ELSEVIER

The development of various non-peptide vasopressin receptor antagonists presents a new area of drug discovery for the treatment of heart failure and hyponatremia of multiple causes.

Arginine vasopressin receptor antagonists (vaptans): pharmacological tools and potential therapeutic agents

Punniyakoti T. Veeraveedu[1,2], Suresh S. Palaniyandi[1,3], Ken'ichi Yamaguchi[4], Yutaka Komai[2], Rajarajan A. Thandavarayan[1], Vijayakumar Sukumaran[1] and Kenichi Watanabe[1]

[1] Department of Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Applied Life Sciences, 265-1 Higashijima Akiha-ku, Niigata City, Niigata 956-8603, Japan
[2] WPI Immunology Frontier Research Center, Osaka University, Suita, Osaka 565-0871, Japan
[3] Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA 94305, USA
[4] Department of Homeostatic Regulation and Development, Niigata University Graduate School of Medical and Dental Sciences, Niigata 951-8510, Japan

Arginine vasopressin (AVP) attracted attention as a potentially important neurohormonal mediator of the heart failure (HF) syndrome and hyponatremic states in humans because AVP influences renal handling of free water, vasoconstriction and myocyte biology through activation of V_2 and V_{1a} receptors. Current research is exploring V_2- and dual V_{1a}/V_2 receptor antagonism for the treatment of hyponatremia, as well as for the congestion and edema associated with chronic HF, because vasopressin receptor antagonists might offer benefits in comparison with conventional loop diuretics. The purpose of this review is to update the current status of experimental and clinical studies with available vasopressin receptor antagonists (conivaptan and tolvaptan) and their potential role in the treatment of HF and hyponatremia of multiple causes.

Introduction

An understanding of the imbalances in the neurohormonal axis has prompted the greatest insights into the pathophysiology and treatment of heart failure (HF) to date. From a cardio renal perspective, neurohormonal imbalances drive much of the sodium and water retention in this disease. These imbalances also contribute to abnormal loading conditions that predispose patients to deteriorations in hemodynamics and circulatory abnormalities. Even when volume is controlled, neurohormonal imbalances drive cellular and molecular processes that cause progression of this syndrome. Therapy for HF today is built around interfering with two

Corresponding author:. Watanabe, K. (nspkoti2001@gmail.com), (watanabe@nupals.ac.jp)

Dr Veeraveedu obtained his master degree in Pharmacy (Pharmaceutical chemistry) in 2002 at Punjabi University, Patiala, India and his PhD in 2008 from Niigata University of Pharmacy and Applied Life Sciences (NUPALS), Japan. After completing post-doctoral research with Professor Kenichi Watanabe at NUPALS he joined the WPI Immunology Frontier Research Center (WPI IFReC), Osaka University, Japan where he has been involving in various research projects and is currently focused on *in vivo* imaging of immune cells from animal models of autoimmune disease using two photon microscopy.

Dr Palaniyandi received Bachelors and Masters Degrees in Pharmacy in India. He received his Doctorate in Pharmaceutical Sciences in 2006 from NUPALS, Niigata, Japan. Currently Dr Palaniyandi is a postdoctoral researcher in the Mochly-Rosen lab at Stanford University School of Medicine, Stanford, CA, USA. His research interests spreads cover the fields of cardiovascular disease, diabetic complications, inflammation and regenerative medicine. He has been actively involved in the drug discovery research throughout his career. Dr Palaniyandi is a recipient of numerous awards including Jay N. Cohn Young Investigator Award (Basic Science) from Heart Failure Society of America.

Ken'ichi Yamaguchi has a PhD in Physiology from Niigata University School of Medicine and is a visiting instructor in the University of Tennessee Health Science Center and a Tenure Lecturer in Niigata University Graduate School of Medical and Dental Sciences. He is a Councilor of the Japan Physiological Society and Neuroendocrine Society, and has been a member of Organizing Committee of World Congress on Neurohypophysial Hormones. His main research interests are the cerebral mechanisms controlling vasopressin secretion from the neurohypophysis and the cardiovascular and fluid regulation by central and peripheral actions of neurohumoral factors such as angiotensin II, catecholamines and amino acid transmitters.

Dr Watanabe obtained his MD in 1974 followed by a Medical PhD from Niigata Medical University in 1985 and a Pharmaceutical PhD from Shizuoka Prefectural University in 1995. Currently, head of the Department of Clinical Pharmacology, NUPALS, Niigata, Japan. Prior to NUPALS, Dr Kenichi Watanabe had held faculty appointments at Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan. His areas of expertise include heart failure, diabetes, hypertension, inflammation, cardiac imaging, cell signaling and metabolic syndrome. He has over

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2010.08.001

neurohormonal systems – the renin–angiotensin–aldosterone system (RAAS) and the sympathetic nervous system (SNS) – with the addition of diuretics as needed for reducing volume expansion. Efforts to further exploit this neurohormonal approach might be warranted. Specifically, the possible contribution of arginine vasopressin (AVP), a nonapeptide hormone, to HF progression has recently been appreciated. Inhibition of AVP seems to have no harmful hemodynamic consequences and might, theoretically, represent a step forward; however, the serum sodium concentration is one of the best predictors of cardiovascular mortality, with hyponatremia patients having a substantially shorter survival period than patients with a normal sodium concentration [1]. It is not known whether neurohormonal activation and a decreased serum sodium level are results of more advanced HF or whether they contribute directly to the progression of the disease.

Hyponatremia (serum sodium < 135 mmol/l) is the most frequent electrolyte disorder encountered in hospitalized patients [2]. It is a state of relative water excess caused by stimulated AVP and fluid intake greater than obligatory losses. Increased AVP secretion leads to decreased electrolyte-free water excretion (aquaresis), with resulting water retention, and can cause dilutional hyponatremia [3–5]. Hyponatremia as it occurs in HF patients is a multifactorial process. The presence of hyponatremia in HF patients correlates with both the severity of the disease and its ultimate outcome [6–8]. Effects of vasopressin via V_1 and V_2 receptors are closely implicated in a variety of water-retaining diseases and cardiovascular diseases, including HF, hyponatremia, hypertension, renal diseases, syndrome of inappropriate secretion of anti-diuretic hormone (SIADH), cirrhosis and ocular hypertension [9–17]. The therapeutic approach to the treatment of hyponatremia in HF has traditionally relied on attempts to improve cardiac function while limiting fluid intake; however, fluid restriction can be very difficult for patients to achieve, is slow to work and does not allow a graded therapeutic approach. In more select circumstances, hypertonic saline, loop diuretics, and/or lithium or demeclocycline have been used. Lithium and demeclocycline act by retarding the anti-diuretic effect of vasopressin but carry with their use the risk of serious renal and/or cardiovascular side-effects [8,18]. More efficient and specific treatments of hyponatremia are needed. In this respect, pharmacologic research has yielded several compounds exhibiting antagonistic qualities at the vasopressin V_2 receptor (V_2R). Among these agents, peptidic derivatives of AVP turned out to have intrinsic anti-diuretic properties *in vivo* when given over days or weeks [19–23]. The development of such agents for use in patients has not been pursued. However, several promising non-peptides, vasopressin receptor antagonists (VRAs, or 'vaptans'), have been described; these agents are conivaptan (YM-087), lixivaptan (VPA-985), satavaptan (SR-121463) and tolvaptan (OPC-41061) [24–29] (Fig. 1). Numerous clinical trials show VRAs to be effective at increasing aquaresis and serum sodium levels in patients with hyponatremia owing to the secretion of SIADH or edema-forming states, such as congestive HF and cirrhosis. This article reviews both preclinical and clinical trial data on the VRAs that have been recently approved for marketing (conivaptan and tolvaptan).

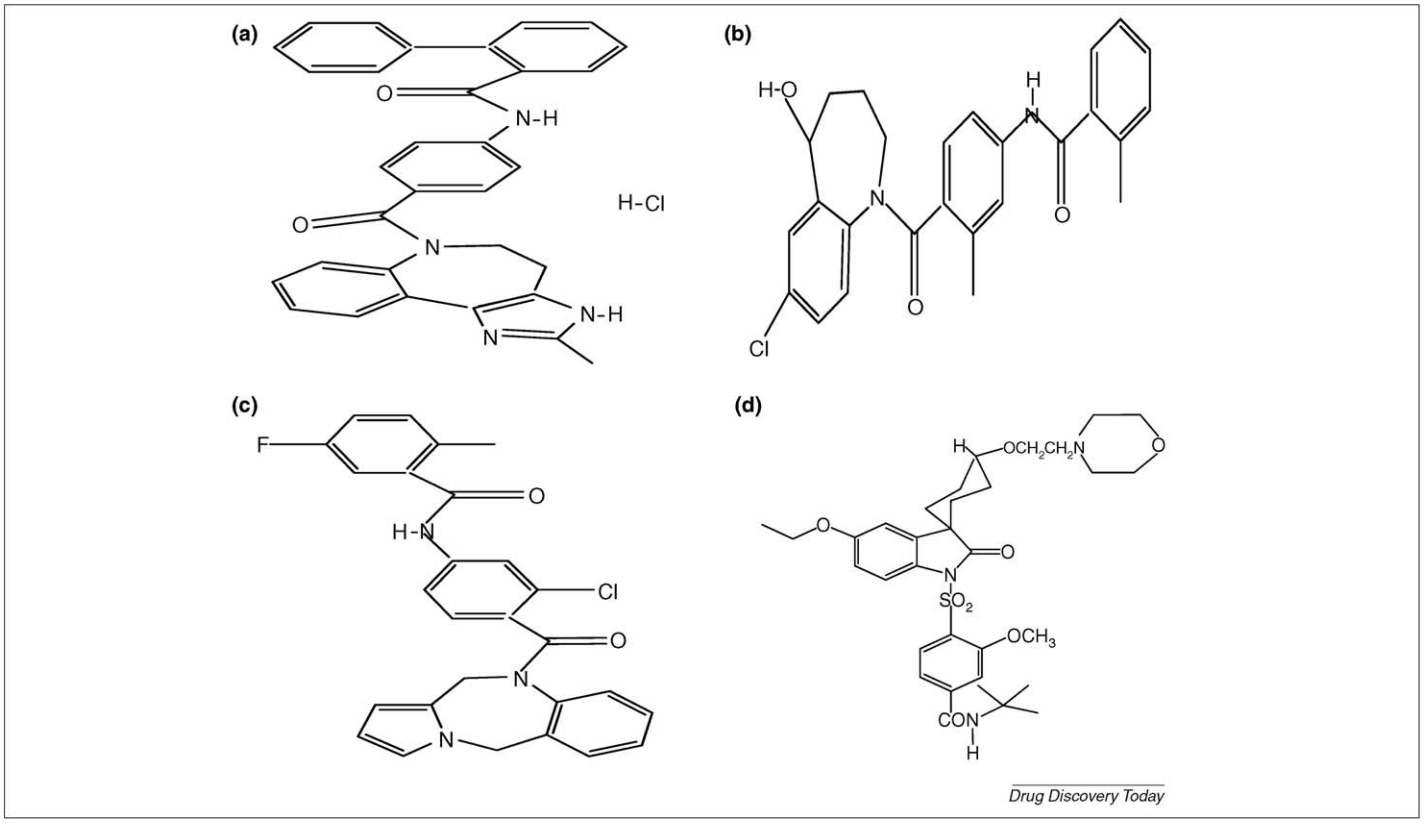

FIGURE 1

Chemical structures of non-peptide AVP receptor antagonists. **(a)** Conivaptan hydrochloride; **(b)** tolvaptan; **(c)** lixivaptan; **(d)** satavaptan.

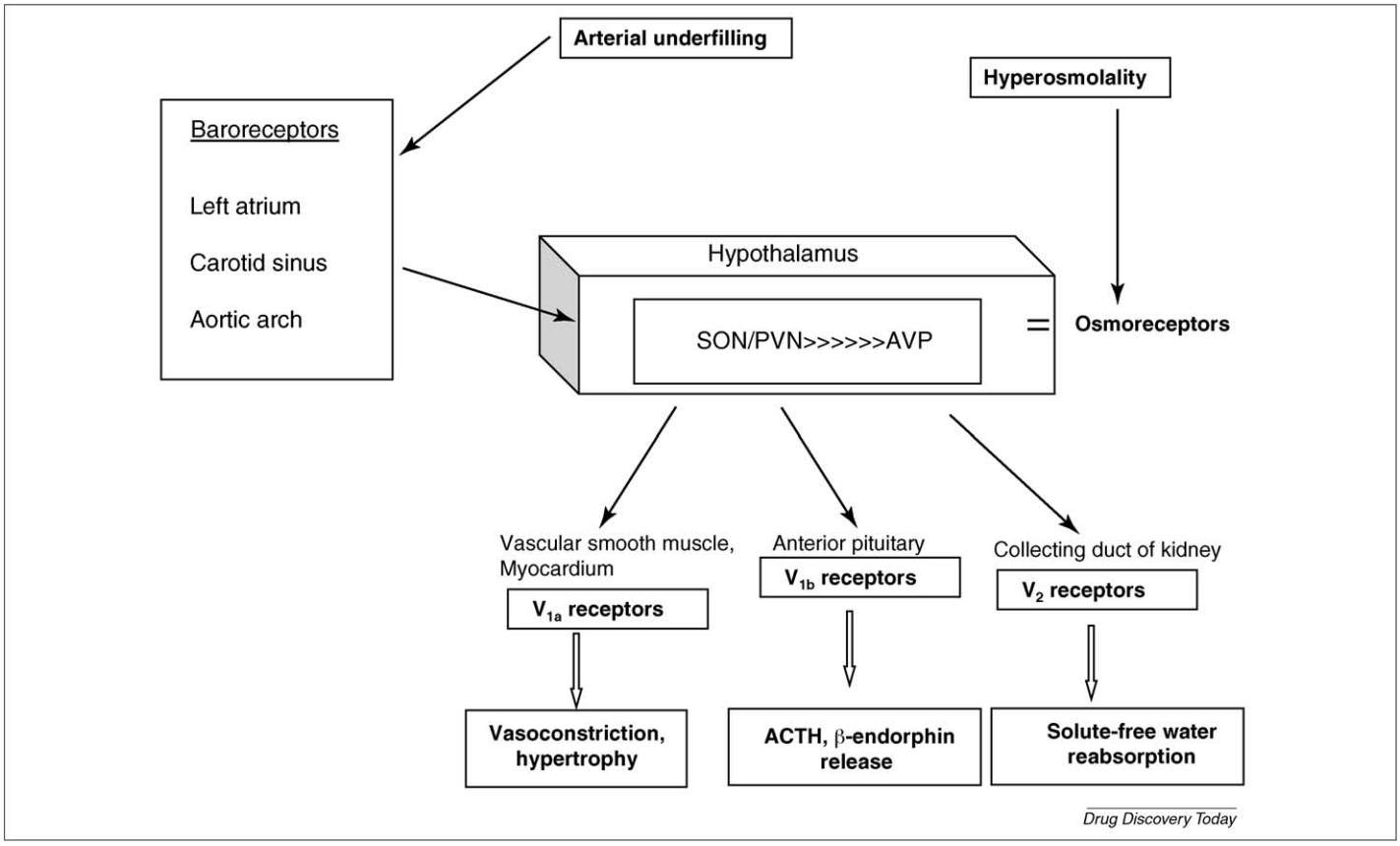

FIGURE 2

Regulation of water balance by AVP. Vasopressin is produced by the supraoptic and paraventricular nuclei of the hypothalamus. It is transported by the neurons projecting into the posterior pituitary gland, where it is stored and released. Activation of V_{1a} receptors located in vascular smooth muscle cells and the myocardium results in vasoconstriction and increased afterload and hypertrophy. The V_2 receptors located primarily in the collecting tubules mediate free water absorption. The V_{1b} receptors are located in the anterior pituitary and mediate adrenocorticotropin hormone (ACTH) and β-endorphin release.

Vasopressin physiology

Vasopressin is synthesized in the neurosecretory cells of the paraventricular and supraoptic nuclei of the hypothalamus and stored in the posterior pituitary gland. The stimuli for vasopressin production and release include low circulating blood volume, increased serum osmolality and/or sodium, and decreased renal perfusion [30,31]. AVP release is stimulated by a decrease in circulating blood volume. A decrease in blood volume causes 'unloading' of pressure-sensitive baroreceptors located in the left atrium, carotid sinus, aortic arch and renal afferent arterioles, which trigger AVP release [32]. AVP release is also stimulated by sensory cells known as osmoreceptors, which are located in the anteroventral third ventricle region of the hypothalamus and detect changes in serum osmolality that either stimulate or inhibit AVP production and release [32–35]. The neurosecretory cells that arise in the hypothalamus project to the posterior pituitary gland, where AVP is initially stored and then released into the circulation. Vasopressin receptors respond to elevated AVP levels by increasing free water reabsorption and vasoconstriction (Fig. 2). Several other stimuli for AVP release have been identified, including norepinephrine and angiotensin II.

Vasopressin receptors and their actions

AVP contributes to HF through several mechanisms because AVP has a complicated set of receptor systems (Table 1). The biological effects of vasopressin are mediated via a family of membrane-bound receptors. In 1979, Michell *et al.* [36] suggested the presence of two distinct receptor subtypes. The vasopressin V_1 receptor subtype is primarily responsible for vasoconstrictor activity, whereas the V_2R subtype regulates anti-diuretic effects. The vasopressin receptors belong to the GTP-binding G-protein-coupled receptor family: the V_1 receptors activate phospholipases via Gq/11, and the V_2Rs stimulate adenylyl cyclase by operating via Gs [37].

The V_1 receptor is further subdivided into V_{1a} and V_{1b} (also called V_3) receptors. Important locations and functions of the receptor subtypes are summarized in Table 1 [38–45]. V_{1a} receptors in myocytes also mobilize endoplasmic reticulum Ca^{2+} stores and potentiate generation of inositol-3-phosphate, which, in turn, improves myocardial inotropic function [38,39]. There are no known direct cardiovascular effects mediated by V_{1b} receptor subtype, which has been implicated in mediating glucagon secretion and promotion of cellular proliferation [42].

V_2R subtypes located primarily in the cells of renal cortical collecting ducts respond to elevated AVP levels by promoting free water reabsorption, which leads to antidiuresis (Table 1). Exaggerated release and action of AVP can produce hyponatremia in the pathological state. The binding of AVP to the V_2Rs on the basolateral membrane of renal collecting ducts leads to an increase in intracellular cyclic 3′5′-adenosine monophosphate (cAMP) by

TABLE 1

AVP receptors and their actions

Receptor	Signaling	Location	Actions	Refs
V_{1a}	G-protein-coupled; IP_3 activation; raises intracellular Ca^{2+}	Vascular smooth muscle cells Cardiomyocytes Hepatocytes Platelets	Vasoconstriction Hypertrophy Glycogenolysis Platelet aggregation	[38,39]
V_{1b} (or V_3)	G-protein-coupled; IP3 activation; decreases intracellular Ca^{2+}	Anterior pituitary Kidney β-Cells in pancreas	Adrenocarticotropin and beta-endorphin release Not well defined Insulin and glucagon secretion	[40–42]
V_2	Adenyl cyclase	Renal collecting ducts Ascending limb of Henle Endothelial cells	Water retention (antidiuresis) Sodium, potassium, and chloride co-transport von Willebrand factor secretion	[43–45]

stimulating adenylyl cyclase activity through Gs protein. This, in turn, regulates renal free water excretion by shuttling aquaporin-2 (AQP 2) water channels from intracellular vesicles into the apical plasma membrane of the renal collecting duct cells, thereby increasing water permeability of the membrane and producing an antidiuresis (Fig. 3), which, in turn, causes a decrease in blood sodium concentration and plasma osmolality [43]. There are additional renal effects of AVP, including modifications of urea reabsorption and sodium transport in the collecting ducts, as well as V_2R-mediated sodium, potassium and chloride co-transport in the ascending limb of Henle [44]. An extrarenal effect mediated by V_2Rs located in the vascular endothelium has been described implicating the secretion of the von Willebrand factor [45] (Table 1). The major actions of AVP in HF are mediated through V_{1a} and V_2Rs.

Role of AVP and its signaling in chronic heart failure

Circulating AVP levels have been found to be elevated significantly in patients with chronic heart failure (CHF) (9.5 pg/ml) compared with healthy controls (4.7 pg/ml) $P < 0.001$, with higher levels found in CHF patients with significant cardiac decompensation and hyponatremia [46–48]. In the Studies of Left Ventricular Dysfunction trial, plasma AVP concentrations increased with the severity of cardiac impairment, with the highest levels in those with overt symptoms of CHF [46]. Furthermore, a multivariate analysis of post-infarction patients in the Survival and Ventricular Enlargement study showed that AVP levels are independent predictors of the combined end points of cardiovascular mortality, development of severe HF or recurrent myocardial infarction [49]. These results confirmed that AVP is overexpressed in the setting of HF. An increase in AVP secretion occurs in response to non-osmotic

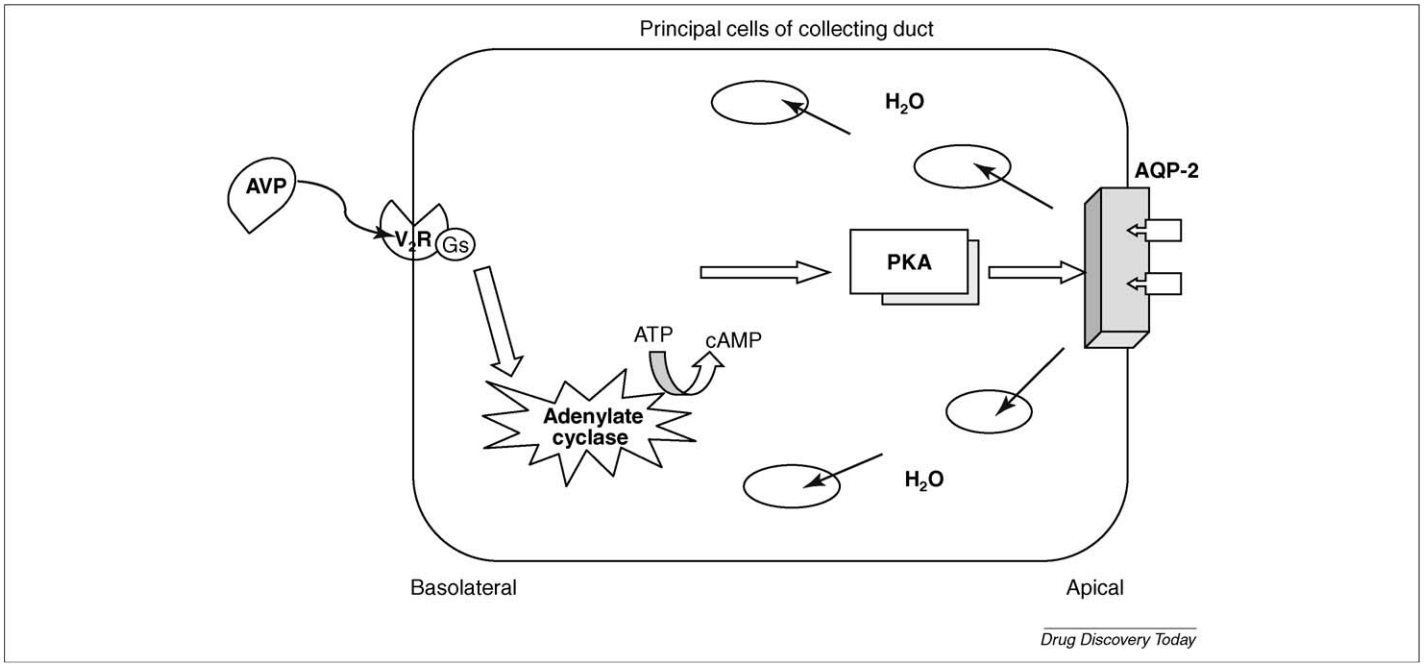

FIGURE 3

AVP signal transduction pathway in the collecting ducts. AVP binds to the V_2 receptor on the basolateral surface of the principal cells of the collecting duct of the kidney. The receptor couples to Gs, a heterotrimeric GTP-binding protein, which then binds to adenylate cyclase, thereby increasing cAMP production. PKA is a multimeric protein, which, when activated by cAMP, phosphorylates the AQP 2 molecule where it is delivered via cytoplasmic vesicles to the apical surface of the collecting duct. The water channels then allow a single file of water molecules to traverse the apical membrane in response to the osmotic gradient where they are returned back to the circulation.

stimuli, such as the low arterial pressure and effective arterial volume characteristic of CHF [5]. Therefore, AVP secretion can be enhanced, despite low plasma osmolality and hypotonicity. Excess AVP activity has several adverse effects on free water reabsorption, cardiac contractility and vascular tone. The increased AVP activity in CHF leads to an increase in the number of AQP 2 channels in the renal collecting ducts [43], and increased expression of AQP 2 messenger RNA has been observed in a rat model of severe CHF [50]. Rats with an elevated left ventricular (LV) end-diastolic pressure (LVEDP) and reduced plasma sodium concentrations had a significant increase in AQP 2 expression compared with rats with mildly compensated CHF without raised LVEDP or reduced plasma sodium concentrations [51]. The increased expression of AQP 2 and the resultant increase in free water reabsorption, even under conditions of low plasma sodium concentrations, illustrates how excess AVP activity contributes to the development of hyponatremia and edema in CHF. It has been hypothesized, therefore, that chronically high levels of circulating AVP not only play an important part in the pathophysiology of HF syndrome but also contribute to the disease's progression.

Hemodynamic effects of AVP

Increases in circulating AVP levels are also associated with hemodynamic abnormalities in patients with CHF [52]. AVP can cause edema and hyponatremia by stimulating V_2Rs, and can increase systemic vascular resistance (SVR) by stimulating V_{1a} receptors [3,21,47]. Intravenous (i.v.) infusions of AVP significantly increased SVR and pulmonary capillary wedge pressure (PCWP) while decreasing cardiac output and stroke volume in CHF patients [52]. The increase in venous blood volume that results from V_2R-mediated water retention can increase preload, leading to an increase in PCWP and LV filling pressure. An increase in afterload as a result of V_{1a} activation and the subsequent arterial vasoconstriction can also contribute to the hemodynamic changes associated with AVP release [4,52]. These mechanisms suggest that excess AVP activity at both V_{1a} and V_2 receptors is responsible for the adverse hemodynamic changes that occur in CHF. In addition, AVP exerts potent mitogenic and hypertrophic effects on vascular smooth muscle cells, primarily through the activation of V_{1a} receptors [53]. *In vitro*, AVP has been found to induce a time-dependent and concentration-dependent increase in the secretion of vascular endothelial growth factor in vascular smooth muscle cells [54]. In addition to its adverse effects on hemodynamics, AVP induces structural changes in the myocardium [38,55–57]. AVP seemed to stimulate myocardial cell hypertrophy by enhancing intracellular Ca^{2+} levels with increased protein synthesis and cellular growth in cultured rat cardiomyocytes [38,55,56]. On exposure to V_{1a} receptor antagonists, the observed hypertrophy was significantly inhibited [56,58]. Thus, high levels of AVP can lead to myocardial fibrosis, hypertrophy and vasoconstriction via the activation of V_{1a} receptors, as well as water retention and hyponatremia via the activation of V_2Rs. The relative contributions of these receptors in the setting of CHF and the consequence of their blockade are unknown.

Limited efficacy of diuretics in HF

Currently, diuretics are the only drugs to reduce the fluid overload that results in congestion in CHF patients. Loop diuretics potently excrete water and electrolytes and, therefore, have been widely prescribed for the treatment of edema associated with CHF, renal disease and hepatic disease [59–61]. Torasemide, a long-acting loop diuretic, has been used to treat both acute and chronic HF and hypertension [60,62]. Comparative studies have shown that in cases of CHF, torasemide exerts more favorable effects on clinical signs of the disease and the functional status of patients than furosemide [63–67]. In animal experiments, torasemide was approximately ten times more potent than furosemide. The increase in urinary potassium excretion caused by torasemide was slight compared to the increase in urinary sodium excretion [61,68,69]. Recently, we have also reported that compared with furosemide, torasemide treatment significantly improved survival rate and LV function and ameliorated the progression of cardiac remodeling in rats with CHF after experimental autoimmune myocarditis [70]. Although rapid symptomatic improvement and a decrease in volume overload are observed with diuretic therapy for HF syndrome, an alternative to diuretics would be welcome given that diuretic therapy also has several disadvantages, including increased neurohormonal activation, worsening renal function and electrolyte disturbances [71–73].

Vasopressin receptor antagonism

The integral role of AVP in regulating sodium and water reabsorption, cardiac contractility and vascular tone, as well as the importance of AVP's activation in the progression of CHF, make it a potential neurohormonal target in the treatment of CHF. Hyponatremia in congestive HF is associated with increased morbidity and mortality, underlying the importance of adequate assessment and treatment of this electrolyte imbalance in patients with CHF. Among HF patients treated with angiotensin-converting enzyme (ACE) inhibitors, diuretics and beta-blockers, even a small decline in serum sodium levels, to 136 mequiv./l or less, was associated with more than twice the risk of 60-day mortality and a significant increase in risk of readmission or death within 60 days compared with serum sodium levels greater than 136 mequiv./l [74]. Increasing the serum sodium concentration during hospitalization improves outcomes; an increase of 2 mequiv./l or greater is associated with a relative reduction in the risk of mortality at 60 days compared with no change in the serum sodium level [75,76]. This suggests the normalization of low serum sodium levels to be another potential mechanism of benefit of V_2R antagonism in HF.

Current options for hyponatremia in CHF include hypertonic saline solutions, loop diuretics, fluid restriction and pharmacological agents such as demeclocycline, lithium carbonate and urea [8,18]. Hypertonic saline solutions must be administered with extreme caution because excessively rapid sodium correction can lead to neurological effects such as central pontine myelinolysis. As described above, use of loop diuretics for congestion is associated with electrolyte abnormalities, which can exacerbate the existing hyponatremia and deteriorate renal function. Fluid restriction is moderately effective and often difficult to implement in a hospital setting. Agents such as demeclocycline and lithium carbonate have potentially serious renal and cardiovascular side-effects [8,18].

The discovery of VRAs has been credited to Manning and Sawyer [77,78]. Initial investigations were carried out using peptide analogs of AVP receptor antagonists. Previous reports on the chronic

TABLE 2

Profile of AVP antagonists for the treatment of heart failure

Agent	Receptor selectivity	Receptor selectivity index in humans (K_i V_{1a}:K_i V_2)	Proposed routes of administration	Urine volume	Urine osmolality	Sodium excretion/ 24 hours	Developed company	Dose	Half-life (hour)
Conivaptan	V_{1a}/V_2	10:1	Oral/IV	↑	↓	↔	Yamanouchi Astellas	40–80 mg	5–12
Tolvaptan	V_2	29:1	Oral	↑	↓	↔	Otsuka	15–60 mg once daily	6–8
Lixivaptan	V_2	100:1	Oral	↑	↓	↔ at low dose ↑ at high dose	Wyeth-Ayerst Cardiokine	50–100 mg twice daily	7–10
Satavaptan	V_2	112:1	Oral	↑	↓	↔	Sanofi Aventis	5–25 mg once daily	14–17

AVP, arginine vasopressin; ↑, increased; ↓, decreased; ↔, no change.

blockade of AVP receptors by peptide V_2R antagonists did not show persistent aquaresis [19,20], and further research with these compounds was limited by poor oral bioavailability, short half-lives, partial agonism and species-specific activity [21–23]. The focus of developmental research subsequently shifted to non-peptide VRAs, OPC-21268 and mozavaptan [9], which showed sufficient oral bioavailability and long-term effects of potential clinical value, further encouraging the development of various non-peptide VRAs. As a result, specific non-peptide antagonists (lixivaptan, satavaptan and tolvaptan), as well as dual V_{1a}/V_2 receptor antagonists (conivaptan), have now been developed and are currently under clinical investigation [23,79] (Fig. 1, Table 2).

The era of blocking AVP to treat HF began with a ten-patient study of advanced HF using an infusion of a V_1 receptor antagonist. In patients with high vasopressin levels, V_1 receptor antagonism was associated with a reduction in SVR consistent with vasodilation and an increase in cardiac output. In patients with low and normal vasopressin levels, however, the VRA tended to have an agonistic rather than an antagonistic effect [80]. The development of non-peptide VRAs with longer half-lives, better oral bioavailability and greater efficacy enabled testing of the hypothesis that interfering with AVP-mediated signaling could be beneficial in HF. It was hoped that non-peptide VRAs could promote symptomatic improvement during acute exacerbations of HF and slow the progression of the clinical syndrome. These agents seemed to be an alternative to loop diuretics. V_{1a} receptor antagonism has the potential to reverse vasoconstriction, improve cardiac output, decrease SVR and lower blood pressure. V_{1a} blockade also could possibly reverse myocardial remodeling. In earlier studies, however, V_{1a} antagonism alone had little clinical benefit. In multiple clinical trials, the effects of vasopressin V_2 antagonists on renal function have been very similar. Specifically, V_2 antagonism results in increased aquaresis without loss of sodium and other electrolytes, increased serum sodium concentration, decreased weight and edema. When selective V_2 antagonists are given, AVP levels become markedly elevated, which is of unclear significance [4,81]. Theoretically, selective V_2 antagonism could increase the interaction of AVP with unblocked V_{1a} receptors causing vasoconstriction, increased afterload, worsening LV function and coronary vasoconstriction; however, these adverse effects have not been noted clinically. V_2 antagonism had similar diuretic potency to furosemide. However, V_2R blockade increases – whereas furosemide decreases – serum sodium levels [82].

As outlined in Table 2, all four non-peptide VRAs produce aquaresis. As a result, VRAs increase urine volume, decrease urine osmolality and raise serum sodium with little or no sodium loss and no compensatory activation of the RAAS. Conivaptan and tolvaptan have been approved by the Food and Drug Administration (FDA) for the management of euvolemic or hypervolemic hyponatremia and are commercially available in the USA. Conivaptan is available for i.v. administration, whereas the other three agents are being developed for oral administration. These agents differ in their degree of specificity for V_{1a} and V_2 receptors (Table 2). The remainder of this article reviews and assesses the available preclinical and clinical trial data for conivaptan and tolvaptan.

VRAs in preclinical studies

VRAs have been fully characterized *in vitro* and *in vivo* (Table 3). Each is a potent antagonist of the targeted receptors, as demonstrated in binding assays and in the expected physiological responses observed in animal studies [24–29]. Conivaptan is a non-peptide VRA with a high affinity for both V_{1a} and V_2 receptors. Conivaptan inhibited specific binding of [^{3}H]-AVP to V_{1a} and V_2 receptors in a concentration-dependent manner [24]. Furthermore, conivaptan markedly blocked AVP-induced cAMP production in cultured renal epithelium cells dependent on its concentration and had no agonistic activities. In rats and dogs, conivaptan has demonstrated both an inhibitory pressor response and an effective aquaretic effect via V_{1a} and V_2 receptor blockade, respectively, without inducing electrolyte excretion, in contrast to furosemide, which produced marked natriuresis and kaliuresis [24,25]. Conivaptan dose-dependently increased urine volume and reduced urinary osmolality without affecting blood pressure [24–26,83]. In dogs with pacing-induced CHF, i.v. conivaptan (0.1 mg/kg) produced significant aquaresis and hemodynamic changes [84]. In anesthetized dogs, the i.v. injection of a bolus of conivaptan (0.1 mg/kg) rapidly attenuated AVP-induced hemodynamic changes [85]. In a rat model of CHF induced by ligating the left coronary artery, conivaptan improved hemodynamics and increased urine volume and decreased urine osmolality without affecting electrolyte excretion [86,87]. It has been suggested that the favorable hemodynamic and aquaretic effects of conivaptan were due to antagonism at the V_{1a} and V_2 receptors, respectively.

TABLE 3

Summary of vasopressin receptor antagonists 'vaptans' in preclinical studies

Agent and Refs	Species	Disease model	Dose (mg/kg) and route	Effects on electrolytes	Effects on hemodynamics and/or neurohormones
Conivaptan		**HF models**			
[84,85]	Dogs	Pacing and AVP-induced congestive HF	0.1 (i.v.)	Marked increase in aquaresis	Marked increase in CO, dP/dt_{max} and decrease in LVEDP and TPR
[86,87]	Rats	MI-induced CHF	0.3–3 (p.o) × single dose 0.03, 0.1 and 0.3 (i.v.)	Dose-dependently produced aquaresis	Conivaptan (3 mg/kg) attenuated LVEDP, lung and RV weights, RVSP, ratio of lung to BW and RAP and improved CO and dP/dt_{max}
[88]	Rats	MI-induced CHF	1 (p.o) plus captopril 50 (p.o) × four weeks	Conivaptan increased water excretion	Combination therapy reduced BP, ANP, RVM, LVM and BW
		Non-HF models			
[24]	Rats	Pithed	0.003–0.03 (i.v.)	Produced dose-dependent aquaresis	Inhibited AVP-induced pressor response
		Dehydrated and conscious	0.01–0.03 (i.v.)		Blocked AVP-induced cAMP production without agonistic activity
[25]	Dogs	Hydrated	0.03–0.3 (p.o) and 0.01–0.1 (i.v.)	Dose-dependently increased UV and decreased U_{osm} with no electrolyte excretion compared to furosemide	Decreased in the pressor effect of exogenous AVP
[26]	Rats	Conscious	0.1, 0.3, 1 and 3 (p.o)	Dose-dependently produced aquaresis	No effect on BP
[83]	Rats	Conscious	0.1–3 (p.o)	Effects are similar as in Ref. [26]	No effect on SBP. Dose-dependently inhibited AVP binding to liver V_{1a} and kidney V_{1a} and V_2 vasopressin receptors over 24 hours
Tolvaptan		**HF models**			
[91]	Dogs	Pacing-induced HF	0.3–10 (p.o) single dose	Produced dose-dependent aquaresis; increased UV, E-CH_2O and decreased U_{osm}; elevated S_{Na}, and S_{osm} versus furosemide (0.3–3 mg/kg)	Unlike furosemide, tolvaptan increases plasma AVP but did not stimulate either sympathetic or RAA system
[94,95]	Rats	Myosin-induced CHF	3 and 10 (p.o) × 28 days	Produced diuresis comparable to furosemide (30 and 100 mg/kg). Other effects were similar as in Ref. [91]. Tolvaptan inhibited AQP 2 protein and its glycosylated form in the kidney of CHF rat	Both had no significant adverse or beneficial effects on cardiac geometry or function. Unlike tolvaptan, furosemide increases PRA, P_{ALD} and P_{cr} levels
[96]	Dogs	Pacing-induced HF	10 (p.o)	Significantly increased UV	Significantly decreased PCWP and increased plasma AVP without any changes in PVR, RBF or GFR
		Non-HF models			
[29]	Rats	Conscious	0.3,1,3 and 10 (p.o.) single dose; 1 and 10 × 28 days	Produced dose-dependent aquaresis and increased S_{osm}, S_{Na} concentrations and urinary AVP excretion	Inhibited [^{3}H]-AVP binding to V_{1a} receptors ($K_i = 325 \pm 41$ nM) and V_2 receptors ($K_i = 1.33 \pm 0.30$ nM), showing higher receptor selectivity ($V_{1a}/V_2 = 244$) than with human receptors
[91,92]	Rats	Conscious	1 and 10 (p.o) single dose	Effects are similar as above in Ref. [91]. Produce an additive effect when combined with furosemide (10 and 100 mg/kg)	Effects are similar as above in Ref. [91]. No additive effect on RAA was seen when combined with furosemide
	Beagles	Conscious	0.3–10 (p.o) single dose		
[98]	Rats	Hyponatremia	1, 3 and 10 (p.o.) × 3 days 0.25–8 (p.o.) (adjusted dose) × 10 days	Produced dose-dependent aquaresis, resulted in a gradual increase in S_{Na} concentration	Improved hyponatremia-driven increases in wet weight and water content in the organs
[112]	Rats	PKD	0.01%, 0.03% and 0.1% in diet (p.o.)	Significantly decreased renal cAMP and kidney weight	Significantly decreased cyst and fibrosis volume, PCNA-positive renal cells and apoptotic indices in PCK rats

Abbreviations: ANP, atrial natriuretic peptide; AQP 2, aquaporin-2; AVP, arginine vasopressin; BP, blood pressure; BW, body weight; CHF, chronic heart failure; CO, cardiac output; E-CH_2O, electrolyte-free water excretion; GFR, glomerular filtration rate; HF, heart failure; HR, heart rate; LVEDP, left ventricular end-diastolic pressure; MI, myocardial infarction; P_{cr}, plasma creatinine; PCNA, proliferating cell nuclear antigen; PCWP, pulmonary capillary wedge pressure; PKD, polycystic kidney disease; P_{ALD}, plasma aldosterone; PRA, plasma renin activity; PVR, peripheral vascular resistance; RAA, renin–angiotensin aldosterone; RBF, renal blood flow; RV, right ventricular; RVM and LVM, right and left ventricular mass; RVSP, right ventricular systolic pressure; RAP, right atrial pressure; SBP, systolic blood pressure; S_{Na}, serum sodium; S_{osm}, serum osmolality; TPR, total peripheral resistance; U_{Na}, urine sodium; UV, urine volume; U_{osm}, urine osmolality; cAMP, cyclic 3′5′-adenosine monophosphate; dDAVP, 1-desamino-8-D-arginine vasopressin; dP/dt_{max}, intraventricular pressure rise.

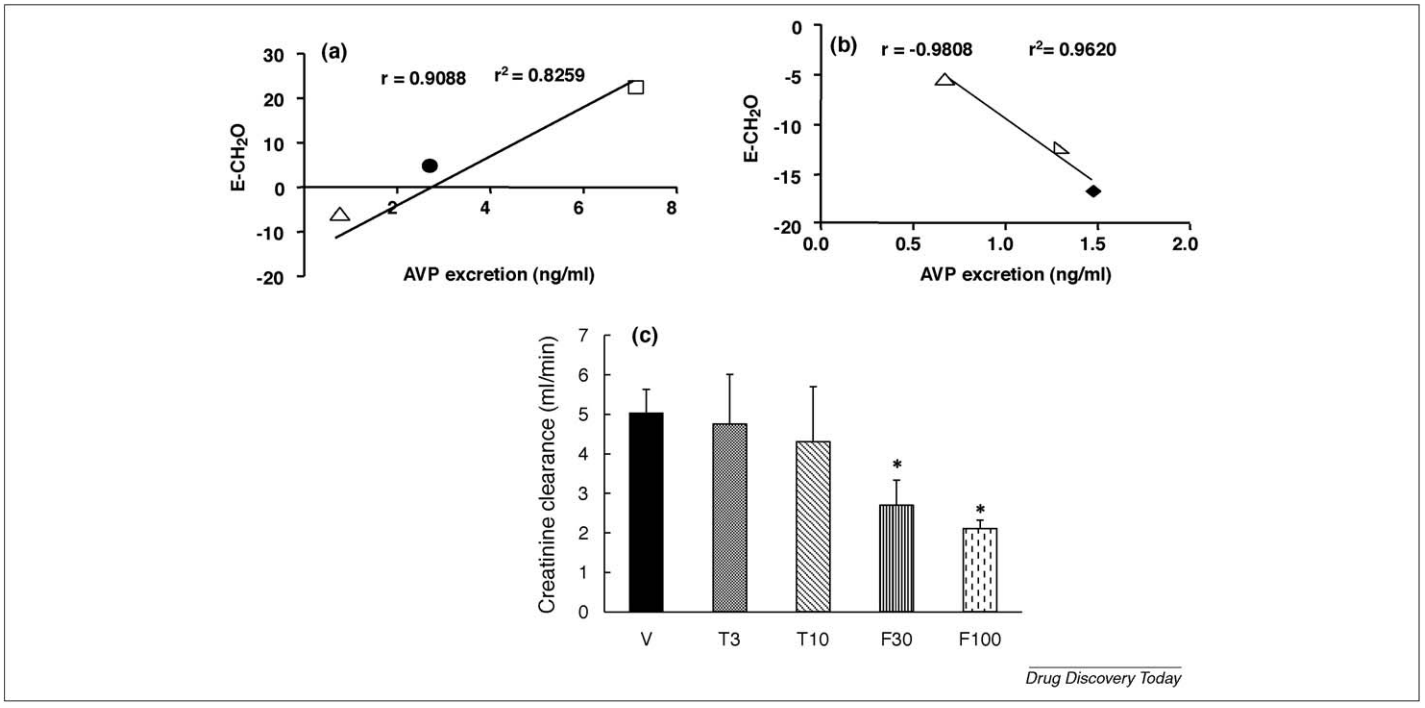

FIGURE 4

(a,b) The correlation between electrolyte-free water excretion (E-CH_2O) and urinary AVP excretion after tolvaptan and furosemide treatment in CHF rats. Δ, CHF rats treated with vehicle; ●, CHF rats treated with tolvaptan (3 mg/kg/day); □, CHF rats treated with tolvaptan (10 mg/kg/day); ◆, CHF rats treated with furosemide (30 mg/kg/day); ◺, CHF rats treated with furosemide (100 mg/kg/day). **(c)** Effect of tolvaptan and furosemide on creatinine clearance in CHF rats. Data are presented as means ± SE. V, vehicle-treated CHF rats; T3, CHF rats treated with tolvaptan 3 mg/kg/day; T10, CHF rats treated with tolvaptan 10 mg/kg/day; F30, CHF rats treated with furosemide 30 mg/kg/day; F100, CHF rats treated with furosemide 100 mg/kg/day. *$P < 0.05$ versus vehicle-treated CHF rats.

In this model, the combination of conivaptan 1 mg/kg and the ACE inhibitor captopril 50 mg/kg was more effective in relieving pulmonary congestion than either drug alone. Moreover, ACE inhibition did not affect the aquaretic response with conivaptan [88] (Table 3). In cultured neonatal rat cardiomyocytes, conivaptan was also demonstrated to dose-dependently inhibit AVP-induced protein synthesis, as measured by decreases in intracellular free Ca^{2+} and mitogen-activated protein kinase (MAPK) activity, suggesting that conivaptan might offer clinical utility in the prevention and regression of cardiomyocyte hypertrophy via blockade at V_{1a} receptor [55]. Thus, in animal models, inhibition of both V_{1a} and V_2 receptors might have a beneficial role in HF.

OPC-31260 was the first non-peptidic V_2R antagonist to be developed and was introduced in 1992 [89]. Since then, two more compounds have become available: VPA-985 (lixivaptan) and SR-121463A (satavaptan) [27,28]. In comparison with OPC-31260, they exhibit a higher affinity and selectivity for V_2Rs, thereby suggesting enhanced efficacy [90]. Tolvaptan, another selective V_2R antagonist, was synthesized through a series of structural conversions of mozavaptan [89]. AVP-binding experiments with this agent revealed a 29:1 (V_2:V_{1a}) receptor selectivity in cloned human receptors and 250 times greater affinity for rat V_2R than rat V_1R [29]. Similar results were obtained in canine membrane preparations [91]. Tolvaptan inhibited production of cAMP, a second messenger of V_2R activation, with no intrinsic agonistic activity [29]. These results indicate that tolvaptan has a potent and selective effect at V_2Rs in humans, rats and dogs. Dose-dependent responses demonstrated in both rats and dogs include increased aquaresis, less urinary loss of sodium than furosemide and no effect on serum creatinine [29,91,92]. Compared with furosemide, serum sodium increased in a dose-dependent manner in animals given tolvaptan. In contrast to loop diuretics, antagonism of V_2Rs seemed not to increase the activation of RAAS [91,92]. On treatment with multiple doses of tolvaptan for 28 days at 1 and 10 mg/kg per os in rats, aquaretic effects remained constant throughout the study period, although effects were more pronounced on the first day of treatment [29]. In animal studies, when administered concomitantly with furosemide, tolvaptan demonstrated an additive diuretic effect, as well as an overall elevation in serum sodium concentrations [91,92] (Table 3).

The aquaretic effect of tolvaptan after repeated administration was evaluated in rats with myosin-induced CHF [93], and its efficacy was compared with that of furosemide, a loop diuretic [94]. Tolvaptan produces diuresis comparable to furosemide in CHF rats. Tolvaptan increased aquaresis (E-CH_2O) and AVP excretion and decreased osmolality without any effect on electrolyte excretion (E-Cosm). Moreover, plasma sodium levels and osmolality were increased significantly in CHF rats. By contrast, furosemide increased urinary sodium excretion and E-Cosm and decreased plasma sodium and E-CH_2O in CHF rats [94] (Table 3). In addition, we observed a negative and positive correlation between urinary AVP excretion and E-CH_2O with furosemide and tolvaptan treatment, respectively (Fig. 4a,b). These results suggest that tolvaptan and furosemide exert an aquaretic and a natriuretic effect, respectively. In addition, neither pharmacological intervention had significant beneficial effects on cardiac geometry or function in CHF rats. In contrast to tolvaptan, furosemide significantly elevated plasma renin activity levels, aldosterone and

TABLE 4

Results of clinical trials on the effects of vasopressin receptor antagonists (vaptans) on hyponatremia and HF

Drug and Refs	Subjects and dosage	Drug effects			
		Diuresis and electrolytes	S_{Na} and/or S_{osm}	BW	Hemodynamics and/or overall benefits
Conivaptan	**Hyponatremia**				
[115]	74 patients (with euvolemic or hypervolemic) (S_{Na} 115–130 mequiv./l). Oral dose at 40 or 80 mg/day for five days	Significant increase in aquaresis and decrease in U_{osm} and U_{Na}	Significant increase in S_{Na} and S_{osm}	Not reported	Changes in neurohormonal parameters were similar among the groups
[116]	84 patients (with euvolemic or hypervolemic) (S_{Na} 115–130 mequiv./l). Single 20 mg bolus followed by continuous infusion of 40 mg/day or 80 mg/day for four days	Significant increase in FWC and decrease in U_{osm}	Significant increase in S_{Na} and S_{osm}	Not reported	Well tolerated
	HF				
[121]	345 patients with CHF (NYHA class II–IV). Oral dose at 10, 20 or 40 mg/day for 12 weeks	Not reported	Not reported	Not reported	No improvement in overall functional capacity, exercise tolerance or quality of life
[122]	170 patients with ADHF. Single 20 mg bolus followed by continuous infusion of 40, 80 or 120 mg/day for two days	Significant increase in UO	Not reported	Decrease in BW	Hemodynamically well tolerated with no change in clinical status
[129]	142 patients with advanced HF (NYHA class III–IV). Single i.v. dose at 10, 20 or 40 mg in addition to standard therapy	Significant increase in UO and decrease in U_{osm}	Not reported	Not reported	Significant reduction in PCWP and RAP without any changes in BP, SVR, CI, or renal function
Tolvaptan	**Hyponatremia**				
[117]	448 patients (with CHF, LC or SIADH) ($S_{Na} < 135$ mequiv./l). Once-daily oral dose started at 15 mg/day, increased to 30 and then 60 mg/day if necessary for 4 and 30 days	Marked increase in UV and decrease in U_{osm}	Significant increase in S_{Na}	Significant decrease in BW	Increases in plasma AVP, improvements in MC
[118]	28 patients (with HF, LC, SIADH) ($S_{Na} < 135$ mmol/l). Once-daily oral dose started at 10 mg/day and increased to 60 mg/day if necessary for 27 days	Marked increase in UV and decrease in U_{osm} and U_{Na} excretion	Significant increase in S_{Na}	Not reported	
[119]	111 patients (with CHF, LC or SIADH) ($S_{Na} < 130$–135 mmol/l). Once-daily oral dose started at 15 mg/day, increased to 30 and then 60 mg/day for a maximum of 214 weeks	Not reported	Significant increase in S_{Na}	Not reported	Well tolerated
	HF				
[124]	254 patients with CHF (NYHA class II–III). 70 (28%) of 254 patients with hyponatremia ($S_{Na} < 136$ mequiv./l). Oral dose at 30, 45 or 60 mg/day for 25 days	Increase in UV and decrease in U_{osm} 24 hours after drug dosing	Normalize S_{Na} levels	Significant reduction in BW 24 hours after drug dosing	Decrease in edema scores with no change in HR, BP, renal function or S_K
[125]	319 patients with ADHF (LVEF $\leq$ 40%). 68 (21%) of 319 patients with hyponatremia ($S_{Na} < 136$ mequiv./l). Oral dose at 30, 60 or 90 mg/day for 60 days	Significant increase in UV	Rapid and sustained increase in S_{Na}	Significant reduction in BW at 24 hours and further decrease in the hospital phase (first ten days)	No change in HR, BP, S_K or renal function in the hospital phase. Degree of worsening HF unchanged at 60 days, but improved mortality rate in the subgroups (i.e. patients with RD or SSC)
[126,127]	4133 patients with ADHF (LVEF $\leq$ 40%) 1124 (27%) of 4133 patients with hyponatremia ($S_{Na} \leq 134$ mequiv./l). Oral dose at 30 mg/day for 60 days in addition to standard care	Increase in UO	Significant increase in S_{Na}	Significant reduction in BW	Greater improvement in GCS, dyspnea and edema. No change in CV mortality, CV death or hospitalization for HF
[130]	181 patients with HF and systolic dysfunction (NYHA class III–IV). Single oral dose at 15, 30 or 60 mg	Increase in aquaresis, UV and decrease in U_{osm}	Not reported	Not reported	Modest reduction in PCWP, PAP, and RAP and modest increase in P_{osm} and S_{Na} with no change in CI, PVR, SVR, S_{cr}, S_K, BUN and vital signs

TABLE 4 (*Continued*)

Drug and Refs	Subjects and dosage	Drug effects			
		Diuresis and electrolytes	S_{Na} and/ or S_{osm}	BW	Hemodynamics and/or overall benefits
[131]	240 patients with HF and systolic dysfunction (LVEF $\leq$ 30%). Once-daily oral dose at 30 mg for up to one year in addition to evidence-based HF therapy	Not reported	Not reported	Not reported	Decrease in mortality and hospital admissions with one year therapy, but no reduction in LV volumes or ejection fractions in patients
[132]	14 patients with mild to moderate HF. Once-daily oral dose at 30 mg for three days, and then crossover for five days total; at day 5, all patients received single oral dose of furosemide 80 mg	Increase UO similar to furosemide	No significant change in S_{Na}	Not reported	In contrast to tolvaptan, furosemide increased U_{Na}, U_K, PRA, and norepinephrine levels and decreased RBF and S_{Na}

Abbreviations: ADHF, acute decompensated heart failure; BP, blood pressure; BUN, blood urea nitrogen; BW, body weight; CHF, chronic heart failure; CI, cardiac index; CV, cardiovascular; CWAs, cirrhotic with ascites; FR, fluid restriction; FWC, free water clearance; GCS, global clinical status; HF, heart failure; HR, heart rate; LC, liver cirrhosis; LV, left ventricular; LVEF, left ventricular ejection fraction; MC, mental component; NYHA, New York Heart Association; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; PRA, plasma renin activity; PVR, pulmonary vascular resistance; P_{osm}, plasma osmolality; RAP, right atrial pressure; RBF, renal blood flow; RD, renal dysfunction; SIADH, syndrome of inappropriate anti-diuretic hormone secretion; S_K, serum potassium; S_{Na}, serum sodium; SSC, severe systemic congestion; SVR, systemic vascular resistance; S_{cr}, serum creatinine; S_{osm}, serum osmolality; U_K, urine potassium; U_{Na}, urine sodium; UO, urine output; U_{osm}, urine osmolality; UV, urine volume.

creatinine concentrations [94]. Creatinine clearance was also significantly decreased in the furosemide-treated rats in comparison with vehicle-treated CHF rats, suggesting that furosemide decreased glomerular filtration rate (GFR) and was not affected by tolvaptan treatment (Fig. 4c). The upregulation of AQP 2 protein in the kidney of CHF rats was inhibited by tolvaptan [95] (Table 3), suggesting that in a rat model of CHF, AVP plays a major part in water retention through the renal V_2R. Tolvaptan dose-dependently increased aquaresis in dogs with HF induced by chronic rapid ventricular pacing. Tolvaptan increased plasma AVP concentrations but did not stimulate either SNS or RAAS despite its potent aquaretic effects [91,96]. Tolvaptan (10 mg/kg) significantly decreased PCWP in parallel with an increase in urine excretion, suggesting a reduction of cardiac preload and pulmonary congestion. Conversely, tolvaptan did not alter peripheral vascular resistance, renal blood flow (RBF) or GFR in HF dogs, suggesting that the compensatory increase in plasma AVP levels by tolvaptan did not aggravate cardiac afterload or renal function in these animals [96] (Table 3). In this model, coadministration of tolvaptan and brain natriuretic peptide resulted in a beneficial profile of renal, neurohumoral and hemodynamic actions, specifically potent diuresis with natriuresis, neutral effect on mean arterial pressure and lack of aldosterone activation [97]. Taking all the results together, tolvaptan might emerge as an important therapeutic option for edematous conditions in CHF, owing to its ability to remove excess water from the body without activating the RAAS or causing serum electrolyte imbalances.

The therapeutic efficacy of tolvaptan was assessed in a rat model of acute and chronic severe hyponatremia by Miyazaki *et al.* [98]. In the acute model, tolvaptan produced dose-dependent aquaresis, which resulted in a gradual increase in the plasma sodium concentration. Consequently, tolvaptan treatment reduced mortality, and at higher doses (3 and 10 mg/kg), no deaths were observed. In the chronic model, tolvaptan gradually increased plasma sodium concentrations to healthy levels without causing abnormal behavior suggesting neurological symptoms or deaths and, in addition, improved hyponatremia-driven increases in wet weight and water content in the organs. It seems, therefore, that tolvaptan should be useful in the management of hyponatremia [98] (Table 3). In addition, VRAs have been shown to markedly increase free water excretion [14,99–108] and reverse the increased AQP 2 expression [106–108] and AQP of collecting duct mRNA [99] in animal models of hyponatremia with water-retaining states such as experimental cirrhosis [99–103], SIADH [14,99,104,105] and glucocorticoid deficiency [106–108]. Administration of AVP V_2R agonist desmopressin induces SIADH, whereas CCl_4 induces cirrhosis, ascites and severe water retention in experimental animals. Furthermore, treatment with VRAs exhibited marked water diuresis, as evidenced by an increase in urinary volume and a fall in urinary osmolality. This treatment also normalizes serum sodium levels and osmolality [14,99–105].

In several recent studies, a V_2R antagonist has been shown to inhibit the development of polycystic kidney disease (PKD) in *cpk* mice and in animal models of autosomal recessive PKD (PCK rats), autosomal dominant PKD (ADPKD) (Pkd2/WS25− mice) and nephronophthisis (*pcy* mice) [13,109–111]. Tolvaptan administered at 0.01–0.1% in diet from the third to the tenth week of age significantly decreased renal cAMP, kidney weight, cyst and fibrosis volume, proliferating cell nuclear antigen-positive renal cells and apoptotic indices in PCK rats, indicating a protective effect against the development of PKD [112] (Table 3). The Ras/MAPK pathway, which is considered to mediate the proliferative response to cAMP *in vitro* [113], is activated in PCK rats and was inhibited by tolvaptan [112]. Furthermore, Nagao *et al.* [114] also confirmed the physiological importance of AVP in the development of PKD by decreasing endogenous AVP levels in water-loaded PCK rats. These results support the importance of AVP and cAMP in the pathogenesis of PKD, confirm the effectiveness in the PCK rat of a V_2 antagonist to be used in ADPKD clinical trials, and suggest that tolvaptan inhibits Ras/MAPK signaling in polycystic kidneys.

VRAs in clinical studies

Trials in hyponatremia

Hyponatremia is challenging to treat, with current approaches having significant limitations. Antagonism of AVP at its receptor is attractive as an approach that directly addresses the pathophysiology. Table 4 summarizes the trials that investigated the use of VRAs in patients with hyponatremia of multiple origins [115–119].

The efficacy and safety of conivaptan were evaluated in two randomized, double-blind, placebo-controlled trials in patients with euvolemic or hypervolemic hyponatremia (serum sodium 115 to <130 mequiv./l) [115,116]. In the first trial, 74 patients received oral conivaptan 40 or 80 mg/day or placebo in two divided doses for five days [115]. Oral conivaptan produced significant increase in serum sodium of 6.4 mequiv./l and 8.2 mequiv./l in the 40 and 80 mg/day group, respectively. The percentage of patients achieving a normal serum sodium level (≥135 mequiv./l) or an increase of at least 6 mequiv./l was 48%, 71% and 82% in the placebo group, and lower and higher conivaptan dose groups, respectively ($P = 0.014$ versus placebo). Despite the efficacy of oral conivaptan in this trial, development of the oral formulation was discontinued because of significant inhibition of the liver enzyme CYP3A4 and elevated plasma levels of other drugs metabolized by this enzyme [115]. To minimize the possibility of drug interactions, the FDA restricted conivaptan's distribution to a parenteral form for short-term in-hospital use.

In the other trial, 84 patients received a 20 mg i.v. loading dose of conivaptan, followed by continuous infusion of conivaptan 40 or 80 mg/day for four days, or placebo [116]. Conivaptan resulted in a significant increase in serum sodium of 6.3 mequiv./l and 9.4 mequiv./l in the 40 and 80 mg/day group, respectively. The percentage of patients achieving a normal serum sodium level (≥135 mequiv./l) or an increase of at least 6 mequiv./l was significantly higher in both conivaptan dose groups ($P = 0.001$ versus placebo), despite the potent aquaretic effect. The rate of the correction of serum sodium concentration was within safe limits without evidence of excessive hypernatremia or feared complications of central pontine myelinolysis from too rapid a correction. Conivaptan was generally well tolerated over the four-day treatment period except for frequent reactions at the site of infusion [116]. Conivaptan has been shown to correct hyponatremia in euvolemic or hypervolemic patients. Its efficacy and safety for short-term use have led to FDA approval of its i.v. form for the correction of hyponatremia in euvolemic and hypervolemic states.

Two recently completed trials, Study of Ascending Levels of Tolvaptan in hyponatremia (SALT)-1 and SALT-2, investigated the effects of tolvaptan on serum sodium levels in patients with euvolemic or hypervolemic hyponatremia associated with CHF, cirrhosis or SIADH [117]. This double-blind multicenter trial randomized 223 patients to receive placebo and 225 patients to receive tolvaptan at an initial dose of 15 mg daily. The dose of tolvaptan was increased to 30 mg daily and then to 60 mg daily, if necessary, on the basis of serum sodium concentrations. Serum sodium concentrations increased more in the tolvaptan group than in the placebo group during the first four days ($P < 0.001$) and after the full 30 days of therapy ($P < 0.001$). During the first week of discontinuation of tolvaptan on day 30, serum sodium concentrations decreased again (hyponatremia recurred). Side-effects associated with tolvaptan included increased thirst, dry mouth and increased urination. In scores on a self-administered questionnaire, subjects with marked hyponatremia who received tolvaptan showed improvements in some cognitive domains of the mental component, including overall well-being and ability to concentrate, but scores on the physical component showed no significant differences between the two groups. Results of this study suggest that in patients with euvolemic or hypervolemic hyponatremia, tolvaptan was effective in increasing serum sodium concentrations at day 4 and day 30 [117]. Of note, patients in the SALT trials were not fluid restricted; however, in one smaller study, compared with placebo plus fluid restriction (1200 ml/day), tolvaptan (10–60 mg/day) seemed to be more effective at correcting hyponatremia in hospitalized patients ($n = 28$) [118].

SALTWATER – a four-year sequential, open-label extension of the randomized, placebo-controlled, double-blind SALT-1 and SALT-2 – assessed the effects of tolvaptan in patients with euvolemic or hypervolemic hyponatremia associated with CHF, cirrhosis or SIADH [119]. In total, 111 patients (38 from SALT-1 and 73 from SALT-2) were randomized to receive either oral tolvaptan (15 mg daily, initially, for a maximum of 214 weeks) or placebo. In the long-term follow-up trial (over a mean follow-up time of 701 days), serum sodium level increased from 130.8 ± 4.4 mmol/l at baseline to above 135 mmol/l after 14 days of treatment and remained within the normal range for the rest of the study [119]. The most common adverse events were similar to those reported in short-term studies of tolvaptan [117]. Results of these studies suggest that short- and long-term administration of tolvaptan maintained serum sodium concentration with an acceptable margin of safety in patients with euvolemic or hypervolemic hyponatremia [117,119]. In May 2009, Tolvaptan (Samsca; Otsuka) was approved by the FDA for the treatment of clinically significant hypervolemic and euvolemic hyponatremia, including patients with HF, cirrhosis and SIADH. Overall, the data surrounding conivaptan and tolvaptan have been consistent in demonstrating favorable effect on serum sodium among patients with hyponatremia of various causes but not in demonstrating effects on overall or disease-specific mortality.

Trials in CHF

Table 4 summarizes the trials that have investigated the use of VRAs in patients with CHF. In an efficacy report that combined three randomized, placebo-controlled trials of conivaptan in patients with CHF ($n = 241$), conivaptan (40 and 80 mg) treatment with one i.v. and two oral formulations was shown to improve serum sodium concentrations from baseline to the end of the study compared with placebo. One interesting finding of the analysis was the slower rate of correction in patients with CHF compared to those without CHF [120].

The ADVANCE (A Dose Evaluation of a Vasopressin Antagonist in Congestive Heart Failure Patients Undergoing Exercise) trial is a multicenter, double-blind, placebo-controlled, randomized study investigating the effects of conivaptan on functional capacity in patients with HF ($n = 345$, New York Heart Association [NYHA] class II–IV and ejection fraction of less than 35%) [121]. Three doses of conivaptan (10, 20 or 40 mg daily) were given orally during the 12-week trial. Results of this study demonstrated that there was no significant improvement in overall functional capacity, exercise tolerance or quality of life among patients receiving conivaptan compared to the placebo group [121].

A dose-ranging pilot study assessing the efficacy and safety of i.v. conivaptan (20 mg loading dose followed by two successive 24-hour continuous infusions of 40, 80 or 120 mg/day) in patients with acute decompensated HF ($n = 170$) found that conivaptan significantly increased urine output, was hemodynamically well tolerated, was not associated with clinically important changes in

vital signs, electrolyte disturbances or cardiac rhythm and had minimal adverse effects. However, no apparent significant change in respiratory symptoms or body weight (BW) was found. There was no evidence of worsening HF in any group [122].

In another pilot study, 24 patients with HF class II/III received 40 or 80 mg *quaque die* of furosemide for six days followed by 20 or 40 mg *quaque die* of conivaptan for an additional three days. The addition of conivaptan was associated with a significant increase in aquaresis with limited natriuresis and kaliuresis, suggesting conivaptan to be useful as an adjunct to furosemide [123].

Tolvaptan has been assessed in several clinical trials for the management of acute and chronic HF. The first double-blind study investigated the effects of three doses of tolvaptan (30, 45 and 60 mg once daily) and a placebo in patients with CHF (mostly NYHA class II and III) for 25 days ($n = 254$) [124]. Patients were not fluid restricted and were maintained on standard therapy. Two hundred and twenty-one (87%) of the 254 patients enrolled completed the study. At day 1, all three doses of tolvaptan were associated with significant reductions in BW that were maintained throughout the study. An increase in urine volume and decrease in urine osmolality was observed with tolvaptan when compared with the placebo at day 1 ($P < 0.05$ for all treatment groups versus placebo). A decrease in edema and a normalization of serum sodium levels in patients with hyponatremia were observed (28% of 254) in the tolvaptan group but not in the placebo group. No significant changes in heart rate (HR), blood pressure, serum potassium or renal function were observed. Compared with the placebo group, edema scores improved, thirst increased and AVP concentrations rose in the active treatment group [124].

In the Acute and Chronic Therapeutic Impact of Vasopressin Antagonist in Congestive Heart Failure trial, which examined decompensated HF patients ($n = 319$), 68 patients (21%) had hyponatremia (serum sodium level < 136 mequiv./l) at randomization [125]. In this trial, three oral, once-daily doses of tolvaptan (30, 60 or 90 mg) or placebo were administered in addition to standard therapy to patients hospitalized with HF (left ventricular ejection fraction, or LVEF, ≤40%). The study drug was administered for up to 60 days. For the first ten days, the patients were hospitalized (inpatient) and for an additional seven weeks (49–51 days), were treated as outpatients. At 24 hours, all tolvaptan groups had significant reductions in BW compared with the placebo group ($P < 0.008$ for all tolvaptan groups versus placebo) and decreased further during the course of hospitalization. The decrease in BW with tolvaptan was not associated with changes in HR, blood pressure, renal function or development of hypokalemia. Compared with the placebo, all tolvaptan doses were associated with significantly higher urine volume on day 1, and this difference was maintained throughout the hospital stay. Tolvaptan produced a rapid and sustained increase in serum sodium levels in patients with hyponatremia. There were no significant differences in worsening HF over the 60-day follow-up period, although a trend toward greater survival was found in the tolvaptan groups compared with the placebo group. In a post hoc analysis, 60-day mortality was lower in tolvaptan-treated patients with renal dysfunction or severe systemic congestion [125].

The Efficacy of Vasopressin Antagonist in Heart Failure Outcome Study with Tolvaptan (EVEREST) program evaluated short- and long-term end points among patients being admitted for acute decompensated HF (LVEF ≤40%), randomized to treatment with tolvaptan at 30 mg daily in addition to routine standard care (including diuretics) or to the placebo plus standard care [126], and followed up on continuing treatment with an assigned study drug after hospital discharge [127]. The study was structured so that the main trial incorporated two short-term trials of symptom assessment for the hospitalization period [126], whereas long-term morbidity and mortality outcome end points were assessed in the well-powered main trial over the longer follow-up period [128]. The phase III EVEREST trial, involving a total of 4133 CHF patients, represents three studies: a long-term outcomes trial evaluating patients after their discharge for a minimum of 60 days of treatment [127] and two identical, embedded short-term pivotal studies that examined tolvaptan compared to placebo over seven days of inpatient care or discharge, whichever came first [126]. In the two short-term studies, tolvaptan treatment yielded significantly greater improvements than the placebo based on the primary end point (study A and B: $P < 0.001$), which was a composite of patient-assessed global clinical status (assessed on a visual analog score), and reductions in average BW at day 7 or discharge. In addition, the use of tolvaptan significantly reduced BW as early as on the first day of treatment in both short-term studies ($P < 0.001$). Improvements in patient-assessed global clinical status at day 7 or discharge were similar between the tolvaptan and placebo groups; however, the changes were numerically in favor of tolvaptan. More patients receiving tolvaptan (76.7% and 72.1% for trial A and trial B, respectively) than the placebo (70.6% and 65.3%, respectively) reported an improvement in dyspnea at day 1 (both trials $P < 0.001$). Edema at day 7 or discharge improved significantly with tolvaptan in trial B ($P = 0.02$) but did not reach a level of significance in trial A ($P = 0.07$) [126].

In the long-term follow-up trial (over a mean of 9.9 months), there was no difference in the end points of all-cause mortality, cardiovascular death or HF hospitalization between the groups ($P = 0.55$) [127]. The long-term tolvaptan data were consistent with those reported in the short-term clinical studies. Specifically, long-term tolvaptan treatment significantly reduced BW at day 1 ($P < 0.001$) compared to the placebo, and the difference in BW numerically favored tolvaptan through week 56. The long-term trial also documented that the tolvaptan-treated patients who enrolled with hyponatremia (serum sodium < 134 mequiv./l) exhibited significantly greater corrections in serum sodium levels at day 7 or discharge, if earlier ($P < 0.001$), and the effect was maintained through 40 weeks of treatment [127]. Tolvaptan caused increased thirst and dry mouth, but frequencies of major adverse events were similar between groups, without excess renal failure or hypotension [126,127].

Overall, the data surrounding conivaptan and tolvaptan suggest that vasopressin antagonism in the setting of CHF has potential medical benefits, including decreasing BW by increasing urine output associated with improvements in symptoms, without causing abnormalities in electrolytes or renal function [122,124–127] and normalizing serum sodium concentrations in hyponatremic HF patients [120,124,125,127] (Table 4). Thus, the use of VRAs, addressing both the pathophysiological state of hyponatremia and the volume overload state simultaneously, could become an important approach in the clinical setting for this challenging group of patients.

Trials in hemodynamics and remodeling in CHF

In a study of 142 patients with advanced HF (NYHA class III or IV) with systolic dysfunction, conivaptan was administered as a single i.v. dose (10, 20 or 40 mg) in addition to standard therapy and compared with a placebo [129]. At 20 and 40 mg, conivaptan significantly reduced PCWP and right atrial pressure compared to the placebo, without producing significant changes in blood pressure, HR, SVR or the cardiac index. There was a significant dose-dependent increase in urine output and decrease in urine osmolality without any significant worsening of hyponatremia or renal function (Table 4). Of interest, no correlation could be established between conivaptan pharmacodynamic effects and baseline AVP or serum sodium levels [129].

Similar hemodynamic and urine output data have recently been reported in a dose-ranging, single-dose administration study of tolvaptan (15, 30 or 60 mg) in which modest reductions in PCWP, pulmonary artery pressure and right arterial pressure, dose-dependent increases in urine output, and decreases in urine osmolality were observed. No significant changes in cardiac index, pulmonary vascular resistance, SVR, serum creatinine, potassium, blood urea nitrogen levels and vital signs were observed after the study drug's administration [130] (Table 4). Serum sodium levels showed a dose-related modest increase. The most commonly reported adverse event was catheter site pain, which occurred in 4.5% and 6.3% of the tolvaptan and placebo-treated patients, respectively. The data reported herein from the ECLIPSE trial suggest that in patients with advanced HF, tolvaptan resulted in favorable but modest changes in filling pressures associated with a significant increase in urine output [130].

The effect of tolvaptan (30 mg/day) on cardiac remodeling was evaluated in the Multicenter Evaluation of Tolvaptan Effect on Remodeling trial in 240 patients with HF and LV systolic dysfunction (LVEF $\leq$ 30%) and signs of volume excess [131]. In this patient sample with very high use of evidence-based background HF therapies, the primary end point (i.e. change from baseline in LV end-diastolic volume index after one year measured by quantitative radionuclide ventriculography) was not statistically significant in the tolvaptan group, although there was a reduction of 1.8 ml/m^2. Overall, there were no obvious effects of tolvaptan on LV volumes or ejection fractions observed over one year of therapy (Table 4). Nevertheless, tolvaptan therapy seemed to be safe and tolerated with no adverse effect on remodeling. In a time-to-event analysis, there was a significant favorable effect of tolvaptan on the composite of mortality or HF hospitalization ($P < 0.03$) [131].

The data from these studies suggest that vasopressin antagonism in patients with HF has favorable hemodynamic effects, possibly mediated via increased urine output [129,130,132] (Table 4). The generally similar results noted for hemodynamics and urine output with single-dose studies with both conivaptan [129] and tolvaptan [130,133] suggest that the predominant effects of vasopressin receptor antagonism in these studies involves effects at the V_2R.

Effects on renal function

In an open-label, randomized, placebo-controlled, single-dose crossover study in patients with mild to moderate CHF designed to assess the effects of tolvaptan and furosemide on renal function and renal hemodynamics [132], tolvaptan (30 mg) was found to increase urine output and RBF. In comparison, furosemide (80 mg) also increased urine output, to a similar extent. Unlike tolvaptan, furosemide increased urinary sodium and potassium excretion and decreased RBF. Tolvaptan, furosemide, and the placebo did not differ with respect to mean arterial pressure, HR, GFR, or serum potassium. Tolvaptan did not significantly change the plasma sodium concentration, whereas furosemide tended to decrease it. However, there were no significant differences among the groups. There were no significant differences with respect to neurohormonal concentrations between tolvaptan and either the placebo or furosemide. Furosemide did cause a significant increase in plasma renin activity and norepinephrine levels compared with the placebo (Table 4). The authors of this study concluded that tolvaptan is an effective aquaretic with no adverse effects on renal hemodynamics, urinary sodium or potassium excretion, serum electrolytes, or neurohormonal systems in patients with mild to moderate HF [132].

In published studies to date [124–127,129,130,132], no significant adverse effects on renal function or electrolyte status have been noted. All these results support the possibility that AVP receptor antagonism might be associated with preserved renal function in HF patients while promoting water loss and relief of congestion.

Future directions

Other potential clinical uses for VRAs are currently being explored, one of which is the treatment of PKD because AVP V_2Rs have been implicated in the pathogenesis of cyst formation and enlargement in PKD patients. Tolvaptan is being evaluated as a potential treatment for PKD after its efficacy was demonstrated in studies with rats with polycystic kidneys. Several clinical studies in PKD have been performed or are currently active. The results of phase II and phase II–III clinical trials suggest that tolvaptan is safe and well tolerated in ADPKD patients. A phase III, placebo-controlled, double-blind study in patients with ADPKD has been initiated and will determine whether tolvaptan is able to prevent cyst growth and progressive loss of renal function in patients with ADPKD [134].

Many unanswered questions remain concerning the potential use of VRAs with regard to HF. Such questions include the potential efficacy of long-term treatment with nonselective receptor antagonism, the use of volume overload associated with HF in the setting of preserved ejection fraction with a nondilated ventricle, the impact of potential sparing of need for short-term versus long-term treatment and dose-adjustment strategies.

Concluding remarks

Despite advances in treatment with neurohormonal antagonists [135], patients with HF continue to progress to more advanced stages of the syndrome, continue to be hospitalized for decompensation and die at an unacceptable rate. This has spurred translational and clinical investigations into the blockade of additional neurohormonal systems. Somewhat unexpectedly, many contemporary trials investigating approaches such as endothelin receptor blockade [136] and cytokine inhibition [137] have shown neutral or unfavorable results. In this light, the 'vaptans' class of VRAs has shown promising results for the treatment of hyponatremia, decompensated HF and HF complicated by hyponatremia.

On the basis of the results of animal studies and multiple clinical trials with two different VRAs (conivaptan and tolvaptan), it is probable that this class will become a mainstay of treatment for euvolemic or hypervolemic hyponatremia. These agents are highly effective in producing a safe and predictable aquaresis via their activity at the V_2R, thereby increasing serum sodium levels in the majority of patients with hyponatremia caused by SIADH, CHF or cirrhosis. In HF patients, consistent increases in urine output, a reduction in BW and an improvement in many signs and symptoms have been seen without unfavorable changes in blood pressure, HR, electrolytes or renal function. The first non-peptide VRAs, conivaptan (a dual V_{1a}/V_2 receptor antagonist) and tolvaptan (a V_2R antagonist) have been shown to induce diuresis and correct euvolemic and hypervolemic hyponatremia and are approved by the US FDA for the treatment of hyponatremia caused by HF, cirrhosis and SIADH as an i.v. infusion and oral administration, respectively. Pivotal trials of lixivaptan for hyponatremia are underway [138]. Treatment of PKD also is being evaluated. Thus far, therapeutic use of VRAs has safely and effectively been shown to reduce BW in patients with decompensated HF and to normalize serum sodium levels in hyponatremic patients. The data from the EVEREST, SALT-1 and SALT-2 trials and the conivaptan data suggest AVP antagonism will become a useful tool for clinicians in specific situations, such as decompensated HF with volume overload, and is potentially an important therapy in the setting of HF with hyponatremia and for hyponatremia of any cause when an improvement in serum sodium concentration is a clinical goal. Additional studies with these agents are needed to provide further insight regarding the nature of the relationship between hyponatremia and poor outcome in CHF.

Acknowledgements

This research was supported by a Yujin Memorial Grant from The Ministry of Education, Culture, Sports, Science, and Technology, Japan, and by a grant from the Promotion and Mutual Aid Corporation for Private Schools, Japan.

References

1 Lee, W.H. and Packer, M. (1986) Prognostic importance of serum sodium concentration and its modification by converting-enzyme inhibition in patients with severe chronic heart failure. *Circulation* 73, 257–267

2 Baran, D. and Hutchinson, T.A. (1984) The outcome of hyponatremia in a general hospital population. *Clin. Nephrol.* 22, 72–76

3 Goldsmith, S.R. (1999) Vasopressin: a therapeutic target in congestive heart failure? *J. Card. Fail.* 5, 347–356

4 Lee, C.R. *et al.* (2003) Vasopressin: a new target for the treatment of heart failure. *Am. Heart J.* 146, 9–18

5 Thibonnier, M. (2003) Vasopressin receptor antagonists in heart failure. *Curr. Opin. Pharmacol.* 3, 683–687

6 Lilly, L.S. *et al.* (1984) Hyponatremia in congestive heart failure: implications for neurohumoral activation and responses to orthostasis. *J. Clin. Endocrinol. Metab.* 59, 924–930

7 Oren, R.M. (2005) Hyponatremia in congestive heart failure. *Am. J. Cardiol.* 95, 2B–7B

8 Sica, D.A. (2006) Hyponatremia and heart failure – treatment considerations. *Congest. Heart Fail.* 12, 55–60

9 Naitoh, M. *et al.* (1994) Effects of oral AVP receptor antagonists OPC-21268 and OPC-31260 on congestive heart failure in conscious dogs. *Am. J. Physiol.* 267, H2245–H2254

10 Palm, C. *et al.* (2001) The role of V2 vasopressin antagonists in hyponatremia. *Cardiovasc. Res.* 51, 403–408

11 Burrell, L.M. *et al.* (1995) Attenuation of genetic hypertension after short-term vasopressin V1A receptor antagonism. *Hypertension* 26, 828–834

12 Burrell, L.M. *et al.* (1994) Blood pressure-lowering effect of an orally active vasopressin V1 receptor antagonist in mineralocorticoid hypertension in the rat. *Hypertension* 23, 737–743

13 Gattone, V.H., 2nd *et al.* (2003) Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. *Nat. Med.* 9, 1323–1326

14 Fujisawa, G. *et al.* (1993) Therapeutic efficacy of non-peptide ADH antagonist OPC-31260 in SIADH rats. *Kidney Int.* 44, 19–23

15 Saito, T. *et al.* (1997) Acute aquaresis by the nonpeptide arginine vasopressin (AVP) antagonist OPC-31260 improves hyponatremia in patients with syndrome of inappropriate secretion of antidiuretic hormone (SIADH). *J. Clin. Endocrinol. Metab.* 82, 1054–1057

16 Bichet, D. *et al.* (1982) Role of vasopressin in abnormal water excretion in cirrhotic patients. *Ann. Intern. Med.* 96, 413–417

17 Lacheretz, F. *et al.* (2000) Effect of SR121463, a selective non-peptide vasopressin V2 receptor antagonist, in a rabbit model of ocular hypertension. *J. Ocul. Pharmacol. Ther.* 16, 203–216

18 Goldsmith, S.R. (2005) Current treatments and novel pharmacologic treatments for hyponatremia in congestive heart failure. *Am. J. Cardiol.* 95, 14B–23B

19 Hofbauer, K.G. *et al.* (1986) Chronic blockade of vasopressin receptors in rats. *J. Cardiovasc. Pharmacol.* 8 (Suppl. 7), S56–S60

20 Mah, S.C. *et al.* (1988) Biologic response to chronic blockade of vasopressin receptors in Sprague–Dawley rats. *J. Pharmacol. Exp. Ther.* 245, 1021–1027

21 Nicod, P. *et al.* (1985) Acute hemodynamic effect of a vascular antagonist of vasopressin in patients with congestive heart failure. *Am. J. Cardiol.* 55, 1043–1047

22 Schrier, R.W. and Martin, P.Y. (1998) Recent advances in the understanding of water metabolism in heart failure. *Adv. Exp. Med. Biol.* 449, 415–426

23 Verbalis, J.G. (2002) Vasopressin V2 receptor antagonists. *J. Mol. Endocrinol.* 29, 1–9

24 Tahara, A. *et al.* (1997) Pharmacological profile of YM087, a novel potent nonpeptide vasopressin V1A and V2 receptor antagonist, *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.* 282, 301–308

25 Yatsu, T. *et al.* (1997) Pharmacological profile of YM087, a novel nonpeptide dual vasopressin V1A and V2 receptor antagonist, in dogs. *Eur. J. Pharmacol.* 321, 225–230

26 Tomura, Y. *et al.* (1999) Pharmacological profile of orally administered YM087, a vasopressin antagonist, in conscious rats. *Clin. Exp. Pharmacol. Physiol.* 26, 399–403

27 Albright, J.D. *et al.* (1998) 5-Fluoro-2-methyl-*N*-[4-(5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepin-10(11*H*)-ylcarbonyl)-3-chlorophenyl]benzamide (VPA-985): an orally active arginine vasopressin antagonist with selectivity for V2 receptors. *J. Med. Chem.* 41, 2442–2444

28 Serradeil-Le Gal, C. *et al.* (1996) Characterization of SR 121463A, a highly potent and selective, orally active vasopressin V2 receptor antagonist. *J. Clin. Invest.* 98, 2729–2738

29 Yamamura, Y. *et al.* (1998) OPC-41061, a highly potent human vasopressin V2-receptor antagonist: pharmacological profile and aquaretic effect by single and multiple oral dosing in rats. *J. Pharmacol. Exp. Ther.* 287, 860–867

30 Schrier, R.W. *et al.* (1979) Osmotic and nonosmotic control of vasopressin release. *Am. J. Physiol.* 236, F321–F332

31 Share, L. (1988) Role of vasopressin in cardiovascular regulation. *Physiol. Rev.* 68, 1248–1284

32 Kamoi, K. *et al.* (1997) Interaction of osmotic and nonosmotic stimuli in regulation of vasopressin secretion in hypoosmolar state of man. *Endocr. J.* 44, 311–317

33 Verbalis, J.G. (2003) Disorders of body water homeostasis. *Best Pract. Res. Clin. Endocrinol. Metab.* 17, 471–503

34 Yamaguchi, K. *et al.* (1985) Plasma vasopressin response to peripheral administration of angiotensin in conscious rats. *Am. J. Physiol.* 248, R249–R256

35 Yamaguchi, K. and Yamada, T. (2006) Involvement of anteroventral third ventricular AMPA/kainate receptors in both hyperosmotic and hypovolemic AVP secretion in conscious rats. *Brain Res. Bull.* 71, 183–192

36 Michell, R.H. *et al.* (1979) Hormonal stimulation of phosphatidylinositol breakdown with particular reference to the hepatic effects of vasopressin. *Biochem. Soc. Trans.* 7, 861–865

37 Birnbaumer, M. (2000) Vasopressin receptors. *Trends Endocrinol. Metab.* 11, 406–410

38 Reilly, B.A. *et al.* (1998) Regulation of protein synthesis in ventricular myocytes by vasopressin. The role of sarcoplasmic/endoplasmic reticulum Ca^{2+} stores. *J. Biol. Chem.* 273, 3747–3755

39 Chandrashekhar, Y. *et al.* (2003) The role of arginine vasopressin and its receptors in the normal and failing rat heart. *J. Mol. Cell. Cardiol.* 35, 495–504

40 Guyton, A.C. (1996) The kidneys and body fluids. In *Text Book of Medical Physiology* (Guyton, A.C. and Hall, J.E., eds), pp. 308–372, WB Saunders

41 Saito, M. *et al.* (2000) Evidence that atypical vasopressin V(2) receptor in inner medulla of kidney is V(1B) receptor. *Eur. J. Pharmacol.* 401, 289–296

42 Folny, V. *et al.* (2003) Pancreatic vasopressin V1b receptors: characterization in In-R1-G9 cells and localization in human pancreas. *Am. J. Physiol. Endocrinol. Metab.* 285, E566–E576

43 Nielsen, S. *et al.* (1999) Physiology and pathophysiology of renal aquaporins. *J. Am. Soc. Nephrol.* 10, 647–663

44 Sands, J.M. *et al.* (1987) Vasopressin effects on urea and H_2O transport in inner medullary collecting duct subsegments. *Am. J. Physiol.* 253, F823–F832

45 Bernat, A. *et al.* (1997) V2 receptor antagonism of DDAVP-induced release of hemostasis factors in conscious dogs. *J. Pharmacol. Exp. Ther.* 282, 597–602

46 Francis, G.S. *et al.* (1990) Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). *Circulation* 82, 1724–1729

47 Goldsmith, S.R. *et al.* (1983) Increased plasma arginine vasopressin levels in patients with congestive heart failure. *J. Am. Coll. Cardiol.* 1, 1385–1390

48 Szatalowicz, V.L. *et al.* (1981) Radioimmunoassay of plasma arginine vasopressin in hyponatremic patients with congestive heart failure. *N. Engl. J. Med.* 305, 263–266

49 Rouleau, J.L. *et al.* (1994) Prognostic value of neurohumoral activation in patients with an acute myocardial infarction: effect of captopril. *J. Am. Coll. Cardiol.* 24, 583–591

50 Nielsen, S. *et al.* (1997) Congestive heart failure in rats is associated with increased expression and targeting of aquaporin-2 water channel in collecting duct. *Proc. Natl. Acad. Sci. U. S. A.* 94, 5450–5455

51 Xu, D.L. *et al.* (1997) Upregulation of aquaporin-2 water channel expression in chronic heart failure rat. *J. Clin. Invest.* 99, 1500–1505

52 Goldsmith, S.R. *et al.* (1986) Hemodynamic effects of infused arginine vasopressin in congestive heart failure. *J. Am. Coll. Cardiol.* 8, 779–783

53 Tahara, A. *et al.* (1999) AVP-induced mitogenic responses of Chinese hamster ovary cells expressing human V1A or V1B receptors. *Pflugers Arch.* 437, 219–226

54 Tahara, A. *et al.* (1999) Vasopressin increases vascular endothelial growth factor secretion from human vascular smooth muscle cells. *Eur. J. Pharmacol.* 368, 89–94

55 Tahara, A. *et al.* (1998) Effect of YM087, a potent nonpeptide vasopressin antagonist, on vasopressin-induced protein synthesis in neonatal rat cardiomyocyte. *Cardiovasc. Res.* 38, 198–205

56 Nakamura, Y. *et al.* (2000) Hypertrophic growth of cultured neonatal rat heart cells mediated by vasopressin V(1A) receptor. *Eur. J. Pharmacol.* 391, 39–48

57 Fukuzawa, J. *et al.* (1999) Arginine vasopressin increases the rate of protein synthesis in isolated perfused adult rat heart via the V1 receptor. *Mol. Cell. Biochem.* 195, 93–98

58 Tahara, A. *et al.* (1997) Effect of YM087, a potent nonpeptide vasopressin antagonist, on vasopressin-induced hyperplasia and hypertrophy of cultured vascular smooth-muscle cells. *J. Cardiovasc. Pharmacol.* 30, 759–766

59 Spannheimer, A. *et al.* (2002) Long-term diuretic treatment in heart failure: are there differences between furosemide and torasemide? *Praxis (Bern 1994)* 91, 1467–1475

60 Blose, J.S. *et al.* (1995) Torsemide: a pyridine–sulfonylurea loop diuretic. *Ann. Pharmacother.* 29, 396–402

61 Friedel, H.A. and Buckley, M.M. (1991) Torasemide. A review of its pharmacological properties and therapeutic potential. *Drugs* 41, 81–103

62 Brater, D.C. (1996) Benefits and risks of torasemide in congestive heart failure and essential hypertension. *Drug Saf.* 14, 104–120

63 López, B. *et al.* (2004) Effects of loop diuretics on myocardial fibrosis and collagen type I turnover in chronic heart failure. *J. Am. Coll. Cardiol.* 43, 2028–2035

64 Müller, K. *et al.* (2003) Torasemide vs. furosemide in primary care patients with chronic heart failure NYHA II to IV–efficacy and quality of life. *Eur. J. Heart Fail.* 5, 793–801

65 Cosín, J. and Díez, J. (2002) Torasemide in chronic heart failure: results of the TORIC study. *Eur. J. Heart Fail.* 4, 507–513

66 Murray, M.D. *et al.* (2001) Open-label randomized trial of torsemide compared with furosemide therapy for patients with heart failure. *Am. J. Med.* 111, 513–520

67 Spannheimer, A. *et al.* (1998) Comparison of therapies with torasemide or furosemide in patients with congestive heart failure from a pharmacoeconomic viewpoint. *Int. J. Clin. Pract.* 52, 467–471

68 Kido, H. and Ohtaki, Y. (2001) *Nippon Yakurigaku Zasshi* 118, 97–105

69 Dunn, C.J. *et al.* (1995) Torasemide. An update of its pharmacological properties and therapeutic efficacy. *Drugs* 49, 121–142

70 Veeraveedu, P.T. *et al.* (2008) Comparative effects of torasemide and furosemide in rats with heart failure. *Biochem. Pharmacol.* 75, 649–659

71 Cooper, H.A. *et al.* (1999) Diuretics and risk of arrhythmic death in patients with left ventricular dysfunction. *Circulation* 100, 1311–1315

72 Domanski, M. *et al.* (2003) Diuretic use, progressive heart failure, and death in patients in the Studies Of Left Ventricular Dysfunction (SOLVD). *J. Am. Coll. Cardiol.* 42, 705–708

73 McCurley, J.M. *et al.* (2004) Furosemide and the progression of left ventricular dysfunction in experimental heart failure. *J. Am. Coll. Cardiol.* 44, 1301–1307

74 Klein, L. *et al.* (2005) Lower serum sodium is associated with increased short-term mortality in hospitalized patients with worsening heart failure: results from the Outcomes of a Prospective Trial of Intravenous Milrinone for Exacerbations of Chronic Heart Failure (OPTIME-CHF) study. *Circulation* 111, 2454–2460

75 Felker, G.M. *et al.* (2004) Risk stratification after hospitalization for decompensated heart failure. *J. Card. Fail.* 10, 460–466

76 Rossi, J. *et al.* (2007) Improvement in hyponatremia during hospitalization for worsening heart failure is associated with improved outcomes: insights from the Acute and Chronic Therapeutic Impact of a Vasopressin Antagonist in Chronic Heart Failure (ACTIV in CHF) trial. *Acute Card. Care* 9, 82–86

77 Sawyer, W.H. and Manning, M. (1973) Synthetic analogs of oxytocin and the vasopressins. *Annu. Rev. Pharmacol.* 13, 1–17

78 Sawyer, W.H. and Manning, M. (1984) The development of vasopressin antagonists. *Fed. Proc.* 43, 87–90

79 Greenberg, A. and Verbalis, J.G. (2006) Vasopressin receptor antagonists. *Kidney Int.* 69, 2124–2130

80 Creager, M.A. *et al.* (1986) Contribution of vasopressin to vasoconstriction in patients with congestive heart failure: comparison with the renin–angiotensin system and the sympathetic nervous system. *J. Am. Coll. Cardiol.* 7, 758–765

81 Goldsmith, S.R. and Gheorghiade, M. (2005) Vasopressin antagonism in heart failure. *J. Am. Coll. Cardiol.* 46, 1785–1791

82 Ohnishi, A. *et al.* (1993) Potent aquaretic agent. A novel nonpeptide selective vasopressin 2 antagonist (OPC-31260) in men. *J. Clin. Invest.* 92, 2653–2659

83 Risvanis, J. *et al.* (1999) *In vivo* and *in vitro* characterisation of a nonpeptide vasopressin V(1A) and V(2) receptor antagonist (YM087) in the rat. *Eur. J. Pharmacol.* 381, 23–30

84 Yatsu, T. *et al.* (1999) Cardiovascular and renal effects of conivaptan hydrochloride (YM087), a vasopressin V1A and V2 receptor antagonist, in dogs with pacing-induced congestive heart failure. *Eur. J. Pharmacol.* 376, 239–246

85 Yatsu, T. *et al.* (2002) Effect of conivaptan, a combined vasopressin V(1a) and V(2) receptor antagonist, on vasopressin-induced cardiac and haemodynamic changes in anaesthetised dogs. *Pharmacol. Res.* 46, 375–381

86 Wada, K. *et al.* (2002) Effect of the vasopressin receptor antagonist conivaptan in rats with heart failure following myocardial infarction. *Eur. J. Pharmacol.* 450, 169–177

87 Wada, K. *et al.* (2005) Intravenous administration of conivaptan hydrochloride improves cardiac hemodynamics in rats with myocardial infarction-induced congestive heart failure. *Eur. J. Pharmacol.* 507, 145–151

88 Naitoh, M. *et al.* (2002) Neurohormonal antagonism in heart failure; beneficial effects of vasopressin V(1a) and V(2) receptor blockade and ACE inhibition. *Cardiovasc. Res.* 54, 51–57

89 Yamamura, Y. *et al.* (1992) Characterization of a novel aquaretic agent, OPC-31260, as an orally effective, nonpeptide vasopressin V2 receptor antagonist. *Br. J. Pharmacol.* 105, 787–791

90 Wong, L.L. and Verbalis, J.G. (2001) Vasopressin V2 receptor antagonists. *Cardiovasc. Res.* 51, 391–402

91 Miyazaki, T. *et al.* (2007) Tolvaptan, an orally active vasopressin V(2)-receptor antagonist – pharmacology and clinical trials. *Cardiovasc. Drug Rev.* 25, 1–13

92 Hirano, T. *et al.* (2000) Effects of the V(2)-receptor antagonist OPC-41061 and the loop diuretic furosemide alone and in combination in rats. *J. Pharmacol. Exp. Ther.* 292, 288–294

93 Kodama, M. *et al.* (1990) A novel experimental model of giant cell myocarditis induced in rats by immunization with cardiac myosin fraction. *Clin. Immunol. Immunopathol.* 57, 250–262

94 Veeraveedu, P.T. *et al.* (2008) Effects of V2-receptor antagonist tolvaptan and the loop diuretic furosemide in rats with heart failure. *Biochem. Pharmacol.* 75, 1322–1330

95 Veeraveedu, P.T. *et al.* (2007) Effects of nonpeptide vasopressin V2 antagonist tolvaptan in rats with heart failure. *Biochem. Pharmacol.* 74, 1466–1475

96 Onogawa, T. *et al.* (2004) Effects of tolvaptan (OPC-41061), a vasopressin V2 receptor antagonist, and furosemide on hemodynamics, renal functions, serum

electrolytes, and neurohormones in dogs with and without heart failure. *Circulation* 110 (Suppl. III), 21
97 Costello-Boerrigter, L.C. *et al.* (2010) Renal and anti-aldosterone actions of vasopressin-2 receptor antagonism and B-type natriuretic peptide in experimental heart failure. *Circ. Heart Fail.* 3, 412–419
98 Miyazaki, T. *et al.* (2005) Therapeutic effects of tolvaptan, a potent, selective nonpeptide vasopressin V2 receptor antagonist, in rats with acute and chronic severe hyponatremia. *Endocrinology* 146, 3037–3043
99 Fujita, N. *et al.* (1995) Role of water channel AQP-CD in water retention in SIADH and cirrhotic rats. *Am. J. Physiol.* 269, F926–F931
100 Tsuboi, Y. *et al.* (1994) Therapeutic efficacy of the non-peptide AVP antagonist OPC-31260 in cirrhotic rats. *Kidney Int.* 46, 237–244
101 Ros, J. *et al.* (2005) Sustained aquaretic effect of the V2-AVP receptor antagonist, RWJ-351647, in cirrhotic rats with ascites and water retention. *Br. J. Pharmacol.* 146, 654–661
102 Fernández-Varo, G. *et al.* (2003) Effect of the V1a/V2-AVP receptor antagonist, Conivaptan, on renal water metabolism and systemic hemodynamics in rats with cirrhosis and ascites. *J. Hepatol.* 38, 755–761
103 Jiménez, W. *et al.* (2000) Long-term aquaretic efficacy of a selective nonpeptide V(2)-vasopressin receptor antagonist, SR121463, in cirrhotic rats. *J. Pharmacol. Exp. Ther.* 295, 83–90
104 Fleeman, L.M. *et al.* (2000) Effects of an oral vasopressin receptor antagonist (OPC-31260) in a dog with syndrome of inappropriate secretion of antidiuretic hormone. *Aust. Vet. J.* 78, 825–830
105 Wada, K. *et al.* (2007) A novel vasopressin dual V1A/V2 receptor antagonist, conivaptan hydrochloride, improves hyponatremia in rats with syndrome of inappropriate secretion of antidiuretic hormone (SIADH). *Biol. Pharm. Bull.* 30, 91–95
106 Ishikawa, S. and Schrier, R.W. (1982) Effect of arginine vasopressin antagonist on renal water excretion in glucocorticoid and mineralocorticoid deficient rats. *Kidney Int.* 22, 587–593
107 Saito, T. *et al.* (2000) Vasopressin-dependent upregulation of aquaporin-2 gene expression in glucocorticoid-deficient rats. *Am. J. Physiol. Renal Physiol.* 279, F502–F508
108 Wang, W. *et al.* (2006) Molecular analysis of impaired urinary diluting capacity in glucocorticoid deficiency. *Am. J. Physiol. Renal Physiol.* 290, F1135–F1142
109 Gattone, V.H., 2nd *et al.* (1999) Developmental expression of urine concentration-associated genes and their altered expression in murine infantile-type polycystic kidney disease. *Dev. Genet.* 24, 309–318
110 Torres, V.E. *et al.* (2004) Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. *Nat. Med.* 10, 363–364
111 Torres, V.E. (2008) Vasopressin antagonists in polycystic kidney disease. *Semin. Nephrol.* 28, 306–317
112 Wang, X. *et al.* (2005) Effectiveness of vasopressin V2 receptor antagonists OPC-31260 and OPC-41061 on polycystic kidney disease development in the PCK rat. *J. Am. Soc. Nephrol.* 16, 846–851
113 Yamaguchi, T. *et al.* (2004) Calcium restriction allows cAMP activation of the B-Raf/ERK pathway, switching cells to a cAMP-dependent growth-stimulated phenotype. *J. Biol. Chem.* 279, 40419–40430
114 Nagao, S. *et al.* (2006) Increased water intake decreases progression of polycystic kidney disease in the PCK rat. *J. Am. Soc. Nephrol.* 17, 2220–2227
115 Ghali, J.K. *et al.* (2006) Efficacy and safety of oral conivaptan: a V1A/V2 vasopressin receptor antagonist, assessed in a randomized, placebo-controlled trial in patients with euvolemic or hypervolemic hyponatremia. *J. Clin. Endocrinol. Metab.* 91, 2145–2152
116 Zeltser, D. *et al.* (2007) Assessment of the efficacy and safety of intravenous conivaptan in euvolemic and hypervolemic hyponatremia. *Am. J. Nephrol.* 27, 447–457
117 Schrier, R.W. *et al.* (2006) Tolvaptan, a selective oral vasopressin V2-receptor antagonist, for hyponatremia. *N. Engl. J. Med.* 355, 2099–2112
118 Gheorghiade, M. *et al.* (2006) Vasopressin v(2) receptor blockade with tolvaptan versus fluid restriction in the treatment of hyponatremia. *Am. J. Cardiol.* 97, 1064–1067
119 Berl, T. *et al.* (2010) Oral tolvaptan is safe and effective in chronic hyponatremia. *J. Am. Soc. Nephrol.* 21, 705–712
120 Verbalis, J. *et al.* (2005) Novel vasopressin V1a and V2 antagonist (conivaptan) increases serum sodium concentration in patients with hyponatremia secondary to congestive heart failure. *J. Card. Fail.* 11 (Suppl. 6), S120
121 Russell, S.D. *et al.* (2003) Rationale for use of an exercise end point and design for the ADVANCE (A Dose evaluation of a Vasopressin ANtagonist in CHF patients undergoing Exercise) trial. *Am. Heart J.* 145, 179–186
122 Goldsmith, S.R. *et al.* (2008) Efficacy and safety of the vasopressin V1A/V2-receptor antagonist conivaptan in acute decompensated heart failure: a dose-ranging pilot study. *J. Card. Fail.* 14, 641–647
123 Tonkon, M. *et al.* (2001) Interaction between furosemide and oral conivaptan (YM087) in patients with heart failure. *Eur. Heart J.* 22, 541
124 Gheorghiade, M. *et al.* (2003) Vasopressin V2-receptor blockade with tolvaptan in patients with chronic heart failure: results from a double-blind, randomized trial. *Circulation* 107, 2690–2696
125 Gheorghiade, M. *et al.* (2004) Effects of tolvaptan, a vasopressin antagonist, in patients hospitalized with worsening heart failure: a randomized controlled trial. *J. Am. Med. Assoc.* 291, 1963–1971
126 Gheorghiade, M. *et al.* (2007) Short-term clinical effects of tolvaptan, an oral vasopressin antagonist, in patients hospitalized for heart failure: the EVEREST Clinical Status Trials. *J. Am. Med. Assoc.* 297, 1332–1343
127 Konstam, M.A. *et al.* (2007) Effects of oral tolvaptan in patients hospitalized for worsening heart failure: the EVEREST Outcome Trial. *J. Am. Med. Assoc.* 297, 1319–1331
128 Gheorghiade, M. *et al.* (2005) Rationale and design of the multicenter, randomized, double-blind, placebo-controlled study to evaluate the Efficacy of Vasopressin antagonism in Heart Failure: Outcome Study with Tolvaptan (EVEREST). *J. Card. Fail.* 11, 260–269
129 Udelson, J.E. *et al.* (2001) Acute hemodynamic effects of conivaptan, a dual V(1A) and V(2) vasopressin receptor antagonist, in patients with advanced heart failure. *Circulation* 104, 2417–2423
130 Udelson, J.E. *et al.* (2008) Acute hemodynamic effects of tolvaptan, a vasopressin V2 receptor blocker, in patients with symptomatic heart failure and systolic dysfunction: an international, multicenter, randomized, placebo-controlled trial. *J. Am. Coll. Cardiol.* 52, 1540–1545
131 Udelson, J.E. *et al.* (2007) Multicenter, randomized, double-blind, placebo-controlled study on the effect of oral tolvaptan on left ventricular dilation and function in patients with heart failure and systolic dysfunction. *J. Am. Coll. Cardiol.* 49, 2151–2159
132 Costello-Boerrigter, L.C. *et al.* (2006) Vasopressin-2-receptor antagonism augments water excretion without changes in renal hemodynamics or sodium and potassium excretion in human heart failure. *Am. J. Physiol. Renal Physiol.* 290, F273–F278
133 Udelson, J.E. *et al.* (2007) Acute hemodynamic effects of tolvaptan, a Vasopressin V2 receptor blocker, in patients with symptomatic heart failure and systolic dysfunction: the ECLIPSE international, multicenter, randomized, placebo-controlled trial. *J. Card. Fail.* 13, 793–794
134 ''TEMPO 3/4 Trial'' Tolvaptan Efficacy and Safety in Management of Polycystic Kidney Disease and Its Outcomes (TEMPO3/4) (http://clinicaltrials.gov/ct2/show/NCT00428948). (accessed 27 October 2009)
135 Hunt, S.A. (2005) American College of Cardiology; American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). American College of Cardiology Website. *J. Am. Coll. Cardiol.* 46, e1–e82
136 McMurray, J.J. *et al.* (2007) Effects of tezosentan on symptoms and clinical outcomes in patients with acute heart failure: the VERITAS randomized controlled trials. *J. Am. Med. Assoc.* 298, 2009–2019
137 Mann, D.L. *et al.* (2004) Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). *Circulation* 109, 1594–1602
138 CardioKine Inc. THE BALANCE study: treatment of hyponatremia based on lixivaptan in NYHA class III/IV cardiac patient evaluation (http://www.clinicaltrials.gov/ct2/show/NCT00578695) (accessed 27 October 2009)