 57 The most prominent V₁-mediated effects on cardiac physiology appear to be coronary vasoconstriction and impaired relaxation. 58 Furthermore, it is plausible that V₁-receptor activation exerts positive inotropic effects, due to increased intracellular calcium levels in cardiac myocytes. 59;60 Besides these direct effects, protein synthesis is also increased by V₁-receptor stimulation, and this phenomenon may be important for the development of cardiomyocyte hypertrophy and cardiac remodelling. 61-63 The relevance of these V₁-mediated effects is emphasized by the assumption that vasopressin possibly serves as a local tissue hormone in the heart. 19

**Figure 4:** Transmembrane topology of the human vasopressin V₁-receptor showing functionally important residues. Amino acids highlighted in black circles are critically involved in agonist binding. Potential glycosylation (on Asn 14, 27 and 196) and palmitoylation (on Cys 365 and 366) sites are also indicated. Adapted from Barberis et al., with permission. 64

In addition to the cardiovascular effects on end organs, the V₁-receptor also mediates many interactions of vasopressin with other neurohormones. The
central pressor action of angiotensin II is partly mediated by an increased release of vasopressin. \(^{65-67}\) Furthermore, \(V_1\)-receptor activation of endothelial cells increases the release of endothelin 1. \(^{68-70}\) In the deoxycorticosterone acetate (DOCA)-salt hypertension model in rats, some of the vascular effects of vasopressin provoking hypertension are mediated by endothelin 1. \(^{71}\) The \(V_1\)-receptor activation is also involved, although indirectly, in the inhibition of Atrial Natriuretic Factor (ANF) secretion in the isolated heart by stimulating NO-release from the endothelium. \(^{72}\) This interaction with endothelium-derived NO causes a reduction of \(V_1\)-mediated vasoconstriction in the rat kidney. \(^{73}\) Furthermore, exogenous vasopressin induces the release of NO, resulting in vasodilation both in dog brain stem arteries and in the human forearm. \(^{74};75\) In certain vascular beds the increase in peripheral resistance may also be diminished by vasodilator prostaglandins from the endothelial cells. \(^{76};77\) Moreover, the prostaglandin-regulated proliferative effects may be induced by \(V_1\)-receptor activation. \(^{78};79\)

### 3.2 \(V_1\)-receptor agonists and -antagonists

In the past decades close to a thousand analogues of vasopressin and oxytocin have been synthesised. \(^{80};81\) These agonists and antagonists have played an important role in the pharmacological identification of the three classical vasopressin receptors. In the past decade several new \(V_1\)-receptor antagonists have been developed. OPC-21268 was the first nonpeptidergic \(V_1\)-antagonist described. \(^{10}\) Although this antagonist was potent in the rat, it failed to induce an inhibition of \(V_1\)-receptor mediated effects in humans. \(^{82};83\) Another nonpeptide antagonist is SR-49059 (relcovaptan), which displays a marked affinity, selectivity and efficacy towards both animal and human \(V_1\)-receptors, without any partial agonistic activity. \(^{84}\) SR-49059 has been studied in women with primary dysmenorrhea, since in such patients the plasma concentrations of vasopressin are known to be elevated. \(^{85};87\) Furthermore the effect of vasopressin on uterine activity is known to increase premenstrually, and a premenstrual rise in the density of \(V_1\)-receptors has been demonstrated. SR49059 proved to be beneficial in the symptomatic treatment of primary dysmenorrhea. In addition SR-49059 was studied in hypertensive patients. The outcomes of these investigations will be discussed in paragraph 4.2. \(^{88}\) In
all human studies so far performed no serious adverse effects of the V₁-antagonist were observed.

![Chemical structures of V₁-antagonists](image)

**Figure 5:** Chemical structures of V₁-antagonists: The quinolinone derivative OPC-21268 and the N-Sulfonyl-indoline derivative SR-49059.

### 3.3 The V₂-receptor

V₂-receptor activation causes second messenger activation, which is comparable to other stimulants of Gₛ-protein coupled receptors. When occupied by an agonist, the V₂-receptor (Fig. 6) catalyses the conversion of GDP into GTP on the Gₛ-protein, with subsequent dissociation of Gₛ into its αₛ and βᵧ subunits. The αₛ subunit stimulates the well-known second messenger system of adenyl cyclase, which catalyzes the generation of cyclic AMP, thus triggering the activation of cyclic AMP-dependent protein kinase (PKA). PKA controls several cellular functions by phosphorylating various substrates. In the principal cells of the entire renal collecting duct, this results in a decreased rate of endocytosis of water channels (aquaporins-2) from the apical membrane. Also the exocytosis-rate of aquaporin-2 containing vesicles to the apical membrane is increased. Furthermore, PKA phosphorylates cyclic-AMP response binding protein, which in its turn increases the transcription rate of the gene encoding aquaporin-2. The increased number of aquaporins on the apical membrane due to V₂-receptor activation results in an increased reabsorption of water by the renal collecting duct.
Figure 6: Signal transduction mechanism mediated by the V2-receptor involved in the regulation Aquaporin-2 translocation to the apical membrane in the collecting duct. Abbreviations: PKA, Protein Kinase A; cAMP, cyclic AMP; AQP2, Aquaporin 2

Vasopressin regulates urine osmolality and volume (Fig. 7) by adapting the permeability to water of the cortical- and medullary-collecting duct to water via the V2-receptor. Furthermore, activation of the V2-receptor induces an increased number of active Na-K-2Cl cotransporters (NKCC2), epithelial sodium channels (ENaC) and renal outer medullary potassium channels (ROMK) in the luminal membrane. 90-93 This results in increased sodium reabsorption and potassium secretion in the thick ascending limb and the cortical and medullary-collecting ducts. Recent studies suggest that vasopressin also decreases extra renal fluid losses via the upregulation of ENaC's in the lung. 94 V2-receptor antagonists are able to prevent this effect. In the terminal inner medullary collecting duct V2-receptor activation increases permeability to urea. This process probably involves a PKA-induced phosphorylation of the vasopressin-regulated urea-transporter A1 (UT-A1). 95
<table>
<thead>
<tr>
<th>Nr.</th>
<th>Location</th>
<th>Receptor</th>
<th>Effect via the activation of the following mechanism.</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>Tall ascending limb</td>
<td>$V_2$</td>
<td>ENaC</td>
</tr>
<tr>
<td>2</td>
<td>Cortical collecting duct</td>
<td>$V_2$</td>
<td>AQP2, NKCC2, ENaC and ROMK</td>
</tr>
<tr>
<td>3</td>
<td>Terminal collecting duct</td>
<td>$V_2$</td>
<td>AQP2 and UT-A1</td>
</tr>
<tr>
<td>4</td>
<td>Medullary interstitial cells</td>
<td>$V_1$</td>
<td>Stimulation of the prostaglandin production</td>
</tr>
<tr>
<td>5</td>
<td>Vasa recta</td>
<td>$V_1$</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>6</td>
<td>Pneumocytes 2 in the lung</td>
<td>$V_2$</td>
<td>ENaC</td>
</tr>
</tbody>
</table>

**Table 1:** Vasopressin-mediated effects in water and electrolyte homeostasis. Abbreviations: AQP2, aquaporin-2; EnaC, epithelial sodium channels; NKCC2, Na-K-2Cl co-transporters; ROMK, renal outer medullary potassium channels; and UT-A1, vasopressin-regulated urea-transporter A1

**Figure 7:** Effects of vasopressin on the Nephron. Numbers refer to those given in table 1.
All $V_2$-mediated effects can be partly counteracted by a $V_1$-receptor mediated production of prostaglandin $E_2$ (Pgs) in the medullary interstitial cells and are thought to play a role at plasma vasopressin levels of 15 pg/ml and higher. Prostaglandin $E_2$ is known to inhibit cyclic AMP in the collecting duct. This explains the well-known antidiuretic side effect of indomethacin and other prostaglandin synthesis-inhibitors (NSAID’s). In addition, physiological elevations of plasma vasopressin levels (up to 8 pg/ml) reduce blood flow to the inner medulla, whereas a constant blood flow in the outer medulla is maintained. This is a $V_1$-receptor dependent effect at the level of the microvessels of the medullary vasa recta. Accordingly, urine osmolality during water restriction is optimised, without any changing of baseline arterial pressure or renal cortical blood flow. This mechanism appears to be of utmost importance because of the high oxygen consumption by the renal cortex.

The $V_2$-receptor is less widely distributed than the $V_1$-receptor. The $V_2$-receptor is especially known for its renal effects, but in the past decade evidence was presented that extra-renal $V_2$-receptor activation may result in vasodilation of several vascular beds.

### 3.4 $V_2$-receptor agonists and antagonists

A well-known vasopressin analogue is 1-deamino-8-D-arginine vasopressin (DDAVP, or Desmopressin). This agonist is about 3000 times more functionally selective for the $V_2$ than for the $V_1$-receptor. $V_2$-receptor activation by desmopressin causes a strong antidiuretic effect. Desmopressin forms the cornerstone in the treatment of all forms of central diabetes insipidus.

It was already demonstrated around nineteen hundred that stress enhanced blood clotting via the increase of plasma adrenaline concentrations. Later it became evident that this effect was caused by an increase in coagulation factor VIII (VIII:C). Besides adrenaline also vasopressin and insulin were able to induce an increase in VIII:C. This increase in VIII:C was comparable in healthy humans and in patients with mild Hemophilia A. The development of the synthetic vasopressin analogue desmopressin, which is virtually devoid of hemodynamic side effects, allowed the application of a blood-transfusion-
free therapy for the prevention and treatment of bleeding in these patients. The source of VIII:C release is not known, but V₂-receptor mediated, since patients with nephrogenic diabetes insipidus are unresponsive to desmopressin. ¹⁰² In addition to the increase of VIII:C, desmopressin increases plasma levels of tissue-type plasminogen activator (t-PA) and von Willebrand factor (vWF) and it enhances platelet adhesion to the vessel wall. ¹⁰³,¹⁰⁴ Endothelial cells release both t-PA and vWF. Only t-PA release can be acutely initiated by desmopressin, whereas the release of the vWF seems to be synthesis dependent. ¹⁰⁵,¹⁰⁶ The fibrinolysis induced by the release of t-PA is generally mild, desmopressin is therefore suitable to reduce the clotting time for clinical purposes.

The following V₂-antagonists are currently under investigation SR-121463, OPC-31260, OPC-41061 (tolvaptan), VPA-985 (lixivaptan), WAY-140288, VP-343, VP-339 and FR-161282. The first 4 compounds are presently undergoing phase II clinical trials. These 4 compounds have been shown to produce a dose dependent aquaresis (increase in urine volume, with a low urine osmolality) and a correction of hyponatremia if present. The major potential clinical indications for the V₂-antagonist are conditions that are associated with hyponatremia and fluid retention, such as congestive heart failure, liver cirrhosis with ascites and the syndrome of inappropriate antidiuretic hormone (SIADH).

YM-087 (conivaptan) is a mixed V₁/V₂-antagonist, with a comparable affinity for the V₁ and V₂ receptor. This compound is under evaluation in phase III clinical trials, in particular for the treatment of hyponatremia associated with congestive heart failure. Currently three other V₁/V₂-antagonists are in the pre-clinical stage of investigation: YM-471, JTV-605 and CL-385004.
Figure 8: Chemical structures of V₂-antagonists and a V₁₂-antagonist: The benzazepine derivatives OPC-31260, OPC-41061 and VPA-985 and the N-Arylsulfonyl-oxindole derivative SR-121463, all selective V₂-antagonists. The benzazepine derivative YM-087 a combined V₁₂-antagonist.
3.5 V₃-receptor (or V₁b-receptor)

Studies of ligand binding profile, coupling to phospholipase C and adenylyl cyclase, revealed a unique pharmacological profile for this pituitary gland receptor. Depending on the level of receptor expression, the V₃-receptor couples to members of the Gₐ₁₁ family, alone or in combination with Gᵢ, and it may also recruit Gₛ. Consequently, the human V₃-receptor displays a pharmacological profile clearly distinct from that of the human V₁- and V₂-receptors. Accordingly, the earlier nomenclature designating this receptor as V₁b, suggesting that this receptor evokes a comparable second messenger activation as the V₁(a)-receptor, has become obsolete.

Stimulation of the V₃-receptor enhances the release of adreno-corticotrophic hormone (ACTH) from the anterior pituitary gland. Furthermore, recent studies have demonstrated that the V₃-receptor is expressed in multiple brain regions and in a number of peripheral tissues, such as the kidney, adrenal medulla, pancreas, thymus, heart, lung, spleen, uterus, and breast. In the human adrenal medulla the V₃-receptor is thought to be involved in the regulation of adrenal function. In the rat pancreas, the V₃-receptor may enhance both insulin and glucagon release. Vasopressin induces insulin and glucagon release in a glucose-dependent manner, the higher the glucose concentration, the stronger the enhancement of vasopressin-induced insulin release, and vice versa. The possible physiological importance of the peripheral V₃-receptor in other organs remains to be established. Recently, the first selective, nonpeptide V₃-antagonist, SSR149415, was described. The developmental status of this compound is still preclinical.

3.6 Recently discovered vasopressin receptor-subtypes

Several new vasopressin-receptors, with varying scientific evidence with regard to their biological activity and functional relevance, are currently under investigation. An example is the vasopressin-activated calcium-mobilizing (VACM-1) receptor. The VACM-1 receptor does not share any structural or sequence homology with the cloned V₁- or V₂-receptors, has one putative transmembrane domain and belongs to the recently discovered gene family
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termed cullins. VACM-1 receptors are localised in endothelial-, renal medullary collecting tubule- and brain cells. The biological role of the VACM-1 receptor so far remains unknown. The VACM-1 receptor has, in analogy with other cullin family members, been implicated in the regulation of the cell cycle. Furthermore, VACM-1-receptor activation is likely to influence intracellular signalling pathways.

Besides the VACM-1 receptor a dual angiotensin II (Ang II)-vasopressin receptor was discovered, by screening of a rat kidney complementary DNA library. This receptor has probably 7 transmembrane regions and seems to be G₅-coupled. It appears to be substantially different from other G-protein coupled receptors. The corresponding mRNA has been cloned in many human peripheral tissues. Functional analysis revealed specific binding to both Ang II and vasopressin, and to Ang II- and vasopressin-induced coupling to the adenylate cyclase second messenger system. Site-directed mutagenesis of the predicted Ang II binding domain obliterated Ang II binding, but preserved vasopressin binding, suggesting the presence of distinct Ang II and vasopressin binding domains.

Recently, the screening of a rat kidney complementary DNA library revealed a "V₆ encoded vasopressin V₁-type receptor", with a high density in vascular smooth muscle cells. Pharmacological studies have suggested the possible existence of another receptor, displaying vasodilator activity after stimulation with vasopressin-derived peptides and exhibiting little or no functional interactions with the classic three vasopressin-receptors. These peptides are structural analogues of V₂ receptor ligands. Indeed, Johns et al. demonstrated a desmopressin-induced vasodilation, which proved insensitive to V₁- or V₂-antagonists, without evoking an increase of cAMP or cGMP levels.
4 Clinical relevance of vasopressin and the related drugs

The present investigation is predominantly concerned with the vascular effects of vasopressin and therefore three cardiovascular syndromes in which vasopressin or its antagonists could play a role will be discussed.

4.1 Shock

Haemorrhagic,\textsuperscript{122} cardiogenic,\textsuperscript{123} and septic shock\textsuperscript{124} are associated with an increase in plasma vasopressin levels. In the early phase these levels are the highest reported for the release of endogenous vasopressin. The plasma levels range between 100 and 1000 pg/ml, compared to the level of 20 pg/ml, which is needed to maximally increase urine osmolality during water deprivation. During the progression of shock, the vasopressin levels are decreasing for so far unexplained reasons.\textsuperscript{125} It has been speculated that inappropriately low plasma levels of vasopressin are, at least partly, related to a depletion of vasopressin stores in the pituitary gland.\textsuperscript{126}

Besides the well-known effectiveness of vasopressin in the treatment of bleeding from oesophageal varices, there exists limited evidence with the systemic use of vasopressin in patients with a severe haemorrhage.\textsuperscript{127} Two randomised clinical trials, in which the therapeutic potential of vasopressin in septic shock was investigated, have been conducted.\textsuperscript{128,129} The first study randomly assigned 10 patients to vasopressin 0.04 U/min or placebo. This treatment resulted in an increased peripheral resistance and blood pressure; moreover, all patients receiving vasopressin were withdrawn from all other catecholamine pressor agents 24 hours later. The second study, randomly assigned 24 patients to a 4-hour double-blinded infusion of noradrenaline or vasopressin. In contrast to noradrenaline, vasopressin decreased the need for conventional therapy and increased urine output and creatinine clearance. There were no signs of myocardial or gastric ischaemia due to the treatment with vasopressin.

In patients subjected to cardiopulmonary resuscitation (CPR) endogenous vasopressin plasma levels are higher in those who survived.\textsuperscript{130} The use of
vasopressin in a CPR setting has theoretically, an advantage over adrenaline, since β-adrenergic receptor-mediated effects do not occur. Accordingly vasopressin does not increase myocardial oxygen consumption and it prevents skeletal muscle vasodilation. Furthermore, in contrast to cathacholamine-induced vasoconstriction, the vasoconstriction elicited by vasopressin is maintained during severe acidosis. This suggests that vasopressin becomes relatively more effective with a longer duration of cardiac arrest. During a short period of experimentally-induced ventricular fibrillation in animals, exogenous vasopressin during CPR increased coronary perfusion pressure, cerebral oxygen delivery, and the blood flow was redistributed in favour of the vital organs.

Two trials have been performed in patients with cardiac arrest comparing vasopressin to adrenaline. One study demonstrated, in patients with out-of-hospital ventricular fibrillation, a significantly better survival for the vasopressin-treated group. Another study evaluated the same drugs in 104 patients with in-hospital cardiac arrest (20% ventricular fibrillation), however, this study failed to detect an advantage for vasopressin over adrenaline. An ongoing large trial (1500 patients) is conducted under the guidance of the European Resuscitation Council in order to study the possible benefits of vasopressin in patients with cardiac arrest.

According to the Guidelines of the American Heart Association, vasopressin may be a more effective than adrenaline in promoting the return of spontaneous circulation in cardiac arrest due to ventricular fibrillation. The evidence from prospective clinical trials in humans is limited but consistently positive (Class IIb). Vasopressin (40 U IV, not repeated) may be substituted for adrenaline as an alternative Class IIb agent (acceptable; fair supporting evidence). The better adverse effects profile of vasopressin may be the major indication for its use. So far, this advice has not yet been incorporated into the guidelines of the European Resuscitation Council.
4.2 Hypertension

The role of vasopressin in the pathogenesis of hypertension is controversial. In animal models of experimental hypertension the role of vasopressin has been extensively investigated. In Spontaneous Hypertensive Rats (SHR), two-kidney one clip hypertension in rats, Dahl's salt sensitive rats receiving a high salt diet, and DOCA-salt induced hypertension in rats, bolus injections of peptidergic- and non-peptidergic V₁-receptor antagonists resulted in a significant decrease in blood pressure. These results suggest that vasopressin is involved in the maintenance of hypertension, at least in these animal models.

Chronic administration of the V₁-receptor antagonist OPC 21268 in an experimental rat model of DOCA-salt induced hypertension significantly prevented the development of hypertension. Treatment with a V₁-receptor antagonist reduced the full development of hypertension in SHR. These results are in contrast with those of others who, in the same model, did not observe a beneficial effect for chronic treatment with a V₁-antagonist. In the two-kidney one clip model of hypertension chronic treatment with V₁- and V₂-antagonists did not demonstrate a role for vasopressin in the maintenance of blood pressure. In another model evaluating the chronic administration of vasopressin antagonists the role of vasopressin seems to be less important. Chronic NO-synthesis inhibition induced hypertension was not influenced by the chronic administration of a V₁-receptor antagonist.

Research in humans concerning the role of vasopressin in hypertension has been performed as well. These studies demonstrated increased plasma vasopressin levels. The major question already asked by Padfield in 1976 is: are the increased levels of vasopressin a cause or an effect of hypertension? V₁-receptor blockade in healthy volunteers does not influence blood pressure, suggesting that vasopressin is not involved in normal blood pressure regulation. Moreover infusion of vasopressin in normal subjects, beyond the levels found in malignant hypertensive patients, did not influence blood pressure. In patients with the syndrome of inappropriate antidiuretic hormone (SIADH) the clinical picture is characterised by fluid retention and low serum sodium levels. However, in patients with SIADH the
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elevation of vasopressin plasma levels do not result in the development of hypertension.\textsuperscript{155-157}

The outcomes of these studies in combination with the results from animal experiments do not support a major role of vasopressin in the development and maintenance of hypertension, although some criticism on this view has been expressed. Ribeiro et al. demonstrated that random sequential blockade of the sympathetic nervous system, the renin-angiotensin system, or the vasopressin system increased the blood pressure dependence of the two unblocked systems in patients with severe hypertension. Although the contribution of the vasopressin system was rather limited, the vasopressin component in blood pressure maintenance was evident.\textsuperscript{158} It is generally accepted that ACE-inhibition in patients decreases vasopressin release,\textsuperscript{153,159} but under certain conditions the sensitivity to vasopressin may be increased under ACE inhibition.\textsuperscript{160} Furthermore, acute blood pressure lowering with sodium nitroprusside or clonidine in hypertensive patients increased vasopressin plasma levels.\textsuperscript{161} Antihypertensive drug treatment at present is mainly based upon suppression of the activities of the sympathetic nervous system and of the renin-angiotensin aldosteron system (RAAS). It can be imagined that the vasopressin "system" may gain importance in the pathophysiology of hypertension, and also as a target for drug treatment.

4.3 Vasopressin in negroid hypertensives

Vasopressin levels appear to increase with age in normotensive subjects, partly as a result of decreased urinary concentrating capacity, and vasopressin is assumed to play a more prominent role in the regulation of blood pressure in the elderly.\textsuperscript{162-166} Ethnic factors also differentiate hypertensive patients in this respect.\textsuperscript{151} African-American hypertensives are known to display increased vasopressin plasma levels compared to age-matched hypertensive Caucasians or African-American normoten-sives.\textsuperscript{167} The involvement of vasopressin in blood pressure control was emphasized by Bakris et al., who found that only in African-American hypertensives a $V_1$-antagonist decreased blood pressure. This effect was not seen in age-matched Caucasians.\textsuperscript{168} African-American hypertensives are known to display a low renin, salt
sensitive form of essential hypertension, which is usually associated with higher levels of vasopressin. Thibonnier et al. evaluated the effect of a single oral dose of the V1-antagonist SR-49059 in 24 patients with hypertension and observed no reduction in blood pressure. However, after a hypertonic salt infusion (a procedure known to increase the release of endogenous vasopressin levels) in the presence of SR49059, the heart rate increase was limited, while the blood pressure peak proved decreased. Although the role of vasopressin in hypertension is certainly not straightforward, in certain categories of patients treatment with vasopressin-antagonists seems worthwhile to be investigated. In hypertension vasopressin-antagonists are thought to be preferably effective against both the V1- and V2-receptor, in order to lower both the peripheral vascular resistance and the circulating volume.

4.4 Congestive heart failure

Several authors have described elevated vasopressin plasma levels in patients with congestive heart failure. Francis et al. reported that patients with asymptomatic left-ventricular dysfunction had elevated plasma vasopressin levels when compared to control patients, whereas patients with symptomatic mild-to-moderate heart failure had even higher vasopressin plasma levels. Rouleau et al. emphasized the prognostic value of vasopressin levels in the Survival and Ventricular Enlargement (SAVE) population of post-myocardial infarction patients with left-ventricular dysfunction. In this study, 1 month after myocardial infarction vasopressin levels were associated with adverse long-term cardiovascular outcomes, including heart failure, recurrent myocardial infarction, and death. In patients with congestive heart failure some studies could not demonstrate a direct relation between cardiac output and serum sodium levels on the one-hand and vasopressin levels on the other. In patients with congestive heart failure, two groups may be distinguished: one group displays relatively low to slightly increased vasopressin levels, showing a 'normal' relationship between vasopressin and osmoregulatory control. Conversely, in the second group, with moderate to highly increased vasopressin levels, no relationship between vasopressin and plasma osmolality is established. In particular parameters reflecting renal function like blood urea nitrogen and serum creatinine levels, proved to be
deteriorated in the second group. Furthermore, plasma sodium levels were strongly decreased in the second group, which is an ominous sign in the prognosis for patients with congestive heart failure.

The possible role of vasopressin was evaluated in several animal models of congestive heart failure. In all studies an increase in vasopressin plasma levels, or an increased vascular sensitivity, was established. Acute administration of a V₁-antagonist induced various hemodynamic effects, ranging from no observed effect at all, to an increase in cardiac output, a decrease in systemic vascular resistance and improved renal function without significant changes in serum electrolytes and hormones. In one study the effect of administration of an ACE inhibitor was comparable to that of a V₁-antagonist with regard to the reduction of peripheral resistance. Furthermore, total peripheral resistance was normalised after the combined administration of an ACE inhibitor and a V₁-antagonist. A selective V₂-receptor antagonist (OPC-31260) induced marked water diuresis, reduced urine osmolality, increased plasma sodium and vasopressin concentrations, and increased plasma renin activity. The combined administration of a V₁ and a V₂-antagonist (OPC-21268 and OPC-31260) has yielded synergistic hemodynamic as well as additive renal and metabolic responses. These results were confirmed by the use of conivaptan, a nonpeptidergic combined V₁/V₂-antagonist.

In the nineteen eighties several studies were conducted to evaluate the response to a peptide V₁-receptor antagonist in patients with advanced congestive heart failure. A decrease of peripheral resistance was observed in 5 to 30 % of the patients. In a randomised, double blind, placebo controlled trial the effect of short-term treatment with conivaptan at a single intravenous dose (10, 20, or 40 mg) was evaluated in 142 patients with congestive heart failure (NYHA III and IV). Conivaptan at 20 and 40 mg significantly reduced pulmonary capillary wedge pressure and right atrial pressure. Furthermore, a dose-dependent increase in urine output was observed.

The possibilities of chronic treatment with V₁-,V₂-, or combined V₁/V₂-antagonists have been evaluated in animal models of congestive heart failure. Chronic V₁-receptor blockade improved left ventricular function as well
as neurohormonal profiles in rapid pacing-induced congestive heart failure in pigs. Chronic treatment with a V$_2$-antagonist provoked a marked water diuresis in a rat model of coronary artery ligation. Dual V$_1$/V$_2$-receptor blockade (conivaptan) induced a decrease in urine osmolality and an increase in urinary volume and plasma vasopressin levels. Furthermore, the right ventricular mass appeared to be reduced. Treatment with the ACE inhibitor captopril did not influence any renal parameter, but as expected it reduced left and right ventricular mass. Combination treatment of both conivaptan and captopril resulted, besides the above-mentioned effects, in a reduction of blood pressure, natriuretic peptide plasma levels, and pulmonary congestion. Therefore, in the management of vasoconstriction and fluid retention in the syndrome of congestive heart failure, treatment with V$_1$ and/or V$_2$ antagonists in addition to ACE-inhibitors could be favourable. Treatment of chronic congestive heart failure in pigs with a V$_1$-receptor antagonism (SR-49059) or Ang II type 1 (AT$_1$)-receptor antagonism (irbesartan), resulted in a reduction of left ventricular end diastolic dimensions, peak wall stress values and noradrenaline levels. Combination treatment by V$_1$- and AT$_1$-receptor blockade furthermore resulted in improved left ventricular and myocyte shortening.
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127. Morales D, Madigan J, Cullinane S, Chen J, Heath M, Oz M et al. Reversal by
vasopressin of intractable hypotension in the late phase of hemorrhagic shock.

128. Malay MB, Ashton RC, Jr., Landry DW, Townsend RN. Low-dose vasopressin in

129. Patel BM, Chittock DR, Russell JA, Walley KR. Beneficial effects of short-term

130. Lindner KH, Haak T, Keller A, Bothner U, Lurie KG. Release of endogenous

administration of vasopressin but not epinephrine maintains coronary perfusion
pressure after early and late administration during prolonged cardiopulmonary

during cardiopulmonary resuscitation: a randomized swine outcome study.

improves vital organ blood flow after prolonged cardiac arrest with

134. Wenzel V, Lindner KH. Employing vasopressin during cardiopulmonary resuscitation

135. Lindner KH, Dirks B, Strohmenger HU, Prengel AW, Lindner IM, Lurie KG.
Randomised comparison of epinephrine and vasopressin in patients with out-of-

Vasopressin versus epinephrine for inhospital cardiac arrest: a randomised

137. Part 6: advanced cardiovascular life support: section 6: pharmacology II: agents

138. de Latorre F, Nolan J, Robertson C, Chamberlain D, Baskett P. European
from the Advanced Life Support Working Group(1) and approved by the Executive

139. Sladek CD, Blair ML, Sterling C, Mangiapane ML. Attenuation of spontaneous

140. Ichikawa I, Ferrone RA, Duchin KL, Manning M, Dzuav VJ, Brenner BM. Relative
contribution of vasopressin and angiotensin II to the altered renal microcirculatory

141. Thibonnier M, Snajdar R, Rapp J. [Regulation of V1-vasopressinergic receptors in

pressure-lowering effect of an orally active vasopressin V1 receptor antagonist in

143. Yu M, Gopalakrishnan V, McNeill JR. Role of endothelin and vasopressin in DOCA-

pressor response in spontaneously hypertensive rats induced by stimulation of
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Aim of the present investigation

The neurohormone vasopressin plays an important role in the maintenance of the circulating volume and as a regulator of the vascular tone. The vasopressin receptor subtypes and their regulating function are reasonably well characterised. The availability of new non-peptidergic vasopressin-antagonists enables us to perform a more detailed analysis of the various effects of vasopressin. Accordingly, the major aim of the present study was to elucidate possible interactions of vasopressin with three other neurotransmitters and modulators associated with the cardiovascular system.

1) The sympathetic nervous is one of the major control mechanisms of the cardiovascular system. Circulatory impairment, in syndromes like congestive heart failure or shock, results in a reflex activation of the sympathetic nervous system. Vasopressin has been demonstrated to potentiate the inhibitory action of the baroreceptor-reflex on the sympathetic nervous system. In addition, several recent studies show that vasopressin may have direct potentiating effects on the perivascular nerve fibres of the sympathetic nervous system. This peripheral facilitation of the sympathetic nervous system has been demonstrated only in single vessel preparations, and it is possibly V₁-receptor mediated. With respect to their pre- or post-synaptic site of action conflicting results have been presented. We have addressed these issues by evaluating the facilitating effect of vasopressin on the sympathetic system in three experimental models. Accordingly, we used the single vessel preparation of the rat mesenteric artery for isometric tension recordings, the intact circulation of the pithed rat to study the effects on the blood pressure, and the forearm-circulation of healthy human volunteers for the local effects on blood flow, respectively. Using the new, relatively selective, vasopressin-antagonists, the receptor characteristics were determined. Furthermore we assessed the location of the vasopressin receptors with respect to the pre-, or postsynaptic sites.

2) It is well established that the endothelium plays a pivotal role in the regulation of the vascular tone. In addition vasopressin interacts with
several local endothelium-dependent factors. To clarify their relative contribution we assessed the interaction of vasopressin with the NO-, the cyclooxygenase-, the lipoxygenase-pathways, endothelium-derived hyperpolarizing factor (EDHF) and the endothelin system, respectively. In these experiments we focussed on the role of these factors in the vasopressin-induced vasoconstriction using the isolated renal artery of the rabbit for isometric tension recordings.

3) In recent years, intensive research has been conducted to analyse the intracellular regulatory pathways and mechanisms that are associated with cardiovascular diseases. The MAPK_{erk1/2} pathway appears to be highly relevant, since it has been associated with the development of congestive heart failure, cardiac hypertrophy and vasoconstriction. The established role of vasopressin in the regulation of the MAPK_{erk1/2} pathway has so far only been investigated in cultured cells. A variety of other G-coupled receptors have demonstrated a MAPK_{erk1/2} pathway dependence in the mediation of vasoconstriction in vascular tissue. Therefore, we focussed on the relation between vasopressin, vasoconstriction, and the MAPK_{erk1/2} pathway. For this purpose, we performed experiments with isolated rat aortae in an organ bath set-up for isometric tension recording in combination with Western blot analysis.