

Vaptans and the Treatment of Water-Retaining Disorders

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Hyponatremia is a frequent and symptomatic electrolyte disorder for which specific treatments have been lacking. Hyponatremia is attributable to nonosmotic vasopressin stimulation and continued increased fluid intake. In the past, peptidic derivatives of arginine vasopressin proved that blockade of vasopressin V-2 receptors served to improve hyponatremia, however, these antagonists had intrinsic agonistic activity, too. In the past decade, random screening of molecules uncovered nonpeptide, orally available vasopressin antagonists without agonistic properties. The agents show competitive binding to the vasopressin V-2 receptor at an affinity comparable with that of arginine vasopressin. Four antagonists have undergone extensive study. Three of these agents—lixivaptan or VPA 985; SR 121 463 B; tolvaptan or OPC 41,061—are specific V-2 antagonists whereas conivaptan or YM 087 is a V-1/V-2 mixed antagonist. In animal and clinical studies all of the agents were able to correct water retention and hyponatremia in a dose-dependent manner. There was no tachyphylaxis, even when the agents were given over many weeks. It is expected that the clinical use of the agents will lead to a major improvement in the treatment of hyponatremia.

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Hyponatremia is a water-retaining disorder that is defined by the presence of a plasma sodium concentration of 136 mmol/L or less. Hyponatremia is by no means rare. An older study¹ reported an incidence of approximately 6% for hospital patients in 1987; however, under the circumstances of present-day medical practice the incidence now may be higher than before in a comparable setting.² Hoorn et al² recently conducted a prospective cohort study in 2,907 consecutive patients admitted to their tertiary care center; they reported a surprising incidence of general hyponatremia of 30%, whereas that of more progressive hyponatremia ($P_{Na} \leq 125$ mmol/L) was 3%.² Hyponatremia is also not infrequent

in outpatients, especially when older patients are considered.³

The relevance of hyponatremia is related to the symptoms it may cause. It was believed previously that chronic mild hyponatremia was an asymptomatic disorder or one that caused lethargy and lack of concentration at best. However, more specific psychomotor testing comparing the same patients in chronic mild hyponatremia of 128 mmol/L and in subsequent normonatremia now has documented impairments of cognition, postural ability, and gait function in chronic mild hyponatremia.⁴ In contrast, acute severe hyponatremia (<48 hours' duration; $P_{Na} \leq 115$ mmol/L) almost always will be followed by major symptoms such as grand mal seizures, coma, permanent brain damage, and even death.⁵ These symptoms are explained at least in part by cerebral edema.

Given the incidence of hyponatremia as it occurs at present and the consequences of it in terms of symptoms and findings, there is a need for effective and specific modalities of treatment. For many years no such treatments had been forthcoming. This appears to be changing (Fig 1). The new approaches are best understood on the basis of the pathophysiology of hyponatremia.

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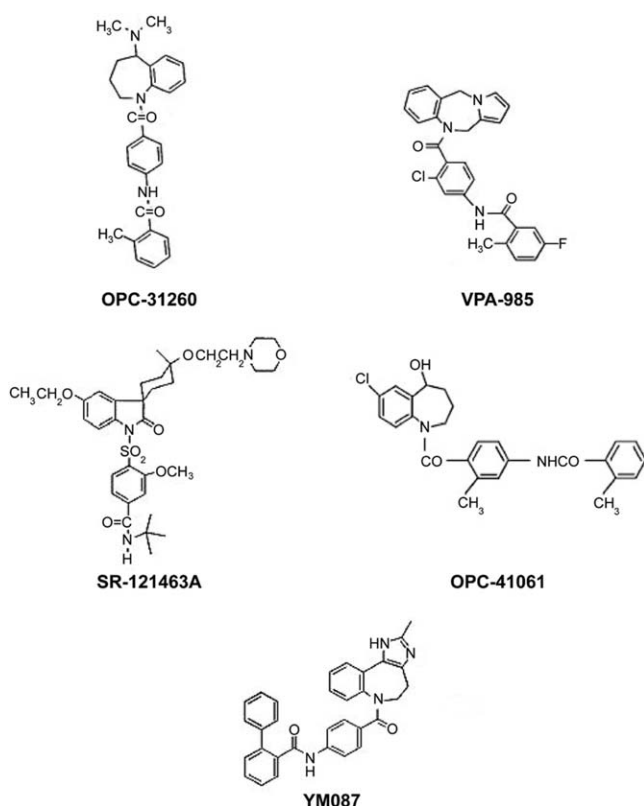


Figure 1 Structure of nonpeptide orally available vasopressin V-2 or V-1/V-2 mixed-receptor (YM 087) antagonists in clinical development.

Role of Vasopressin in Hyponatremia

Long before the advent of precise methods for the measurement of vasopressin in plasma,⁶ antidiuretic hormone strongly was suspected for being instrumental in hyponatremia.⁷ Leaf and Mamby⁷ gave a standard oral water load to hyponatremic patients. They noted a failure of the urinary osmolality and the urinary volume to change in response to the water load whereas hyponatremia worsened. The investigators used a bioassay to detect antidiuretic hormone; they found an increase of it that remained unchanged by the water load.⁷ A comparable set of changes was seen when an oral water load was applied in conjunction with a parenteral dose of vasopressin in human beings.⁸ Later, similar changes were reproduced in normal laboratory animals.^{9,10} Infusions of hypotonic fluid or of antidiuretic hormone alone did not change the plasma sodium concentration.⁹ However, infusion of both components together was followed by stable hyponatremia and this occurred in a dose-dependent fashion.⁹ A negative sodium balance was not required for hyponatremia.⁹ When animal models devoid of endogenous vasopressin were tested such as hypophysectomized animals or the Brattleboro rat, maneuvers ordinarily resulting in water retention and hyponatremia ceased to do so.^{11,12} After the introduction of the radioimmunoassay for the measurement of vasopressin in 1973,⁶ studies were performed to determine

the prevailing levels of vasopressin in animal models of hyponatremia. Such studies have been reported in hyponatremic states of congestive cardiac failure,¹³ cirrhosis,¹² glucocorticoid deficiency,¹⁴ and mineralocorticoid deficiency.¹⁵ Collectively they showed measurable (ie, stimulated) antidiuretic hormone (ADH) concentrations despite the hyponatremia-associated hypo-osmolality. Similar measurements also have been obtained in patients with hyponatremia (eg, in cardiac failure,¹⁶ cirrhosis,¹⁷ nephrotic syndrome,¹⁸ hypothyroidism,¹⁹ syndrome of inappropriate antidiuretic hormone [SIADH], and other conditions). In virtually all of these hyponatremic states stimulated vasopressin was documented. The causes of the failure of hypo-osmolality to suppress antidiuretic hormone in hyponatremia have been addressed and this was performed in edematous conditions and in extracellular volume depletion. Summarizing the evidence, baroreceptors in the arterial circulation sensing low cardiac output in heart failure and extracellular volume depletion or low peripheral vascular resistance in cirrhosis are held responsible for overriding osmotic suppression of ADH by hyponatremia.²⁰ Taken together there is overwhelming evidence for a pivotal although not exclusive role of nonosmotically stimulated vasopressin in water-retaining disorders resulting in hyponatremia.

Standard Recommendations for the Treatment of Hyponatremia

The role of vasopressin in hyponatremia suggests that an effective treatment of the electrolyte disorder should result if it were possible to inhibit vasopressin release from the pituitary or its effect in the collecting duct of the nephron. The first suggestion—inhibition of vasopressin release—is an option in laboratory animals²¹ but has no role in the treatment of hyponatremic patients. The second concept was conceived years ago and analogs of the arginine vasopressin molecule with antagonistic properties were developed.²² These agents proved the concept and turned out to be very useful in conducting research studies.²³ However, their peptidic nature, the requirement for their parenteral delivery, and their associated agonistic properties precluded any clinical use in the treatment of hyponatremia. Antagonists in rats were found to be agonists in human beings. This later was found to be caused by different molecular structures of the receptors that are responsible for different affinities for agonists and antagonists in human and rat species.²⁴

Consequently, in the absence of specific treatments, surrogate approaches have been used in the past. To counteract pathologic water accumulation a standard recommendation is to impose a water restriction of 0.8 L/d or less. This measure will be effective if the patient is able to adhere to it.²⁵ Water restriction is slow to work. It also is difficult to sustain at least in part because of an inherent thirstiness of hyponatremic patients.¹ The possibility of removing excess water in hyponatremia by pharmacologic induction of a state of nephrogenic diabetes insipidus also was considered. This is

Table 1 Overview of Selected Properties of Nonpeptide Orally Available Competitive Vasopressin-Receptor Antagonists

Compound	OPC-31260	SR-121463A	VPA-985	OPC-41061	YM-087
Generic name			Lixivaptan	Tolvaptan	Conivaptan
Route of application	Oral	Oral	Oral	Oral	Oral
Derivative of	Pyrazino-benzodiazepine	Benzamide	Benzodiazepine	Benzazepine	Benzazepine
K _i , in rats (nmol/L) (V-2 receptor)	21.7*	1.42*	0.48†	1.33‡	3.04–V2R§
K _i , in human beings (nmol/L) (V-2 receptor)	25.4	4.1	0.60	0.43	Not determined
Selectivity index (K _i V1a/V2)	10	112	Not determined	Not determined	0.15
Urine volume	↑	↑	↑	↑	↑
Urine osmolality	↓	↓	↓	↓	↓
Na ⁺ - excretion/24 h	Unchanged	Unchanged	Unchanged at LD (↑ at HD)	Unchanged at LD (↑ at HD)	Unchanged
K ⁺ - excretion/24 h	Unchanged	Unchanged	Unchanged at LD (↑ at HD)	Not determined	Unchanged
Serum (Na ⁺) response	↑	↑	↑	↑	↑
Agonistic activity	None	None	None	None	None
Clinical trials conducted	Yes	No	Yes	Yes	Yes

*Data from Ghali et al.⁴⁷†Data from Saito et al.⁴⁰‡Data from Soupart et al.⁴⁹§Data from Gerbes et al.⁴³

achievable in principle by agents such as demeclocycline hydrochloride²⁵ or lithium carbonate,²⁶ yet both agents proved to yield unpredictable enhancement of renal water clearance and they were fraught with adverse effects. Yet another suggestion that was made for the hyponatremia of SIADH holds that urea in the form of capsules is therapeutic.²⁷ Urea if given in sufficient quantities induces osmotic diuresis and water excretion.²⁷ However, urea capsules have not become widely popular. In the treatment of SIADH, an elegant therapeutic maneuver consists of prescribing a loop diuretic at a high dose and replacing any sodium lost in the urine by quantitative infusion of hypertonic saline.²⁸ This measure keeps sodium balance even. Because loop diuretics are very effective in SIADH and induce a high-volume low-sodium diuresis, this option is helpful and frequently used; however, it is cumbersome. It requires frequent urinary measurements and the calculation of volumes and sodium concentrations in the infusate. Miscalculations are inevitable. Taken together the present modes of therapy of hyponatremia are nonspecific, ineffective, and laborious. It is expected that novel treatments (Fig 1; Table 1) will antagonize the renal V-2 vasopressin receptor specifically, thereby addressing and resolving these issues.

The Renal Vasopressin V-2 Receptor

Vasopressin has been known to possess antidiuretic and vasopressor properties.²⁹ Initial studies showed that cyclic adenosine monophosphate (cAMP) was the second messenger of vasopressin action in kidney tubules and amphibian bladder. In contrast, in hepatocytes and vascular smooth

muscle cells vasopressin brought about an increase in the cytosolic concentration of Ca²⁺ and the breakdown of phosphatidylinositol.²⁹ Accordingly, it was proposed that the 2 receptors be named as previously established for β-receptors: V-2 vasopressin receptor for the cAMP-related renal collecting duct form, and V-1 vasopressin receptor for its Ca²⁺-related hepatic and smooth muscle cell counterpart.³⁰

The renal vasopressin V-2 receptor is expressed on the basolateral membrane of collecting duct principal cells. Vasopressin target cells usually contain large numbers of spare receptors.²⁹ The binding of vasopressin to the V-2 receptor initiates the coupling of the cholera-toxin-sensitive G-protein G_s to the receptor, activation of adenylate cyclase, production of cAMP, and activation of protein kinase A. The latter promotes an insertion of preformed subapical aquaporin-2 water channels into the apical cell membrane. They function as selective water-conducting channels according to the prevailing osmotic gradient from the tubular lumen to the medullary interstitium.³¹ Agonist binding to the receptor also initiates an enhanced synthesis of aquaporin-2 messenger RNA and protein.³² Occupancy of the receptor by vasopressin promotes receptor phosphorylation by G-protein-coupled receptor kinases and subsequent internalization.³³ A second mechanism of signal termination probably is related to extracellular cleavage of the agonist/V-2-receptor complex by a cellular metalloproteinase ectoenzyme.³⁴

Research involving molecular biology has elucidated vasopressin V-2 receptor structure. Cloning the complementary DNA and sequencing and expression of the protein have been accomplished.³⁵ They showed the V-2 receptor to be a typical G-protein-coupled receptor of 7 hydrophobic transmembrane domains (Fig 2). Studies of mutagenesis of the

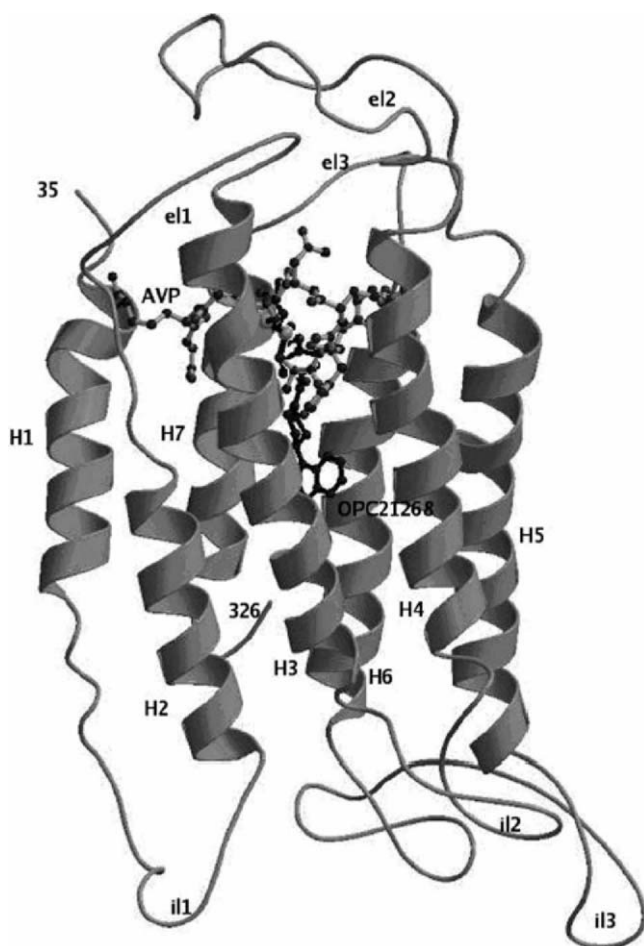


Figure 2 The structure of oral vasopressin antagonists imitates the structure of the native hormone arginine vasopressin (AVP) and these antagonists interfere with the binding pocket of AVP.⁵² Superposition of the models of AVP and OPC 21,268, a V1a antagonist, in ball-and-stick representation, as docked onto the V2R model shown as a ribbon diagram. The loops are labeled e1, e2, and e3 for the extracellular loops, and i1, i2, and i3 for the intracellular loops. The binding sites for agonist and antagonist are distinct with partial overlap. The figure was generated with the programs MolScript⁵³ and Raster3D⁵⁴ and reproduced with permission from Shoham et al.⁵⁵

receptor delineated some of its functional domains. It was observed that the first and second extracellular loops of the receptor protein are involved in ligand binding, whereas the third intracellular loop is a mediator of G-protein coupling.³⁵ Mutations of the human V-2 vasopressin receptor gene cause X-chromosome-linked congenital diabetes insipidus. Numerous mutations of the gene have been identified.³⁶ The signal transduction pathways of the vasopressin V-1 receptor and of the V-3 receptor (also called *V-1b receptor*), which involve Ca^{2+} and phosphatidylinositol in both cases, also have been described.³¹

Random screening for new chemical compounds recently has yielded a number of nonpeptide molecules that antagonize the V-2 receptor at a high degree of affinity. The new agents are available orally and have been called *vaptans* (Fig 1, Table 1).

OPC 31,260

In 1992, Yamamura et al.³⁷ reported a new compound with aquaretic properties found by random screening. The agent, called OPC 31,260, was a benzazepine derivative with a chemical name of [5-dimethylamino-1-(4-[2-methyl benzoylamino] benzoyl)-2,3,4,5-tetrahydro-1H-benzazepine]. In preparations of rat liver (V-1-receptor containing) and kidney (V-2-receptor containing) cell membranes, OPC 31,260 caused competitive displacement of [³H]-AVP from V-2 receptors at an inhibitory concentration 50 (IC_{50}) of $1.4 \pm 0.2 \times 10^{-8}$ mol/L, whereas the affinity of OPC 31,260 to the V-1 receptor was approximately 2 orders of magnitude lower than that to the V-2 receptor. In other words, OPC 31,260 was relatively specific for the V-2 receptor. OPC 31,260, when infused into water-loaded anesthetized rats in doses ranging from 10 to 100 $\mu\text{g}/\text{kg}$, inhibited the antidiuretic effect of exogenously administered AVP, but it failed to show any intrinsic agonistic (antidiuretic) property. Oral administration also was effective and doses of 1 to 30 mg/kg of OPC 31,260 given to normal water-loaded rats dose-dependently increased urine flow several-fold, whereas urinary osmolality decreased by more than 75% for more than 6 hours. Compared with furosemide, OPC 31,260 decreased urinary osmolality more and increased urinary sodium excretion rates less when doses were titrated to yield comparable urinary volumes.³⁷ Hence the term *aquaretic* for OPC 31,260.

When the agent was tested *in vivo*, where it was used in the treatment of experimental SIADH in rats showing plasma sodium concentrations of 119 mmol/L, it promptly corrected the serum sodium level to 134 mmol/L in 12 hours at a dose of 5 mg/kg orally.³⁸ The aquaretic effect in the experiment consisted of an increase of the daily urinary volume from 9.6 mL to 28 mL, whereas urinary osmolality decreased from 1,800 to 500 mOsm/kg in response to OPC 31,260.³⁸ However, the urinary sodium concentration rate did not change. The effect of OPC 31,260 also was studied in water-retaining rats with cirrhosis³⁹ induced by carbon tetrachloride. After oral administration of OPC 31,260 at a dose of 5 mg/kg the previously observed impaired excretion of an oral water load no longer was present.³⁹ Instead the cirrhotic rats now excreted more of an oral water load and at a lower urinary osmolality than did controls.³⁹ In normal hydrated human beings an intravenous dose of OPC 31,260 of up to 1.0 mg/kg promoted the formation of hypotonic urine for 4 hours, yielded a positive free-water clearance, and left the Na^+ excretion rate unchanged.⁴⁰ Plasma osmolality, plasma sodium concentration, and plasma ADH all increased in response to OPC 31,260, but blood pressure, heart rate, and plasma potassium concentration remained unaltered.⁴⁰ Although OPC 31,260 was never released for general use in the treatment of hyponatremia, it was the first orally available specific V-2 vasopressin antagonist and as such it had paradigmatic importance.

VPA 985, Lixivaptan

In 1998, a report of an orally active V-2 receptor antagonist was published by Chan et al.⁴¹ It was called VPA 985 or

lixivaptan. Its chemical descriptive term is 5-fluoro-2-methyl-N-[4-(5H-pyrrolo [2,1-c] [1,4]benzodiazepine-10 [11 H]-yl carbonyl)-3 chlorophenyl] benzamide. The preclinical pharmacology showed VPA 985 to inhibit the binding of AVP to V-2 receptors at a high degree of affinity.⁴¹ In V-2-receptor preparations from rat and dog renal medullary tissue K_i values of 0.48 ± 0.03 nmol/L and 0.82 ± 0.03 nmol/L for the VPA 985-related inhibition of agonist binding were observed. The level of inhibition of the human V-2 receptor by VPA 985 also was investigated. Studies of membranes obtained from cultured murine fibroblasts expressing the human V-2 receptor yielded a value of the inhibitor constant K_i value of 0.60 ± 0.02 nmol/L, comparable with the data in dogs and rats. In Scatchard analysis of AVP binding to cloned human V-2 receptors the presence of VPA 985 caused the dissociation constant K_d to change from 0.79 ± 0.03 nmol/L to 1.04 ± 0.04 nmol/L, whereas the maximum binding capacity (B_{max}) remained unaltered. These data suggested that VPA 985 was a competitive inhibitor of AVP binding to V-2 receptors.

Generation of the second messenger cAMP also was investigated. In murine fibroblasts expressing the human V-2 receptor AVP stimulated cAMP production. The maximal effect occurred at 1 nmol/L concentration of AVP and the EC_{50} was measured at 53.3 ± 4.4 pmol/L. Addition of VPA 985 had no effect on baseline cAMP generation; however, the IC_{50} was 6 ± 1 nmol/L. In membranes prepared from rat kidney medulla VPA 985 completely inhibited the increase in cAMP that normally is induced in this preparation by $0.05 \mu\text{mol/L}$ AVP.

The binding of VPA 985 to V-1 receptors was investigated. In membrane preparations obtained from rat hepatocytes and human platelets K_i values of 82 ± 15 nmol/L and 5.5 ± 12 nmol/L were measured. These K_i values were approximately 2 orders of magnitude higher than those described previously for V-2 receptors. These results showed that VPA 985 is approximately 100-fold more selective for the V-2 receptor as compared with the V-1 receptor. The absence of an effect of VPA 985 on basal cAMP generation indicated that the agent had no partial agonistic activity of its own.

The effects of VPA 985 in vivo in laboratory animals were studied. The agent was given to conscious AVP-pretreated ($0.4 \mu\text{g/kg}$ intraperitoneally) and water-loaded (30 mL/kg orally) rats in doses of 1, 3, and 10 mg/kg orally. This treatment increased volumes of excreted urine over those of AVP-treated controls by 187%, 529%, and 667%.⁴¹ In the same experiment, urinary osmolality decreased from 1,307 mOsm/kg in AVP-treated controls to 629, 366, and 302 mOsm/kg in the groups receiving the 3 doses of VPA 985 in addition to AVP. In a comparison study of the same type the V-2 antagonist OPC 31,260 was given instead of VPA 985 using identical doses (VPA 985 compared with OPC 31260). In this experiment the urinary volumes increased by 156%, 264%, and 447% whereas urinary osmolality decreased from 1,252 mOsm/kg to 857, 517, and 418 mOsm/kg. The findings showed that VPA 985 was more potent than OPC 31260 on a per weight basis. Similar observations also were made in conscious water-loaded dogs that had received pretreatment

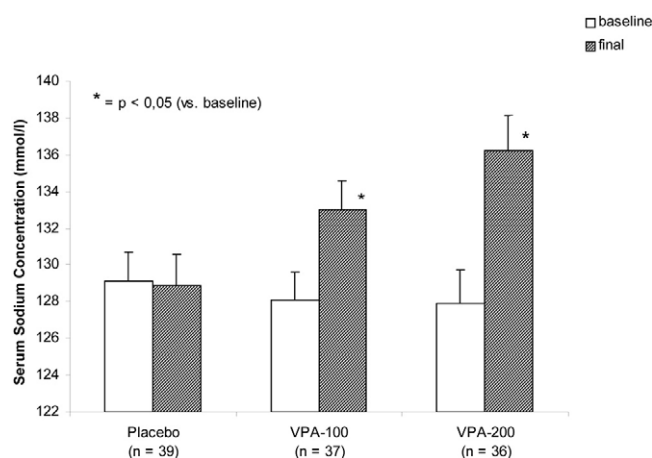


Figure 3 Increase of the serum sodium concentration in lixivaptan (VPA 985)-treated patients versus placebo controls. VPA-100 indicates that this group of patients received lixivaptan 100 mg/d in 2 divided doses. The final day of study usually was day 7 of protocol. * $P < .05$ versus baseline. □, Baseline; ▨, final.

by AVP. Finally, VPA 985 was given to homozygous Brattleboro rats with hereditary central diabetes insipidus. The dose was 10 mg/kg orally and VPA 985 was given twice daily over 5 days. In this important experiment there was no evidence of agonist effects of VPA 985; instead persistence of antagonistic activity was shown. In contrast, when an older peptidic antagonist called SK&F 101 926 was used in the same experimental set-up both agonistic and antagonistic properties were shown.⁴¹ All of these observations laid the ground for clinical studies involving VPA 985 in hyponatremia.

The antagonist was the first one to undergo extensive clinical trials. Observations were made in 112 patients with hyponatremia between 115 and 132 mmol/L in a prospective, randomized, double-blind, placebo-controlled study.⁴² Eligibility required that patients were characterized by hyponatremia related to cirrhosis, congestive cardiac failure, or SIADH.⁴³ Hyponatremia in the context of moderately severe renal insufficiency was excluded, as was that occurring in volume-contracted states, severe hyperglycemia, hypothyroidism, and adrenal insufficiency.⁴² Patients with severe pulmonary dysfunction, those with cardiac failure in New York Heart Association class IV, and cirrhotic patients in Child-Pugh stage C likewise were excluded. Study patients received VPA 985 orally in single doses of 50 mg or 100 mg twice per day, or placebo for a maximum of 7 days or until normalization of serum sodium level had been accomplished. Study patients were allowed a daily fluid intake of 1,000 mL including liquids contained in food and medications. VPA 985 increased the serum sodium concentration (Fig 3). The agent normalized the serum sodium level in 35% of patients receiving the low dose (50 mg twice per day) and in 53% of patients receiving the high dose (100 mg twice per day) of VPA 985. In placebo controls only 13% of hyponatremic patients normalized their serum sodium level. In fact, the mean serum sodium concentration of placebo controls decreased in the study (Fig 3). These changes were associated with a commensurate increase of serum osmolality in both

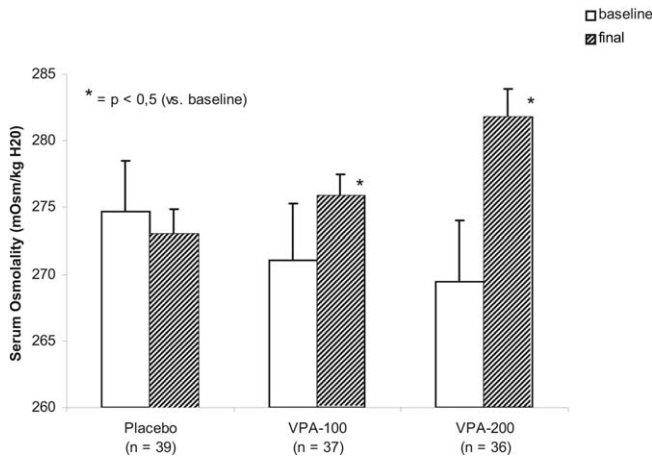


Figure 4 Increase of the serum osmolality in lixivaptan (VPA 985)-treated patients versus placebo controls. **P* < .05 versus baseline. □, Baseline; ▨, final.

groups receiving VPA 985, but serum osmolality decreased in placebo controls (Fig 4). Patients responding to treatment by VPA 985 showed evidence of a response within the first 72 hours after the beginning of treatment. Patients with SIADH responded significantly better than those with cirrhosis in terms of their improvement of hyponatremia to a given dose of VPA 985 (Fig 5). The antagonist caused a brisk increase of the daily urinary volumes; on day 1 of treatment patients receiving the high dose of VPA 985 (100 mg twice per day) excreted 3.1 ± 0.4 L of urine versus 1.1 ± 0.25 L in controls (Fig 6). Although the increased urine flow in treated patients lessened over the next 5 days, it remained significantly increased over that of placebo control throughout (Fig 6). VPA 985 decreased the urinary osmolality from 420 ± 50 mOsm/kg at baseline to 190 ± 36 mOsm/kg on day 1 (high dose of 100 mg twice per day) and urinary dilution was maintained thereafter at the low level until the end of the study (Fig 7). Free-water clearance, which had been negative

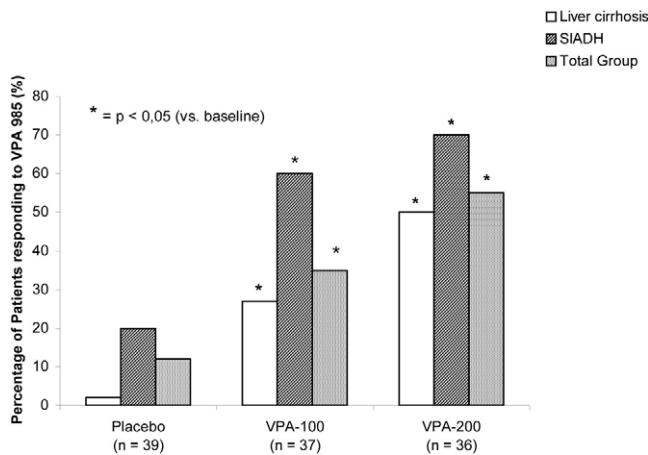


Figure 5 Percentage of patients responding to lixivaptan (VPA 985). The overall response rate in patients with SIADH was significantly higher than in patients with cirrhosis. **P* < .05 versus baseline. □, Cirrhosis; ▨, baseline; ▩, total group.

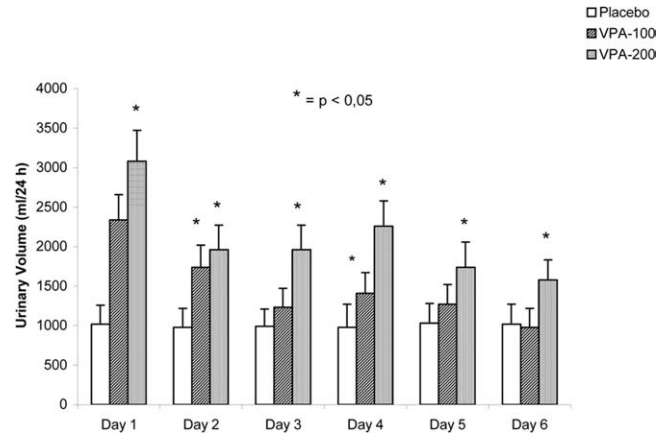


Figure 6 Daily urinary volumes excreted in patients receiving lixivaptan (VPA 985) in 2 doses (50 mg twice per day or 100 mg twice per day) and placebo controls. The *P* value was calculated by comparison with the corresponding data for placebo controls on the same day. **P* < .05. □, Placebo; ▨, VPA-100; ▩, VPA-200.

at baseline, remained negative in placebo controls but turned positive in both groups receiving VPA 985. There was also a dose-related increase of the plasma vasopressin concentration in VPA 985-treated groups and vasopressin approximately tripled in response to high-dose VPA 985 (baseline, 1.55 ± 0.2 pg/mL; end of study, 5.4 ± 0.5 pg/mL). Weights decreased by 1.1 ± 0.5 kg in the high-dose treatment group. Thirst sensation increased after VPA 985.⁴³ In terms of drug safety there was no difference in reported adverse events between the 2 VPA 985 groups and placebo controls. Vital signs (supine blood pressure and pulse rate) remained unaltered. Taken together it was concluded that VPA 985 is able to correct hyponatremia in the circumstances studied and that it is safe.

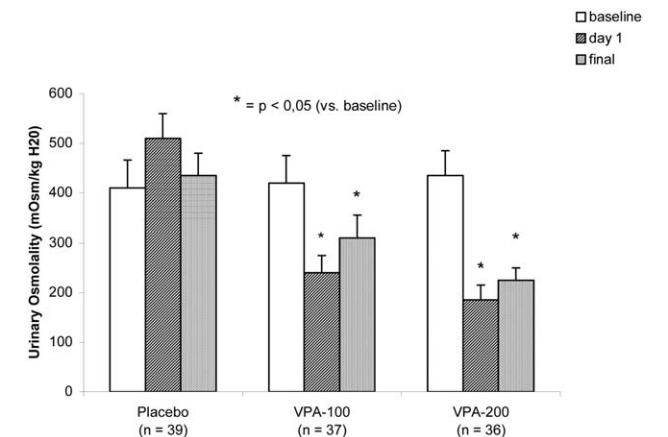


Figure 7 Urinary osmolality in patients receiving lixivaptan (VPA 985) in 2 doses (50 mg twice per day or 100 mg twice per day) and placebo controls. Measurements were performed at baseline before treatment with lixivaptan, on day 1 of treatment, and on the final day of treatment, usually day 7 of protocol. **P* < .05 versus baseline. □, Baseline; ▨, day 1; ▩, final.

YM 087, Conivaptan

Described in 1997, conivaptan is the first V-1 (V_{1a})/V-2 combined vasopressin-receptor antagonist.⁴⁴ It is an orally available nonpeptide agent described chemically as N-[4-[1, 4, 5, 6-tetrahydro-2-methyl-6-imidazo [4,5-d][1] benzazepinyl carbonyl] phenyl][1, 1'-biphenyl]-2-carboxamide monohydrochloride. Its preclinical pharmacology has been studied.⁴⁵ Inhibition by YM 087 of vasopressin binding was tested. The concentration of YM 087 that reduced specific vasopressin binding 50% (IC_{50}) was 2.2 ± 0.1 nmol/L in rat liver V-1 (V_{1a}) receptors and 0.4 ± 0.1 nmol/L in rat kidney V-2-receptor preparations. Thus, YM 087 binds to V-1 (V_{1a}) and V-2 receptors with an affinity resembling that of the natural ligand, arginine vasopressin. Scatchard plots showed a concentration-dependent increase of the apparent K_d of liver V-1 (V_{1a}) receptor binding sites in response to YM 087 but no effect on B_{max} . Comparable observations also were made for the K_d and B_{max} in kidney V-2-receptor binding sites. These findings indicate that YM 087 is a competitive inhibitor at the V-1 (V_{1a}) and the V-2 receptor. In experiments in vivo the time course of the antagonism after a single oral dose of YM 087 was estimated. It was found that 3 mg/kg of YM 087 inhibited specific binding of labeled vasopressin to V-1 receptors of liver tissue and V-2 receptors of kidney for up to 24 hours; a smaller dose of antagonist (0.1 mg/kg) still was effective as an inhibitor for 0.5 hours at V-1 receptors and 16 hours at V-2 receptors. The lasting effects indicate that YM 087 may be suitable for once-a-day use.

In vivo YM 087 was given orally to rats over 7 days. It was found to cause dose-dependent effects on urine volume and concentration. Urinary volumes increased by 100% to 500% depending on the dose of YM 087, and fluid intake increased proportionately; at the same time urinary osmolality was reduced by 55% to 80%, but urinary sodium excretion rate remained unaltered. The plasma sodium concentration increased by 5 mmol/L during the 7 days. Despite its properties as a V-1 (V_{1a}) antagonist, YM 087 had no effects on systolic blood pressure and pulse rate. However, an increased endogenous vasopressin concentration was noted after YM 087. The effects to lower the urinary osmolality persisted when YM 087 was given for as long as 5 weeks; there was no indication of tachyphylaxis nor were any agonistic properties noted when YM 087 was given for such a period of time.⁴⁵

Acute pharmacokinetic and pharmacodynamic effects have been described in normal human volunteers.⁴⁶ A single oral dose of 60 mg or a single intravenous dose of 50 mg increased the urine flow rate from approximately 100 to 700 mL/h during the peak effect at 2 hours after dosing. Simultaneously urinary osmolality decreased from approximately 600 to 90 mOsm/kg. The changes were associated with an increase of plasma osmolality from 283 ± 1.3 mOsm/kg to 288 ± 1 mOsm/kg. The endogenous vasopressin concentration increased as well.

Recently several clinical trials of YM 087 have been conducted.⁴⁷ All studies were double blind, randomized, and placebo controlled. Eligible patients were those with a serum sodium concentration between 115 and 130 mmol/L in the

setting of congestive cardiac failure or SIADH. Patients with significant renal insufficiency, hyperglycemia, hypothyroidism, or adrenal insufficiency were excluded, as were patients with cirrhosis and primary volume-depletion states. YM 087 was given intravenously at dose of 40 or 80 mg/d by continuous infusion over 4 days. Fluid intake was limited to 2 L/d. YM 087 increased the serum sodium concentration significantly and in a dose-dependent manner in cardiac failure and SIADH alike. In the high-dose YM 087 treatment the serum sodium level increased by 8 mmol/L from a baseline of 125 mmol/L. Primarily in the first 3 days of treatment YM 087 increased the urinary flow rate significantly, diluted the urine excreted, and brought about a positive free-water clearance. The urinary sodium excretion rate did not change in response to YM 087. The serum potassium level remained stable. Several patients in the treated groups reported thirst as an adverse event. Adverse events were similarly frequent in the control and treated groups. The most frequently reported adverse events were headache and postural hypotension. The serum vasopressin concentration increased in the high-dose conivaptan group. It was concluded that YM 087 was an efficient agent for the treatment of hyponatremia in cardiac failure and SIADH and that it was without significant adverse effects.

SR 121 463, Satavaptan

In 1996 Serradeil-Le Gal et al⁴⁸ described SR 121 463 A as an orally active specific V-2 vasopressin antagonist with prolonged half-life and an absence of agonistic properties. This nonpeptide molecule has a chemical designation of (1-[4-(N-tert-butyl-carbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy) cyclohexane] indol-2-one, fumarate). In V-2-receptor containing cell membranes from the renal medullary tissue of several species including rat, dog, and human beings, SR 121 463 A showed high competitive affinity for V-2 receptors in nanomolar concentrations and K_i values were between 0.70 and 4.1 nmol/L. In contrast, binding of the agent to human V-1 (V_{1a}) and V-3 (V_{1b}) receptor required concentrations that were more than 2 orders of magnitude higher than those for binding to V-2 receptors, indicating very low affinity to V-1 receptors. Functional assays of adenylate cyclase activation confirmed the high affinity of SR 121 463 A to V-2 receptors. In these assays in human kidney cell membranes, SR 121 463 A antagonized AVP-elicited cAMP generation at a K_i concentration as low as 0.26 ± 0.04 nmol/L.⁴⁸

Experiments in vivo in the rat using doses of SR 121 463 A between 0.1 and 1.0 mg/kg orally have been performed. They increased maximal urine flow at 2 hours after dosing by 2- to 5-fold, whereas urinary osmolality decreased from $1,380 \pm 42$ mOsm/kg in controls to 310 ± 20 mOsm/kg in the high-dose group. The duration of the aquaretic effect was dose dependent. In the high-dose treatment group (1 mg/kg) the increase of urine flow persisted for 12 hours. When the agent was given at a dose that increased urinary volume by approximately 150% (antagonist dose, 0.3 mg/kg) there was only a small increase of the urinary sodium excretion rate (ie, from

884 ± 48 μmol/24 hours to 1,026 ± 100 μmol/24 hours); doses of furosemide or HCTZ causing similar increases of the urinary volume augmented the sodium excretion rate significantly more (ie, by an additional 25% and 70% beyond the increase seen with SR 121 463 A). Whereas the V-2 antagonist left kaliuresis unchanged, furosemide and HCTZ increased it. The vasopressin-deficient Brattleboro rat was used to search for any possible agonistic properties. Treatment with SR 121 463 A was given over 8 days at a high dose of 10 mg/kg/d. There was no water retention; rather the urinary osmolality decreased somewhat.⁴⁸ This observation excluded intrinsic agonistic properties.

Clinical studies in patients with SIADH have been conducted.⁴⁹ In a phase III protocol 34 hyponatremic patients received 25 or 50 mg of SR 121 463 B once a day orally for 5 to 23 days. The study was placebo controlled, double blind, randomized, and it was performed in several centers. A fluid restriction to 1.5 L/d was prescribed; however, patients receiving SR 121 463 B drank up to 30% more than that as the study progressed. The antagonist increased the urinary volume from 1.5 to 2.5 L/d in the high-dose group and this response was maintained for the first 5 days of the double-blind part of the study. The urinary osmolality decreased by more than 50% and the free-water clearance became positive for the duration of the study. The hyponatremia corrected from 127 ± 5 mmol/L to 140 ± 6 mmol/L in the high-dose group. An excessively rapid correction rate (>12 mmol/L/12 h) was seen in approximately 10% of treated patients. Additional observations showed the urinary sodium excretion rate to be numerically lower than controls in treated patients whereas the potassium excretion was unchanged. There was no weight loss. Endogenous AVP more than doubled in the high-dose group. Thirst tended to increase. Approximately 10% of patients did not seem to respond to the agent in a discernable way. A few of the patients participated in a long-term open-label continuation of the study. In this protocol it was shown that normonatremia could be maintained for at least 1 year of treatment with SR 121 463 B. Drug safety was judged to be adequate.⁴⁹

OPC 41061, Tolvaptan

Tolvaptan is an oral V-2 antagonist undergoing clinical study at the present time. It first was described in 1998.⁵⁰ Its chemical designation is 7-chloro-5-hydroxy-1-[2-methyl-4-(2-methylbenzoylamino) benzoyl]-2, 3, 4, 5-tetrahydro-1H-1-benzazepine. In preparations of V-2 receptors expressed in transfected HeLa cells competitive receptor binding was shown and the binding affinity to V-2 receptors was 1.8 times higher than that of the natural ligand arginine vasopressin.⁵⁰ Tolvaptan was found to be 29 times more selective for the human V-2 receptor than for human V-1 (V_{1a}) receptors. Biochemical observations further strengthened the binding data; in these studies nanomolar concentrations of tolvaptan inhibited AVP-induced cAMP generation.⁵⁰ However, concentrations of tolvaptan as high as 10 μmol/L did not stimulate any cAMP production by themselves. This excluded the possibility of intrinsic agonistic effects.

In vivo studies in rats used doses between 0.3 and 10 mg/kg orally in single or multiple doses to evaluate the physiologic response to tolvaptan.⁵⁰ Within 2 hours after dosing the urine output increased approximately 12-fold, urinary osmolality decreased by approximately 75%, and natriuresis increased modestly.⁵⁰ In additional experiments lasting 28 days the response to a daily dose of tolvaptan of 1 or 10 mg/kg was tested. The experiments proved an unmitigated effectiveness of tolvaptan to induce aquaresis throughout the entire 28 days—even though the analysis of the B_{max} of V-2 receptors in these kidneys was found to decrease by approximately 25%. Endogenous concentrations of AVP increased in response to tolvaptan. Surprisingly, the plasma renin activity and plasma aldosterone decreased in the high-dose group.^{50,51} Additional in vivo studies were made to test the effects of a combination of loop diuretic and tolvaptan. It was observed that furosemide and tolvaptan had additive effects.⁵¹ Although furosemide alone increased the electrolyte excretion rate, the addition of tolvaptan led to a further increase of the urinary flow rate together with an additional degree of dilution of the urine excreted.⁵¹

Two clinical trials to study the efficiency and safety of tolvaptan in the treatment of hyponatremia—Sodium Assessment with Increasing Levels of Tolvaptan in Hyponatremia (Salt) I and Salt II—recently have been concluded. The 2 trials were placebo controlled, randomized, and double blind, and incorporated more than 200 participating patients in each trial. Inclusion in the trials required the presence of chronic mild to moderate hyponatremia in the settings of cardiac failure, cirrhosis, or SIADH. Tolvaptan was given in a single oral dose, and the investigators were able to determine the dose depending on the response of hyponatremia (tolvaptan or placebo, 15–60 mg). The duration of treatment was 30 days during the double-blind phase, followed by an open label continuation phase of up to 1 year of tolvaptan. In contrast to previous studies the protocol did not prescribe a fluid restriction; most of the treatment was given on an outpatient basis. Some of the results of the Salt II trial were presented recently in a preliminary form (Late-Breaking Trials Session, Annual Meeting of the American Society of Nephrology, Philadelphia, PA, November 8–13, 2005). It was shown that tolvaptan corrected hyponatremia consistently and safely.

Conclusions

Over the past decade pharmacologic research has succeeded in developing at least 4 highly potent, nonpeptide, orally available, specific vasopressin receptor antagonists for the treatment of water retention and hyponatremia. All of them are devoid of intrinsic agonistic effects and none of them was subject to tachyphylaxis. Lixivaptan or VPA 985, SR 121 463 B and tolvaptan, or OPC 41,061 are V-2 specific, conivaptan or YM 087 is a V-1/V-2 mixed-receptor antagonist. All 4 antagonists proved effective in animal experiments and clinical studies as aquaretics increasing free-water clearance without a proportionate sodium diuresis. Therapeutic doses in patients ranged from 15 (tolvaptan) to 200 mg (lixivaptan)

per day. All of the agents were able to correct hyponatremia. Serum potassium concentrations remained unchanged. Endogenous plasma vasopressin increased in all observations. None of the agents was able to suppress thirst, and drinking of fluid increased in general. Despite the aquaresis, weight decreased only minimally by no more than 1 kg during the 5 to 30 days of study reported. All agents appeared to be safe; overly rapid correction of hyponatremia or treatment overshooting into the hypernatremic range were exceptions. It was possible to conduct treatments with antagonists on an ambulatory basis in outpatients. The agents were effective even when no fluid restrictions were imposed. Taken together these new agents are very promising for the treatment of hyponatremia. Future studies will try to determine the benefit to patients from vasopressin antagonists in settings such as hyponatremia in cardiac failure, hyponatremia in hepatic encephalopathy, and mild hyponatremia in the elderly with SIADH.

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