Review

Science Review: Vasopressin and the cardiovascular system part 1 – receptor physiology

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Published online: 26 June 2003 *Critical Care* 2003, **7**:427-434 (DOI 10.1186/cc2337)
This article is online at http://ccforum.com/content/7/6/427

Abstract

Vasopressin is emerging as a rational therapy for vasodilatory shock states. Unlike other vasoconstrictor agents, vasopressin also has vasodilatory properties. The goal of the present review is to explore the vascular actions of vasopressin. In part 1 of the review we discuss structure, signaling pathways, and tissue distributions of the classic vasopressin receptors, namely V_1 vascular, V_2 renal, V_3 pituitary and oxytocin receptors, and the P_2 class of purinoreceptors. Knowledge of the function and distribution of vasopressin receptors is key to understanding the seemingly contradictory actions of vasopressin on the vascular system. In part 2 of the review we discuss the effects of vasopressin on vascular smooth muscle and the heart, and we summarize clinical studies of vasopressin in shock states.

Keywords adrenergic agents, antidiurectic hormone, cardiac inotropy, hypotension, nitric oxide, oxytocin, physiology, potassium channels, receptors, septic shock, smooth muscle, vasoconstriction, vascular, vasodilation, vasopressin

Introduction

Arginine vasopressin (hereafter referred to as vasopressin), also known as antidiuretic hormone, is essential for survival, as attested by its teleologic persistence. Oxytocin- and vasopressin-like peptides have been isolated from four invertebrate phyla and the seven major vertebrate families, representing more than 120 species [1]. Therefore, the ancestral gene encoding the precursor protein appears to antedate the divergence of the vertebrate and invertebrate families, about 700 million years ago [2]. Virtually all vertebrate species possess an oxytocin-like and a vasopressin-like peptide, and so two evolutionary lineages can be traced. The presence of a single peptide, vasotocin ([Ile3]-vasopressin or [Arg8]-oxytocin), in the most primitive cyclostomata supports the notion that primordial gene duplication with subsequent mutations gave rise to the two lineages [2].

Vasopressin is essential for cardiovascular homeostasis. The vasopressor effect of pituitary extract, first observed in 1895, was attributed to the posterior lobe of this gland [3]. It was not until 18 years later that the antidiuretic effect of neurohypophyseal extract was demonstrated [4,5]. After isolation and synthesis of vasopressin in the 1950s, it was proven that the same hormone in the posterior pituitary possessed both antidiuretic and vasopressor effects [6,7]. The importance of vasopressin in osmotic defense is fundamental. Indeed, the antidiuretic effect of vasopressin has been exploited clinically for over half a century to treat diabetes insipidus. Only recently has vasopressin emerged as a therapy for shock states, renewing interest in the cardiovascular effects of vasopressin.

Shock states induce an increase in vasopressin levels from 20- to 200-fold [8–12]. These supraphysiologic levels cause

ACTH = adrenocorticotropic hormone; DAG = diacylglycerol; DDAVP = 1-deamino-8-D-arginine vasopressin; GPCR = G-protein-coupled receptor; GRK = G protein-coupled receptor kinase; OTR = oxytocin receptor; PKC = protein kinase C; P_2R = P_2 purinergic receptors; V_1R = V_1 vascular receptor; V_2R = V_2 renal receptor; V_3R = V_3 pituitary receptor.

profound vasoconstriction and help to maintain end-organ perfusion [13,14]. Prolonged shock is associated with a fall in vasopressin levels [15–18], probably due to depletion of vasopressin stores [19,20], and may contribute to the refractory hypotension that is seen in advanced shock states. Paradoxically, vasopressin has also been demonstrated to cause vasodilation in some vascular beds [21–28], distinguishing this hormone from other vasoconstrictor agents.

The present review explores the vascular actions of vasopressin. First, a discussion of the signaling pathways and distribution of vasopressin receptors is necessary to gain an understanding of the seemingly paradoxic vasodilatory and vasoconstrictor actions of vasopressin. We discuss the structural elements responsible for the functional diversity found within the vasopressin receptor family. In part 2 of our review, we explore the mechanisms of vasoconstriction and vasodilation of the vascular smooth muscle, with an emphasis on vasopressin interaction in these pathways. We review the seemingly contradictory studies and some new information regarding the actions of vasopressin on the heart. Finally, we summarize the clinical trials of vasopressin in vasodilatory shock states and comment on areas for future research.

Overview of vasopressin

Structure of the hormone and the genes

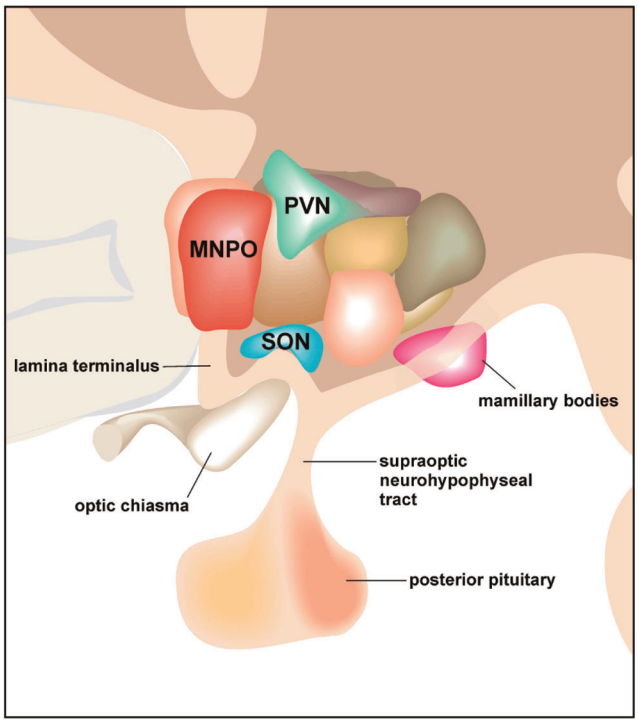
Vasopressin is a nonapeptide with a disulfide bridge between two cysteine amino acids [29] and is synthesized by the magnocellular neurons of the hypothalamus [30] (Fig. 1). Although oxytocin differs from vasopressin by only one amino acid (80% homology), they have clearly divergent physiologic activity. Vasopressin is involved in osmotic and cardiovascular homeostasis, whereas oxytocin is important in parturition, lactation, and sexual behavior.

Oxytocin and vasopressin are encoded by separate genes but they lie on the same chromosome, at 20p [31], separated by a segment of DNA only 12 kilobases long [32]. The similarities in structure as well as the close apposition are suggestive of recent gene duplication [33]. Despite ample documentation of cell-specific expression and physiologic regulation of the vasopressin gene, there is striking lack of progress in identifying transcription factors that act on the vasopressin promoter [34].

Structure of the receptor

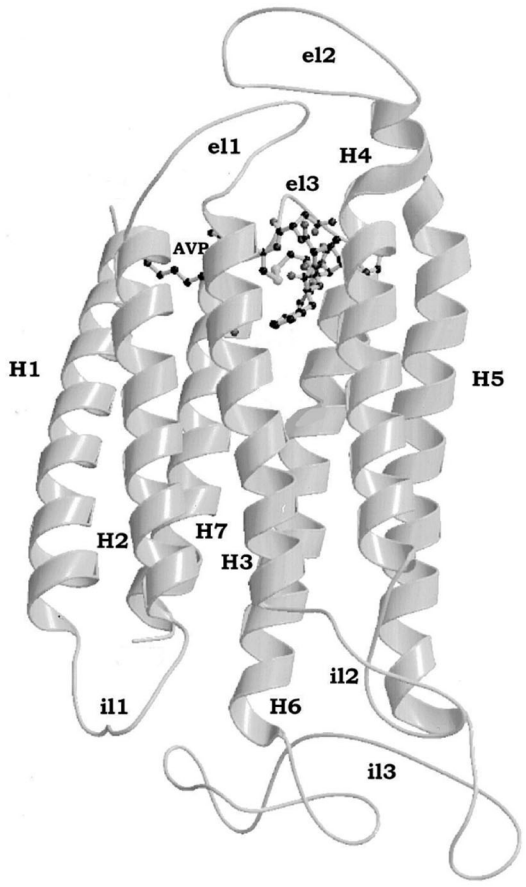
The actions of vasopressin are mediated by stimulation of tissue-specific G-protein-coupled receptors (GPCRs), which are currently classified into V_1 vascular (V_1R), V_2 renal (V_2R), V_3 pituitary (V_3R) and oxytocin (OTR) subtypes [35] and P_2 purinergic receptors (P_2R) [36]. The GPCRs are comprised of seven hydrophobic transmembrane α-helices joined by alternating intracellular and extracellular loops, an extracellular amino-terminal domain, and a cytoplasmic carboxyl-terminal domain (Fig. 2) [29]. The actions of vasopressin are signaled through pathways that are similar to extracellular agents such as hormones (glucagon, luteinizing hormone, and epinephrine [adrenaline]), neurotransmitters (acetylcholine, dopamine, and serotonin) and chemokines (interleukin-8). Local mediators signal to the four main G protein families to regulate cellular machinery such as metabolic enzymes, ion channels, and transcriptional regulators [37]. The extracellular signals are routed to specific G proteins through distinct types of receptors. For example, epinephrine's signal is transmitted through the β-adrenergic receptor coupled to G_i, and the α_1-adrenergic receptor coupled to G_q and G_{11}. Many important hormones, including epinephrine, acetylcholine, dopamine, and serotonin, interact with the G_i pathway, which is characterized by inhibition of adenylyl cyclase [37].

Figure 1



Hypothalamic nuclei involved in vasopressin control. The hypothalamus surrounds the third ventricle ventral to the hypothalamic sulci. The main hypothalamic nuclei subserving vasopressin control are the median preoptic nucleus (MNPO), the paraventricular nuclei (PVN), and the supraoptic nuclei (SON), which project to the posterior pituitary along the supraoptic–hypophyseal tract. Afferent nerve impulses from stretch receptors in the left atrium (inhibitory), aortic arch, and carotid sinuses (excitatory) travel via the vagus nerve, and neural pathways project to the PVN and the SON. These nuclei also receive osmotic input from the lamina terminalis, which is excluded from the blood–brain barrier and is thus affected by systemic osmolality. Vasopressin is synthesized in the cell bodies of the magnocellular neurons located in the PVN and SON. The magnocellular neurons of the SON are directly depolarized by hypertonic conditions (hence releasing more vasopressin) and hyperpolarized by hypotonic conditions (hence releasing less vasopressin). Finally, vasopressin migrates (in its prohormone state) along the supraoptic–hypophyseal tract to the posterior pituitary, where it is released into the circulation. Used by permission from *Chest* [95].

Figure 2



Vasopressin docking and transmembrane topology of the human V_1 vascular receptor (V_1R). A model of arginine vasopressin (AVP), as bound to the human V_1R, is depicted. Vasopressin is shown in ball-and-stick representation and the receptor is shown in ribbons. The intracellular loops of the receptor are labeled il1, il2, and il3, and the extracellular loops are labeled el1, el2, and el3. The transmembrane segments are labeled H1–H7. Reprinted from Thibonnier M, Coles P, Thibonnier A, Shoham M: **Molecular pharmacology and modeling of vasopressin receptors.** *Prog Brain Res* 2002, **139**:179-196. © 2002, with permission from Elsevier [96].

Agonist stimulation of vasopressin receptors leads to receptor subtype-specific interactions with G-protein-coupled receptor kinases (GRKs) and protein kinase C (PKC) through specific motifs that are present in the carboxyl termini of the receptors [38]. Guanine nucleotide-binding proteins (G-proteins) are signal transducers, attached to the cell surface membrane, that connect receptors to effectors and thus to intracellular signaling pathways [39]. Functional characterization of the G-proteins, including G_s, $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$ [37], indicates that a single receptor can activate multiple second messenger pathways through interaction with one or more G-proteins [40–42].

Vasopressin's signal is transmitted through both G_s and $G_{q/11}$ subtypes [37]. The G_s pathway is characterized by inhibition of adenylyl cyclase, leading to increased levels of cAMP that in turn connects to multiple cellular machines, including ion channels, transcription factors, and metabolic enzymes. Both β-adrenergic receptors and vasopressin receptors regulate G_s protein signaling. The $G_{q/11}$ pathway is the classical pathway that is activated by calcium-mobilizing hormones and stimulates phospholipase-β to produce the intracellular messengers inositol trisphosphate and diacylglycerol (DAG) [37]. Inositol trisphosphate triggers the release of calcium from intracellular stores and DAG recruits PKC to the membrane and activates it. The α-subunit of G_q also activates the transcription factor nuclear factor-κB [43].

The V_1 receptor

The V_1R gene is located on chromosome 12 and maps to region 12q14-15 [44]. Functionally, the V_1R activates G-proteins of the $G_{q/11}$ family. The α-subunits regulate the activity of the β-isoforms of phospholipase C [29]. A variety of signaling pathways is associated with the V_1R, and these pathways include activation of calcium influx, phospholipase A_2, phospholipase C, and phospholipase D [45].

V_1Rs are found in high density on vascular smooth muscle and cause vasoconstriction by an increase in intracellular calcium via the phosphatidyl–inositol-bisphosphonate cascade. Cardiac myocytes also possess the V_1R and are discussed in part 2 of the review. Additionally, V_1Rs are located in brain, testis, superior cervical ganglion, liver, blood vessels, and renal medulla [46]. The exact physiologic role of vasopressin in many of these diverse tissues remains unknown.

Platelets express the V_1R, which upon stimulation induces an increase in intracellular calcium, facilitating thrombosis [47]. However, there appears to be tremendous variability in the aggregation response of normal human platelets to vasopressin [48]. Based on kinetic studies and the effects of PKC inhibition on the aggregation response to vasopressin, significant heterogeneity in the aggregation response of normal human platelets to vasopressin has been demonstrated, which is probably related to a polymorphism of the platelet V_1R [49].

V_1Rs are found in the kidney, where they occur in high density on medullary interstitial cells, vasa recta, and epithelial cells of the collecting duct. Vasopressin acts on medullary vasculature through the V_1R to reduce blood flow to inner medulla without affecting blood flow to outer medulla [50]. V_1Rs on the luminal membrane of the collecting duct probably exerted through V_{1a} receptors located on luminal membrane limit the antidiuretic effects of vasopressin [50]. Interestingly, cyclosporine A induces upregulation of V_1R mRNA in vascular smooth muscle [51], increasing the number of V_1Rs by twofold [52], which could be a key mechanism by which cyclosporine A causes both hypertension and reduced glomerular filtration. Addition-

ally, vasopressin selectively contracts efferent arterioles [53], probably through the V_1R, but not the afferent arteriole. This selectivity, which is not shared by catecholamine vasopressors, would tend to increase glomerular filtration, probably accounting for the paradoxic increase in urine output observed when this antidiuretic hormone is administered to patients in vasodilatory shock [54,55].

There is considerable interspecies variation in the V_1R. For instance, although rat and human vasopressin are identical, the human V_1R is only 80% homologous with the rat V_1R [1]. This must be kept in mind when interpreting animal studies aimed at interpreting receptor subtypes based on the use of specific receptor inhibitors.

The V_2 receptor

The V_2 R differs from the V_1R primarily in the number of sites susceptible to N-linked glycosylation; the V_1R has sites at both the amino-terminus and at the extracellular loop, whereas the V_2R has a single site at the extracellular amino-terminus [56]. Despite structural similarities, the V_2R differs functionally from the V_1R. Mutagenesis experiments involving the V_1R and V_2R have confirmed that the short sequence at the amino-terminus of the cytoplasmic tail confers V_2 receptor–G_s coupling selectivity. The efficiency of V_2R–G_s coupling can be modulated by the length of the central portion of the third intracellular loop [57], whereas the second intracellular loop of the V_1R is critically involved in selective activation of $G_{q/11}$ [58].

The well known antidiuretic effect of vasopressin occurs via activation of the V_2R. Vasopressin regulates water excretion from the kidney by increasing the osmotic water permeability of the renal collecting duct – an effect that is explained by coupling of the V_2R with the G_s signaling pathway, which activates cAMP [59]. The increased intracellular cAMP in the kidney [60,61] in turn triggers fusion of aquaporin-2-bearing vesicles with the apical plasma membrane of the collecting duct principal cells, increasing water reabsorption [62]. Vasopressin regulates water homeostasis in two ways: regulation of the fast shuttling of aquaporin 2 to the cell surface and stimulation of the synthesis of mRNA encoding aquaporin 2 [63]. Most cases of diabetes insipidus can be explained by mutations in the V_2R gene, which is located on chromosome region 10q28 [64]. For example, an Arg137→His mutation in the V_2R abolishes coupling to the G_s protein, causing a complete phenotype of nephrogenic diabetes insipidus [65].

It has been postulated that the V_2R is also expressed in endothelium because the potent V_2R agonist 1-deamino-8-D-arginine vasopressin (DDAVP) causes both release of von Willebrand factor and vasodilation [21]. Previous studies of the localization and distribution of different vasopressin receptors have been hampered by the use of nonselective radioligands such as [^{3}H]arginine vasopressin, which binds to all types of V_1R and V_2R, certain OTRs, and neurophysins. When selective V_1R and V_2R radioligands with *in vitro* autoradiography were used to study V_1R and V_2R binding sites, no binding was demonstrated on endothelium or liver, where DDAVP might influence clotting factor release, or in the brain, spinal cord, sympathetic ganglia, heart or vascular smooth muscle – regions where DDAVP might cause vasodilation [46]. Specific binding was only identified in the kidney, which is consistent with the known distribution of antidiuretic V_2Rs on renal collecting tubules.

The V_3 receptor

The human V_3R (previously known as $V_{1b}R$) is a G-protein-coupled pituitary receptor that, because of its scarcity, was only recently characterized. The V_3R gene maps to chromosome region 1q32 [66]. The 424-amino-acid sequence of the V_3R has homologies of 45%, 39%, and 45% with the V_1R, V_2R, and OTR, respectively [67]. However, the V_3R has a pharmacologic profile that distinguishes it from the human V_1R and activates several signaling pathways via different G-proteins, depending on the level of receptor expression [68]. Interestingly the V_3R is also is over-expressed in adrenocorticotropic hormone (ACTH)-hypersecreting tumors.

More than one G-protein appears to participate in signal transduction pathways linked to V_3Rs, depending on the level of receptor expression and the concentration of vasopressin [69]. For instance, vasopressin causes secretion of ACTH from the anterior pituitary cells in a dose-dependent manner through activation of PKC [70] via the $G_{q/11}$ class [68]. Other cellular responses, including increased synthesis of DNA and cAMP, which are important in the induction and phenotype maintenance of ACTH-secreting tumors, are mediated through recruitment of several pathways, including G_s, G_i, and $G_{q/11}$ [68]. The V_3R has been inferred to exist in the pancreas [71] on the basis of antagonist studies; however, this conclusion may be suspect because significant homology exists between the V_3R and the V_1R [59].

The oxytocin receptor

The OTR can be considered a 'nonselective' vasopressin receptor. The OTR has equal affinity for vasopressin and oxytocin, whereas the V_1R has a 30-fold higher affinity for vasopressin than for oxytocin [72]. OTRs are functionally coupled to $G_{q/11}$ class binding proteins, which stimulate the activity of phospholipase C [73]. This leads to the generation of inositol trisphosphate and 1,2-DAG. Inositol trisphosphate triggers calcium release from intracellular stores, whereas DAG stimulates PKC, which phosphorylates unidentified target proteins [73]. A variety of cellular events are initiated in response to an increase in intracellular calcium. For example, the forming calcium–calmodulin complexes trigger activation of neuronal and endothelial isoforms of nitric oxide synthase. Nitric oxide in turn stimulates the soluble guanylate cyclase to produce cGMP, leading to vasodilation. In smooth muscle cells, the calcium–calmodulin system triggers the activation of myosin light chain kinase activity, which initiates smooth muscle con-

traction (e.g. in myometrial or mammary myoepithelial cells) [74]. In neurosecretory cells, rising calcium levels control cellular excitability, modulate their firing patterns, and lead to transmitter release. Further calcium-promoted processes include gene transcription and protein synthesis.

OTRs have been localized to a variety of reproductive and nonreproductive tissues [73]. Importantly, OTRs exist in high density on vascular endothelium, mediating nitric oxide dependent vasodilation [75]. Recently, the oxytocin/OTR system has been discovered in the heart. Activation of cardiac OTR stimulates the release of atrial natriuretic peptide, which is involved in natriuresis, regulation of blood pressure, and cell growth [76]. Embryonic stem cells exposed to oxytocin exhibit increased atrial natriuretic peptide mRNA and abundant mitochondria, and express sarcomeric myosin heavy chain, which is consistent with promotion of cardiomyocyte differentiation [77].

Purinergic receptors

Recently, vasopressin was demonstrated to act on the P_2 class of purinoreceptors (P_2Rs) [36]. P_2Rs also belong to the seven-transmembrane-domain GPCR superfamily. ATP released from platelets and damaged cells bind endothelial P_2Rs [78]. ATP can act on either of the two subclasses of purinoceptors, namely $P_{2\gamma}$ and P_{2v}. In both cases, activation of phospholipase C leads to mobilization of intracellular calcium stores. This binding stimulates phospholipase A_2 and nitric oxide synthase, resulting in increased synthesis and release of prostacyclin and nitric oxide, respectively, and causing vascular smooth muscle vasodilation [78].

Purinoreceptors may also have an important role in cardiac contractility. ATP released by platelets, endothelial cells, and damaged myocardium activates the P_2R, causing a large increase in cytosolic calcium and myocyte contractile amplitude [79]. ATP is also released as a cotransmitter with norepinephrine from sympathetic nerve endings and acts in a synergistic manner with β-adrenergic agents, increasing myocardial contractility [80]. In contrast to β-adrenergic agents, inotropy is not accompanied by a positive chronotropic effect. It is speculated that P_2R agonist-stimulated increase in contractility could occur without the expense of a rate-related increase in myocardial oxygen demand [79].

Recently, vasopressin was shown to exert cardiac effects through activation of P_2Rs expressed on cardiac endothelium. Intracoronary infusion of vasopressin-dextran (confines vasopressin to the intravascular space) and vasopressin at maximal concentration in isolated perfused guinea pig hearts caused coronary vasoconstriction and negative inotropy – effects that were blocked with vasopressin antagonists and P_2R antagonist [36]. Caution must be exercised in interpreting this study because activation of P_2Rs and increased levels of ATP normally increase inotropy. Furthermore, the same experiments performed in isolated perfused rat hearts demonstrated positive inotropy – an effect that was blocked by P_2R antagonists [36]. Further study is necessary to ascertain the significance of vasopressin P_2R activation in the human heart, but the discovery that vasopressin acts on P_2Rs is intriguing.

A number of pharmacologic observations have suggested the existence of vasopressin receptor/OTR subtypes beyond the five described above [72]. These include receptors for the metabolites of vasopressin and oxytocin (VP4-9 R and OT4-9 R) [72], and a cAMP-coupled vasopressin receptor with a V_1-like pharmacologic profile termed V_{2b} [81]. A novel 'vasotocin-like' receptor subtype has also been proposed [82].

Vasopressin/oxytocin receptor downregulation

Upon ligand binding, GPCRs undergo activation followed by a decrease in receptor responsiveness (desensitization). Agonist-dependent desensitization of these receptors can reduce their signaling responsiveness to maximum stimulation by up to 70–80% [83]. Receptor desensitization occurs when activated receptors become phosphorylated and bind to β-arrestin proteins, inhibiting further interaction with G-proteins [84,85]. Receptor responsiveness is also limited by the degradation of cAMP by phosphodiesterases. β-Arrestins coordinate both phosphorylation of receptors and the rate of cAMP degradation by phosphodiesterases [85].

Exposure to vasopressin leads to desensitization of the V_1R, which occurs quickly and is accompanied by sequestration of receptors inside the cell [59]. The V_1R can also be desensitized by angiotensin II [86]. Compared with V_1Rs and β_2-adrenergic receptors, which are known to recycle and resensitize rapidly, the V_2R recycles and resensitizes slowly [87]. Mutagenesis experiments demonstrate that the interaction of β-arrestin with a specific motif in the GPCR carboxyl-terminal tail dictates the rate of receptor dephosphorylation, recycling, and resensitization [87,88]. The clinical importance of vasopressin desensitization of the vasopressin receptor/OTR family in human disease states is currently unknown.

Despite the clinical importance of the vasopressin receptors and OTRs, little is known about the mechanisms by which they undergo internalization and desensitization. Agonist activation of all vasopressin receptor/OTR subtypes leads to a specific physical association of the receptors with GRKs and/or PKC, following different time courses that are specific to the receptor subtype [38]. The pattern of interaction with GRKs and PKC is also unique to each vasopressin receptor subtype and occurs at the level of their carboxyl-termini [38].

Vasopressin is known to modulate the effect of other vasoactive agents [89,90] – an interaction that may be explained by arrestin trafficking. Isoproterenol-dependent internalization

of β_2-adrenergic receptors is specifically blocked (>65% inhibition) by vasopressin-induced activation of V_2Rs coexpressed at similar levels [42]. β_2-Adrenergic receptors caused no detectable effect on V_2R internalization in the same cells. There is evidence to suggest that this nonreciprocal inhibition of endocytosis is mediated by receptor-specific intracellular trafficking of β-arrestins [42]. Interestingly, interaction of vasopressin with arrestins and resistance of vasopressin receptors to downregulation may explain the reported ability of vasopressin to bypass desensitized myocardial adrenergic receptors in an experimental model of congestive heart failure [91]. The clinical importance of vasopressin upregulation of adrenergic receptors in critically ill humans is an important area for further study.

Conclusion

During the past 10 years, considerable progress has been made in our understanding of vasopressin receptor structure and function. The physiologic significance of the various receptors has been elucidated by the development of specific agonists and antagonists, particularly by Dr Maurice Manning's group [92–94]. An understanding of the molecular basis of receptor function will greatly aid in the development of new molecules with high selectivity for the different subtypes of receptors, and will have potential therapeutic significance, not only for conditions as diverse as hypertension, diabetes insipidus and premature labor, but also in vasodilatory shock with organ dysfunction. In part 2 of the review, we discuss the interaction of vasopressin with its various receptors in vascular smooth muscle and the heart, and its potential utility in vasodilatory shock states.

Competing interests

None declared.

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