Sodium Excretion in Response to Vasopressin and Selective Vasopressin Receptor Antagonists

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ABSTRACT

The mechanisms by which arginine vasopressin (AVP) exerts its antidiuretic and pressor effects, via activation of V2 and V1a receptors, respectively, are relatively well understood, but the possible associated effects on sodium handling are a matter of controversy. In this study, normal conscious Wistar rats were acutely administered various doses of AVP, dDAVP (V2 agonist), furosemide, or the following selective non-peptide receptor antagonists SR121463A (V2 antagonist) or SR49059 (V1a antagonist). Urine flow and sodium excretion rates in the next 6 h were compared with basal values obtained on the previous day, after vehicle treatment, using each rat as its own control. The rate of sodium excretion decreased with V2 agonism and increased with V2 antagonism in a dose-dependent manner. However, for comparable increases in urine flow rate, the V2 antagonist induced a natriuresis 7-fold smaller than did furosemide. Vasopressin reduced sodium excretion at 1 μg/kg but increased it at doses >5 μg/kg, an effect that was abolished by the V1a antagonist. Combined V2 and V1a effects of endogenous vasopressin can be predicted to vary largely according to the respective levels of vasopressin in plasma, renal medulla (acting on interstitial cells), and urine (acting on V1a luminal receptors). In the usual range of regulation, antidiuretic effects of vasopressin may be associated with variable sodium retention. Although V2 antagonists are predominantly aquaretic, their possible effects on sodium excretion should not be neglected. In view of their proposed use in several human disorders, the respective influence of selective (V2) or mixed (V1a/V2) receptor antagonists on sodium handling in humans needs reevaluation.


The mechanisms by which arginine vasopressin (AVP) exerts its antidiuretic and its pressor effects are relatively well understood. On the one hand, AVP improves water conservation by increasing the permeability to water of the renal collecting duct (CD), an effect mediated by the V2 receptors (V2R) and permitted by the insertion in the luminal membrane of principal cells of preformed aquaporin 2 (AQP2) molecules. This allows more water to be reabsorbed when these ducts traverse the hyperosmotic medulla. On the other hand, AVP increases blood pressure (BP) by inducing a vasoconstriction through its binding to V1a receptors (V1aR) expressed in vascular smooth muscle cells. For these two different effects, in vivo studies are in good agreement with the expectations based on results obtained in vitro.

In contrast, the experiments intended to study the effects of AVP on sodium handling in vitro or in vivo provide results that are difficult to reconcile. In the isolated microperfused CD, V2R activation increases sodium transport,1 an effect that should reduce sodium excretion in vivo; however, in a number of studies, AVP infusion in animals and humans...
has been shown to induce an increase in sodium excretion.\(^2\)\(^–\)\(^9\) It is usually assumed that AVP might contribute to some forms of hypertension by its vasoconstrictive effects, but an increase in sodium excretion, if it occurred in normal life, should more likely contribute to lower BP.

In an attempt to resolve these conflicting results, we undertook a series of experiments in conscious undisturbed rats to obtain precise dose-response curves to AVP and to selective agonists and antagonists of V1aR or V2R. Each rat served as its own control, receiving on separate days either the vehicle or the drug(s), and the urine produced in the next 6 h was analyzed. This allowed us to evaluate separately the respective influence of V1aR and V2R activation on urine concentration and sodium excretion and their interactions at different physiologic and supraphysiologic levels of AVP. These new results should be of special interest because nonpeptide vasopressin receptor antagonists are now proposed in hyponatremia with heart failure and cirrhosis, two conditions with severe water and sodium retention,\(^10\),\(^11\) in polycystic kidney disease,\(^12\) and possibly in some forms of salt-sensitive hypertension.\(^13\)

**RESULTS**

For most experiments, the changes induced by the various treatments are presented as experimental day/basal day ratios (Exp/Basal). One rat group always received the vehicle(s) alone on both day 1 and day 2 as an additional control for assessing possible day-to-day variations. Absolute values obtained for the basal day in the various experiments are shown in Table 1.

**Dose-Response Curves of the V2R Agonist and Antagonist (Experiments A and B)**

The V2R agonist 1-desamino 8-d-arginine vasopressin (dDAVP) showed the expected antidiuretic action, inducing a marked decline in urine flow rate (V) and a rise in urine osmolality (Uosm). The V2R antagonist showed the expected aquaretic action (Figure 1). In addition, both drugs also influenced solute excretion rates. Urea excretion rate fell with increasing V2 agonism, as already widely known. Sodium excretion rate declined, and potassium excretion rate increased by increasing V2 agonism, as already widely known. Sodium excretion rate was modestly reduced along with the modest diuretic and natriuretic influence of endogenous AVP. These changes suggest that the V1aR antagonism suppressed a significant decrease in V and sodium excretion occurred in some but not all rats with 10 mg/kg BW (data not shown). These changes to reach statistical significance in most of the dose groups; however, the transtubular potassium gradient (TTKG), an index of the active secretion of potassium in the distal nephron, rose only with dDAVP (Figure 1).

**Dose-Response Curve of AVP and Effects of the V1aR Antagonist on the Response to AVP (Experiments C and D)**

When given at a dose of 1 \(\mu g/kg\), the natural hormone AVP increased Uosm and reduced V by approximately 35% (Figure 2). The difference for Uosm did not reach significance because of large interindividual variation in the response. A tendency for a dose-dependence of the antidiuretic response is visible when considering the control and the two lowest doses (thin line), but this effect disappears with higher doses (Figure 2). Sodium excretion rate was modestly reduced along with the antidiuretic effect at 1 \(\mu g/kg\) but rose markedly with higher doses of AVP, whereas urea excretion fell. The highest dose increased potassium excretion rate and TTKG by 40% (Figure 2).

To evaluate whether the loss of the antidiuretic effect of AVP and the appearance of a natriuretic effect at higher doses are due to V1aR stimulation, we conducted additional experiments with co-administration of AVP 15 \(\mu g/kg\) BW and a selective V1aR antagonist. In preliminary experiments, the effects of the V1aR antagonist given alone at various doses (0.1, 1, and 10 mg/kg BW) were evaluated. Dose-dependent significant increases in Uosm were seen with all three doses, whereas significant decreases in V and sodium excretion occurred in some but not all rats with 10 mg/kg BW (data not shown). These changes suggest that the V1aR antagonism suppressed a modest diuretic and natriuretic influence of endogenous AVP. In experiment D, two doses of AVP were tested. AVP at 3 \(\mu g/kg\) was antidiuretic, lowering V by 27% \((P < 0.01)\) and increasing Uosm by 33% \((P < 0.001)\), and induced no change in sodium excretion rate. With a five-fold higher dose (15 \(\mu g/kg\)), the antidiuretic effect was completely lost and a marked natriuretic

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**Table 1. Basal values observed on day 1 in groups of rats used in the various experiments**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>(n)</th>
<th>BW (g)</th>
<th>V (ml/h)</th>
<th>Uosm (mOsm/kg H(_2)O)</th>
<th>Na Excretion ((\mu)mol/h)</th>
<th>K Excretion ((\mu)mol/h)</th>
<th>Urea Excretion ((\mu)mol/h)</th>
<th>TTKG</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>64</td>
<td>356</td>
<td>0.58 ± 0.02</td>
<td>1436 ± 51</td>
<td>57.5 ± 3.0</td>
<td>111 ± 4</td>
<td>431 ± 14</td>
<td>8.36 ± 0.13</td>
</tr>
<tr>
<td>B</td>
<td>35</td>
<td>281</td>
<td>0.48 ± 0.03</td>
<td>1723 ± 84</td>
<td>51.0 ± 3.7</td>
<td>124 ± 7</td>
<td>394 ± 20</td>
<td>9.34 ± 0.16</td>
</tr>
<tr>
<td>C</td>
<td>48</td>
<td>300</td>
<td>0.53 ± 0.03</td>
<td>1643 ± 74</td>
<td>71.5 ± 3.1</td>
<td>126 ± 5</td>
<td>385 ± 17</td>
<td>9.36 ± 0.14</td>
</tr>
<tr>
<td>D</td>
<td>70</td>
<td>285</td>
<td>0.60 ± 0.03</td>
<td>1609 ± 49</td>
<td>81.1 ± 4.3</td>
<td>133 ± 5</td>
<td>462 ± 20</td>
<td>9.02 ± 0.14</td>
</tr>
<tr>
<td>F</td>
<td>72</td>
<td>274</td>
<td>0.51 ± 0.02</td>
<td>1363 ± 52</td>
<td>51.8 ± 2.5</td>
<td>105 ± 4</td>
<td>387 ± 16</td>
<td>9.48 ± 0.13</td>
</tr>
</tbody>
</table>
Luminal effects of AVP in the renal CD have been described. This prompted us to evaluate urinary AVP concentration (UAVP) that should, at least approximately, vary in proportion to its concentration in the lumen of the CD. Twenty-four-hour dehydration increased UAVP approximately 20-fold. Administration of AVP 3 μg/kg BW increased UAVP to a similar extent, whereas dDAVP did not change it (Table 2). A five-fold higher dose of AVP (15 μg/kg BW) increased UAVP close to five times more. This high luminal AVP concentration was associated with an increase in diuresis and natriuresis (as in experiment D) and a decline in Uosm such that V was higher and Uosm was lower than those observed in rats receiving a smaller dose of AVP or in water-deprived rats. Several previous studies already observed this simultaneous increase in sodium excretion and urine flow rate after AVP infusions. In these experiments, it would have been difficult to evaluate the mean plasma AVP (PAVP) level reached during 6 h (duration of the urine collection). Measurements of the AVP excretion rate provide more integrated information during this period. Previous studies showed that urinary AVP excretion rate is roughly proportional to the plasma level when osmolar excretion remains stable. Thus, given the results presented in Table 2, it may be assumed that administration of 1 to 3 μg/kg BW AVP increased PAVP to levels similar to those seen after 24 h of water deprivation, whereas the dose of 15 μg/kg BW increased PAVP to higher values, reached only during exogenous vasopressin infusion.

**Effects of the V2R Antagonist on Sodium and Water Excretion during the Whole 24 h (Experiment B)**

Selective V2R antagonists are usually reported to increase markedly urine output without affecting solute excretion. Because a significant increase in sodium excretion rate was observed in these experiments, it was interesting to determine whether this change was short-lived or sustained. Collection of urine for the 18 h after the initial 6-h collection and calculation of the antagonist’s effects during these two aggregated periods showed that the diuresis but not the natriuretic effect remained detectable over 24 h (Figure 4). Most probably, thirst and thus fluid intake increased during the whole duration of V2R blockade and resulting aquaresis. After the initial loss of sodium, compensatory sodium retention occurred in the subsequent hours, bringing back sodium balance to zero. A similar compensation also occurred for potassium and urea. Note that with the highest dose of the antagonist, sodium retention occurred during the 6- to 24-h period despite a persisting increase in aquaresis (Figure 4). It is conceivable that the natriuretic effect induced by the V2 antagonist could be sustained over the whole 24 h, along with the aquaretics, if the rats could compensate the initial salt loss (that probably stimulates their salt appetite) by having access to a source of salt independent of the food, as they do for water.

**Urine AVP Concentration in Different Experimental Conditions (Experiment E)**

Luminal effects of AVP in the renal CD have been described. This prompted us to evaluate urinary AVP concentration (UAVP) that should, at least approximately, vary in proportion to its concentration in the lumen of the CD. Twenty-four-hour dehydration increased UAVP approximately 20-fold. Administration of AVP 3 μg/kg BW increased UAVP to a similar extent, whereas dDAVP did not change it (Table 2). A five-fold higher dose of AVP (15 μg/kg BW) increased UAVP close to five times more. This high luminal AVP concentration was associated with an increase in diuresis and natriuresis (as in experiment D) and a decline in Uosm such that V was higher and Uosm was lower than those observed in rats receiving a smaller dose of AVP or in water-deprived rats. Several previous studies already observed this simultaneous increase in sodium excretion and urine flow rate after AVP infusions. In these experiments, it would have been difficult to evaluate the mean plasma AVP (PAVP) level reached during 6 h (duration of the urine collection). Measurements of the AVP excretion rate provide more integrated information during this period. Previous studies showed that urinary AVP excretion rate is roughly proportional to the plasma level when osmolar excretion remains stable. Thus, given the results presented in Table 2, it may be assumed that administration of 1 to 3 μg/kg BW AVP increased PAVP to levels similar to those seen after 24 h of water deprivation, whereas the dose of 15 μg/kg BW increased PAVP to higher values, reached only during exogenous vasopressin infusion.
Comparison of V2R Antagonist and Furosemide (Experiment F)

Because the V2R antagonist induced an increase not only of water but also of sodium excretion, we performed an additional experiment designed to compare these effects with those of a classical diuretic in the same experimental setting. Figure 5 shows the results obtained in 56 rats studied in parallel and receiving various doses of either furosemide or V2R antagonist (results of 14 time-control rats receiving only the vehicles are not included). The changes in sodium or potassium excretion are plotted as a function of the simultaneous changes in water excretion (both changes expressed as Exp/Basal). For both drugs, changes in sodium and in water excretion were always associated (both regression lines cross the 1 \times 1 point), but the slopes of the regression lines for the sodium-to-water relationship differed markedly: 1.36 for furosemide versus 0.19 for the V2R antagonist ($P < 0.001$). Potassium excretion rate rose with both drugs but less intensely with the V2R antagonist (slope = 0.058) than with furosemide (slope = 0.099), although the difference did not reach significance ($P = 0.097$). Note that the potassium excretion rate decreased (Exp/Basal < 1) when the aquaretic effect was less than three-fold above basal.

DISCUSSION

AVP is mostly known for its effect on water conservation. Its possible effects on sodium excretion in usual life are rarely addressed. This study evaluated the dose-dependent influence of AVP on sodium excretion in vivo in conditions as close as possible to normal life, and dissociated the respective roles of V1aR and V2R. The main findings are that V2R effects are strictly antinatriuretic, whereas V1aR effects are natriuretic above a certain threshold of hormone level. For levels of the endogenous hormone prevailing in normal life, the widely known antidiuretic effects of AVP may be associated with antinatriuretic effects of variable intensity.

One of the advantages of this study is that all experiments were conducted in conscious, unrestrained rats without any pretreatment. There was no previous oral water load or intravenous infusion of iso- or hypotonic fluid, maneuvers that induce acute perturbations of the fluid balance. Rats underwent no anesthesia and surgery that are known to stimulate potent endogenous AVP secretion. The use of highly selective nonpeptide V1aR or V2R antagonists with relatively long half-life and the building of dose-response curves allowed a better evaluation of AVP effects within the range occurring in usual physiologic and pathophysiologic situations. Finally, each rat was its own control, a favorable situation given the large interindividual variability of urine flow rate and osmolality and of the response to AVP. That food and fluid intakes were not measured represents a limitation in interpreting some of the results. dDAVP is known to have some affinity for V1b receptors, but this affinity is much lower in rats than in humans. This experimental design was not intended to address the possible V1b receptor-mediated effects.

V2R Effects

Experiments performed with the V2R agonist and antagonist confirm that V2R-mediated effects are not only antidiuretic but also antinatriuretic. This is consistent with observations made in isolated CD or in various mammalian or amphibian cell culture models. In these tissues, AVP, applied to the basolateral side, increases amiloride-sensitive sodium transport, an effect mediated by the epithelial sodium channel (ENaC).
V2R are also expressed in the thick ascending limb of Henle’s loop, but previous studies suggested that the influence of AVP on sodium transport in this segment is negligible in normal conditions. 

Micropuncture experiments in rats receiving SR121463A confirmed that the aquaretic effect was located downstream of the early distal tubule and thus did not involve the thick ascending limb. 

In humans, acute dDAVP administration also induced a two-fold reduction in sodium excretion rate that was observed in healthy individuals and in individuals with nephrogenic diabetes insipidus as a result of mutations of AQP2 but not in those with mutations of the V2R. 

In the isolated, erythrocyte-perfused kidney (a model in which good oxygenation allows an efficient urine concentration), dDAVP induced a five-fold decrease in the fractional excretion of sodium. 

Noteworthy, in the present experiments, the antidiuretic effects of dDAVP reached their maximum for low doses, whereas the effects on solute excretion rate rose progressively with increasing doses (Figure 1). This suggests a very sensitive influence of V2R activation on AQP2 and resulting water permeability in the CD and a less sensitive action on ENaC and on the urea transporter UT-A1, in agreement with in vitro findings. 

Most studies describing the effects of selective nonpeptide V2R antagonists in experimental animals or humans concluded that these drugs behave as pure “aquaretics” with no effect on electrolyte excretion. Why was the natriuretic effect of aquaretics not disclosed in previous studies? As shown in Figure 4, the natriuretic effects observed here were not apparent in 24-h urine because compensatory sodium retention occurred after the initial loss. In two previous studies in conscious rats receiving daily injections of a V2R antagonist, no effects on 24-h sodium excretion were reported, but distinct and significant three-fold increases in sodium excretion rate are visible (although not commented on) in the first 4 or 6 h after daily dosing in figures of both articles (Figure 6 in reference25 and Figure 2 in reference26). A significant three- and five-fold increase in sodium excretion with 10 and 30 but not with 1 and 3 mg/kg BW was also reported in the previous studies of the first nonpeptide V2R antagonist, OPC-31260. VPA 985, another V2R antagonist, increased sodium excretion significantly in patients with hyponatremia and cirrhosis as-cites28,29 but not in patients with the syndrome of inappropriate secretion of antidiuretic hormone. SR121463A was also shown to increase sodium excretion chronically in rats with cirrhosis. 

In agreement with the natriuretic effect of V2R antagonists, it is interesting to note that the fractional excretion of sodium was 50% higher in healthy individuals when they were studied during high oral hydration (likely reducing their endogenous AVP level) than when they received a low fluid intake and that the excretion of a sodium load was two-fold faster. 

Even if aquaretics increase sodium excretion to some extent, it should be kept in mind that their natriuretic effect remains far smaller than their aquaretic effect, at variance with the action of classical diuretics, as shown here in Figure 5. For
Figure 4. Dose-dependent effects of the V2R antagonist on urine flow rate and osmolality and on sodium excretion rate in the first 6 h after drug administration, in the subsequent 18 h (6 to 24 h), and in the whole 24 h during the experimental day (basal day not shown; Experiment B, n = 4 to 6 rats per dose). Paired t test, experimental versus basal: *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 5. Influence of the V2R antagonist (○ and dotted line, n = 27) or furosemide (□ and solid line, n = 29) at various doses (0.1, 0.3, 1.0, 10.0, and 30.0 mg/kg BW for furosemide and 0.1, 0.3, 1.0, and 10.0 mg/kg BW for the V2R antagonist) on sodium (top) and potassium (bottom) excretion rates as a function of the simultaneous changes in fluid excretion rate (urine flow rate). Results are expressed as Exp/Basal for each rat, and regression lines are shown. For both the abscissa and the ordinate, a value of 1 means no change. The equations of the regression lines and corresponding correlation coefficients are as follows. For sodium with furosemide y = 1.360x – 0.54; r = 0.88, P < 0.001. For sodium with the V2R antagonist y = 0.189x + 0.82; r = 0.82, P < 0.001. For potassium with furosemide y = 0.099x + 0.88; r = 0.45, P < 0.05. For potassium with the V2R antagonist y = 0.058x + 0.86; r = 0.77, P < 0.001.

The effects of the V2R agonist and antagonist on potassium handling deserve some comments. Although both drugs have opposite effects on all other variables, they both increased potassium excretion rate. These puzzling results are easily explained by two widely known previous observations. First, stimulation of V2R is known to increase potassium secretion by the CD.35,36 This is confirmed here by the dose-dependent increase in TTKG seen in rats receiving dDAVP (Figure 1, bottom left). Second, large increases in V are known to increase potassium secretion by a flow-dependent mechanism.37 As seen in Figure 1 (bottom right), this physical effect is not due to an increase in potassium secretion, because there is no significant increase in TTKG. Of note, the influence of dDAVP on potassium secretion was also seen in healthy humans in some38 but not all studies.23

V1aR Effects

In agreement with a number of previous studies of rats, dogs, sheep, and humans, we observed that AVP increased sodium excretion.2–9 The dose-response curve performed in this study shows that this effect occurred only for high doses (15 and 50 μg/kg BW) that probably increased PAVP levels distinctly above those involved in water conservation, even after 24 h of water deprivation, as judged here by the urinary AVP data (Table 2). With lower doses (0.1 and 1 μg/kg BW), likely corresponding to the range of usual osmotic stimul,
the antidiuretic effects of AVP were accompanied by a small but significant reduction in sodium excretion, as with dDAVP. Because the natriuretic effect of AVP 15 μg/kg BW was largely abolished by the co-administration of the selective V1αR antagonist, these experiments clearly establish that the natriuresis induced by AVP is mostly due to V1αR-mediated actions.

Several possible renal and nonrenal mechanisms may account for this natriuretic effect. V1αR and V2R are expressed in multiple sites throughout the body and throughout the kidney itself. Although these experiments cannot disclose the mechanisms involved in the observed responses or the organs/tissues/cell types contributing to these changes, we can briefly mention some possible pathways. (1) In several previous studies, the AVP-induced natriuresis was assumed to result from the volume expansion induced by fluid loads given orally and/or intravenously before and/or during AVP administration. The authors proposed that this expansion induced the release of another mediator inhibiting renal tubular sodium transport.43,44; however, our experiments show that AVP increased sodium excretion even in the absence of any fluid load. (2) Prostaglandins are known to reduce sodium transport in the CD5 and to increase medullary blood flow,45,46 two effects that each will contribute in different ways to increased sodium excretion. Actually, prostaglandins have been shown to contribute to the AVP-induced natriuresis.9 AVP stimulates prostaglandin production in interstitial medullary cells,40 which express V1αR,43 and in the cortical CD.40 V1αR in the CD are mostly located in the luminal membrane14,15; therefore, urinary, rather than peripheral AVP could be involved here. Of note, AVP concentration is known to be higher in the CD lumen and in the medullary interstitium than in peripheral blood.17 (3) Finally, the natriuretic effects of AVP could be due to pressure-natriuresis resulting from the vasopressor effects of the hormone, either within the whole circulation or selectively within the kidney vasculature.

Integration of V2R and V1αR Effects

This functional in vivo dissection of the respective influence of V1αR and V2R on sodium handling by the kidney provides for the first time several clues for a better understanding of the integrated actions of AVP in vivo. Combined effects mediated by V2R and V1αR can be predicted to vary greatly according to the levels of plasma and urinary AVP. They will provide different sets of water and sodium responses. The changes in sodium excretion as a result of the algebraic sum of V2 and V1αa effects over progressively increasing plasma levels of AVP are represented schematically in Figure 6. V2R antinatriuretic and V1αR natriuretic effects are depicted as sigmoid curves with different thresholds (B for V2R and C for V1αR effects) and different AVP levels inducing the maximum effects for each receptor type (B’ and C’, respectively).

In the range of very low plasma levels of AVP, only the V2 effects on water permeability of the CD are apparent, and AVP will reduce V without any effect on sodium excretion rate (Figure 6, range A to B). Such purely antidiuretic action is visible in the study of Andersen et al.,44 who gave subpicomolar doses of AVP to individuals undergoing water diuresis. Although these doses did not increase PAVP above the detection limit of the assay, V fell and Uosm rose significantly without any change in sodium excretion rate. Another example is found in individuals with central diabetes insipidus, in whom dDAVP induced a marked decline in V independent of any change in sodium excretion rate (see Figure 4 in reference23). This action of AVP in the low range of plasma concentrations can induce the reabsorption of very large amounts of solute-free water.17

When PAVP rises a little more (beyond B in Figure 6), V2R effects stimulate sodium reabsorption and should thus reduce sodium excretion rate in addition to reducing V, as seen in healthy humans who received an infusion of AVP at a dose of 25 μg/min per kg BW44 or with dDAVP23 and in the isolated rat kidney perfused with a medium containing erythrocytes and dDAVP.24 This sodium-retaining effect probably explains why, in normal individuals (with normal fluid and food intake) who provided multiple urine samples, sodium excretion rate declined in parallel with V in the samples in which Uosm exceeded 600 mOsm/kg H2O but was not altered in those under this limit despite wide differences in V (from 60 to 300 ml/h).45 This is in agreement with in vitro data showing that the effect of AVP of sodium transport in isolated perfused rat CD requires higher levels of peritubular AVP than the effect on water permeability.17

With further increases in PAVP, V1αR-dependent natriuretic effects appear and increase progressively, but the net effect of AVP is still antinatriuretic (PAVP between C and M in Figure 6). Finally, with much higher levels of AVP, such as those induced by exogenous AVP infusion at a rate higher than 5 μg/kg, the V1α natriuretic effects overcome the V2 effects, leading to increased natriuresis (PAVP beyond point M in Figure 6). We want to underline that the dose of AVP inducing either negative or positive effects on sodium excretion was quite variable among rats and experiments between 1 and 5 μg/kg BW (data not shown). This variability may be due to different sensitivities of the two receptors and/or of subsequent signal transduction, and to differences in AVP concentrations in luminal fluid of the CD and/or in medullary tissue (determining V1α effects) with respect to peripheral AVP concentration (determining V2 effects).

The secretion of AVP is not known to depend on sodium intake. As discussed previously,20,23 the V2R-dependent stimulation of sodium reabsorption likely serves the purpose of fluid conservation rather than that of regulating sodium excretion. A stronger sodium reabsorption in the AVP-sensitive distal nephron, which expresses both ENaC and AQP2, will drive more water out of the lumen and will thus increase the concentration of all other solutes but sodium. Thus, AVP will help conserve water at the expense of a less efficient sodium excretion. V1αR-dependent effects that increase sodium excretion may be viewed as a safeguard mechanism limiting the risk for too intense sodium retention. An imbalance between V1αR
and V2R sensitivity or signal transduction may thus influence the ability of the kidney to excrete sodium and may, indirectly, be responsible for inappropriate sodium retention and resulting increase in BP. Actually, it has long been known that highly selective peptide and nonpeptide inhibitors of V1aR block exogenous AVP’s pressor action but that they have little effect on the basal levels of arterial BP.46 Thus, under normal conditions of cardiovascular homeostasis and appropriate extracellular fluid volume, AVP and its V1aR seem to play only a minor role in maintaining normal cardiovascular function. In contrast, several observations link BP and the antidiuretic V2R-dependent actions of AVP. Long-term dDAVP infusion in normal rats has been shown to increase BP by approximately 10 mmHg.47,48 Salt-sensitive hypertension-prone Sabra rats exhibit higher AVP mRNA, higher P_{AVP}, and twofold higher U_{osm} and lower V than their salt-resistant counterparts.49 In humans, significant negative correlations were observed between 24-h V and BP.13,50

In the usual range of P_{AVP}, the antidiuretic effect of AVP is possibly associated with an antinatriuretic effect of variable intensity depending on the level of urine concentration. This will delay the excretion of sodium and could thus result in some degree of sodium and volume retention, which could favor an increase in BP. The natriuretic effects induced by the V1aR stimulation may counteract this tendency. Thus, instead of the usually assumed pressor effect, V1a activation within the physiologic range may actually reduce the risk for V2R-induced sodium retention. Moreover, the balance between V1aR- and V2R-mediated actions in cells possessing the two types of receptors (e.g., cortical CD) may be further amplified by a down-regulation of the V2R induced by a V1a-mediated pathway.51

That V1aR effects counteract V2R tubular effects could explain several puzzling observations. (1) In pathologic models in which AVP levels are known to be elevated (nitric oxide-deprived hypertensive rats and diabetic rats), the long-term administration of a selective V1aR nonpeptide antagonist worsened BP and albuminuria and did not prevent or even aggravated diabetes-related vascular damage.52 (2) Several other studies suggested that V1a effects attenuate or counteract several direct and indirect V2R-dependent effects in normal rats and in rats with renal failure.54–57 (3) In patients with chronic renal failure, the increased urinary excretion of AVP per remaining nephron correlated positively and more significantly than for any other hormone with the fractional excretion of sodium, suggesting that luminal AVP contributed to ensure an appropriate natriuresis through V1aR-mediated luminal effects.58 (4) Interestingly, mice with deletion of the V1aR exhibit a lower BP as a result not of a reduced vasoconstriction but of a lower extracellular fluid volume.59

In summary, these results provide a better knowledge of the respective V2R- and V1aR-dependent effects of AVP on sodium excretion in rats. Whether a similar balance between V1aR- and V2R-dependent effects occurs in humans remains to be determined. These results are potentially important in view of the proposed use of AVP antagonists in several human disorders.10–12 In patients with hypervolemic hyponatremia as a result of heart failure or cirrhosis, the additional effect of V2R antagonism on sodium excretion may be beneficial and may decrease diuretic use. V2R antagonists may also be beneficial in some forms of salt-sensitive hypertension; however, more precise information is required regarding V1a and V2 effects in humans to know whether selective rather than mixed antagonists are more appropriate in each of these disorders.

**CONCISE METHODS**

**Animals and General Procedures**

Adult male Wistar rats (Charles River, L’Arbresle, France) of initial body weight 230 to 240 g were housed individually in metabolic cages and maintained on standard rat chow and tap water ad libitum.
with free access to tap water and powdered food (A03; Safe, Villemeisson/Orge, France) at all times before and during the experiments (except for one rat group in experiment E, discussed later). They were accustomed to the cages for 5 d before the experiments. All experiments were carried out in conscious, undisturbed rats and involved no previous water load, no instrumentation, and no pretreatment. Each experiment included 24 to 36 rats studied in parallel on two successive days. On day 1 (basal), rats were administered an injection at time 0 (approximately 10 a.m.) of the vehicle(s) only, and on day 2 (experimental) with AVP and/or various drugs (see details that follow). Urine was collected for 6 h starting just after the injection(s).

Urine volume was determined gravimetrically, assuming the density of urine was equal to unity. \( U_{\text{osm}} \) was measured with a freezing point osmometer (Roebling, Berlin, Germany) and urinary concentration of sodium and potassium by flame photometry (IL 943, Instrument Laboratory, Lexington, MA). Urine urea concentration was measured with a standard kit (Urea Kit S 1000; BioMérieux, Lyon, France).

In each experiment, on the basis of values obtained during the basal day, the rats were divided into several groups of equivalent urine volume and osmolality \( (n = 4 \text{ to } 6 \text{ per group}) \) to test, during the experimental day, the effects of the various doses of hormones or drugs in groups of rats with equivalent urine concentrating activity. Usually, three or four independent experiments were performed in the same series of rats at 1-wk intervals (to allow washout from the preceding experiment). All animal procedures were conducted in accordance with the European guidelines for the care and use of laboratory animals.

**V2R Agonist dDAVP: Dose-Response Curve (Experiment A)**

dDAVP (Minirin; Ferring Pharmaceuticals AB, Malmö, Sweden) is a selective peptidic V2R agonist.\(^6^6\) The dDAVP solution was emulsified in oil (1:9 vol/vol) to prolong its bioavailability, and injected subcutaneously (under the skin of the back of the neck) in seven rat groups in oil (1:9 vol/vol) to prolong its bioavailability, and injected subcutaneously, as described for dDAVP (600 \( \mu \)l emulsion/kg BW), in five groups of rats. The concentration of AVP in the aqueous phase was designed to deliver 0.0 (vehicle alone), 0.1, 1.0, 15.0, or 50.0 pg/kg BW (corresponding approximately to 0.1, 1.0, 15.0, or 50.0 nmol/kg BW). As in previous studies,\(^5^4,5^7\) the dose range for AVP was approximately five times higher than that for dDAVP because of the faster degradation of AVP in vivo.

**Natural AVP Plus V1aR Antagonist SR49059 (Experiment D)**

For evaluation of the role of V1aR in the response to the natural hormone AVP, rats of this series were administered AVP (15 \( \mu \)g/kg BW, same procedure as in Experiment C) without or with the concomitant administration of the selective nonpeptide V1aR antagonist SR49059 (Relcovaptan; Sanofi-Aventis) at a dose of 10 mg/kg BW, shown in previous studies to block the pressor response to AVP.\(^6^4\) This drug was dissolved in DMSO and cremophor (each 10% of final volume) and then in saline, and was injected intraperitoneally (1 ml/kg BW). For comparison, additional rats received vehicle alone, the V1aR antagonist alone, AVP at a lower dose (3 \( \mu \)g/kg BW), or dDAVP (0.6 \( \mu \)g/kg BW).

**Urinary AVP Concentration in Various Situations (Experiment E)**

To evaluate the concentration of AVP prevailing in the lumen of the CD in different situations, we performed additional experiments in which urine was collected for 6 h, as in the other experiments, under various experimental conditions, including injection of isotonic saline, injection of dDAVP 0.6 \( \mu \)g/kg BW, injection of AVP 3 or 15 \( \mu \)g/kg BW, and 24-h dehydration. Urine volume and \( U_{\text{osm}} \) were measured, and aliquots of urine were stored at \(-20^\circ\text{C}\) for further AVP assay. \( U_{\text{AVP}} \) was measured as described previously.\(^6^5\) The antibody used does not recognize dDAVP.

**Comparison of V2R Antagonist and Furosemide Effects (Experiment F)**

Classical diuretics and aquaretics (V2R antagonist) increase \( V \) by different mechanisms. To compare the respective effects of the two types of drugs on water and sodium excretion, we performed an additional experiment in which different groups of rats received either furosemide (Lasix; Sanofi-Aventis) or the V2R antagonist (prepared and injected as in Experiment B) at various doses (0.0, 0.1, 0.3, 1.0, 10.0, and 30.0 mg/kg BW for furosemide and 0.0, 0.1, 0.3, 1.0, and 10.0 mg/kg BW for the V2R antagonist). Urine was collected for 6 h after the injections.

**Statistical Analysis**

\( V \) and the excretion rate of the different solutes were calculated according to standard formulas. The TTKG, reflecting the intensity of potassium secretion, was calculated by dividing the urine/plasma potassium concentration ratio by the urine/plasma osmolality ratio.\(^6^6\) Plasma osmolality was arbitrarily considered to be 300 mOsm/kg H\(_2\)O and plasma potassium concentration 5 mmol/L in all rats.

Results are expressed as means \( \pm \) SEM. In Experiments A through D, each rat was its own control. Thus, for each variable, the Exp/Basal
provides a quantitative estimate of the changes induced by the hormone and/or drug. The statistical significance of these changes was evaluated by paired t test comparing results of the experimental day with those of the basal day in each rat. In Experiment E, comparison between the various groups was performed by one-way ANOVA followed by Fisher post hoc test. In Experiment F, linear regressions were calculated between the changes in sodium or potassium excretion and those in water excretion.

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DISCLOSURES

None.

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