Review

Antidiuretic action of vasopressin: quantitative aspects and interaction between V1a and V2 receptor-mediated effects

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Abstract

(1) Vasopressin (VP), or antidiuretic hormone, is secreted in response to either increases in plasma osmolality (very sensitive stimulus) or to decreases in plasma volume (less sensitive stimulus). Its normal plasma level is very low (about 1 pg/ml, i.e. 10⁻¹² M), close to the detection limit of present immunoassays, and distinct antidiuretic effects are observed after infusion of small undetectable amounts of VP. (2) This antidiuretic action results from three main effects of VP on principal cells of the collecting duct (CD) mediated by occupancy of peritubular V2 receptors. (i) Increase in water permeability along the entire CD (via AQP2). (ii) Increase in urea permeability in only the terminal inner medullary CD (via UT-A1). (iii) Stimulation of sodium reabsorption, mainly in the cortical and outer medullary CD (via ENaC). VP also acts on medullary vasculature (V1a receptors) to reduce blood flow to inner medulla without affecting blood flow to outer medulla. Besides these actions, all concurring to increase urine osmolality in different and additive ways, other VP effects, probably exerted through V1a receptors located on luminal membrane, tend to limit the antidiuretic effects of the hormone. They induce the formation of prostaglandins which reduce V2-dependent cAMP accumulation in these cells and thus partially inhibit all three V2 effects. (3) Because urine is first diluted along the nephron before being concentrated in the medulla, VP is required, not only for urine concentration, but first for re-equilibration of tubular fluid osmolality with plasma osmolality, a step taking place in the renal cortex, and achieved through the reabsorption of large quantities of water (more than what is subsequently reabsorbed in the medulla to concentrate urine). Accordingly, VP effects on urine flow-rate are not linear. Small changes in plasma VP in the low range of urine osmolality will induce wide changes in urinary flow-rate, whereas in the upper range of urine osmolality larger changes in plasma VP induce much more limited further reduction in urine flow-rate. (4) Most likely, the different effects of VP require different levels of VP concentration to occur and are thus recruited successively with progressive rise in VP secretion.

Keywords: Hemodynamics; Hormones; Membrane permeability/physics; Receptors

1. Introduction

The neurohypophyseal hormone vasopressin (VP) or antidiuretic hormone (ADH) is crucial for the regulation of water conservation in the body. This is mainly due to the capacity of this hormone to increase the permeability to water in the last portion of the nephron in a regulated fashion, thus enabling the reabsorption of up to 10% of the filtered water. When the hormone is absent or unable to activate its antidiuretic receptors, diuresis and consequently water needs are enhanced about tenfold (a condition called diabetes insipidus, DI).

The present review addresses quantitative aspects regarding the antidiuretic action of vasopressin and its different components in the kidney and other organs, in healthy subjects and/or mammals in general. Differences

Abbreviations: VP, Vasopressin; ADH, Antidiuretic hormone (=vasopressin); dDAVP, deamino-8-arginine vasopressin; DI, Diabetes insipidus; V1aR, V1bR, V2R, VP receptors V1a, V1B and V2; PVP, Plasma vasopressin concentration; Pomo, Plasma osmolality; Uomo, Urine osmolality; Cuo, Solute-free water clearance; Tuw.o, Solute-free water reabsorption; GFR, Glomerular filtration rate; TAL, Thick ascending limb of Henle’s loop; DCT, Distal convoluted tubule; CNT, Connecting tubule; CD, Collecting duct; CCD, Cortical collecting duct; IMCD, Inner medullary collecting duct; OM, Outer medulla; IM, Inner medulla; AQP2, Aquaporin 2; ENaC, Epithelial sodium channel; UT-A1, Urea transporter A1.

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between species, strains and genders are also addressed but pathological situations are not considered here. The long-term consequences of sustained high VP concentration have been recently reviewed in other papers [1,2]. The abbreviations used here for kidney structures are those recommended by the International Union of Physiological Sciences [3]. Two remarks concerning the name of the hormone and its abbreviation: (1) vasopressin (VP) is the most commonly used name for this hormone although it plays little role in the control of vascular resistance and blood pressure in vivo. In contrast, it plays a crucial role in the control of water excretion. The term antidiuretic hormone (ADH) would thus be more appropriate and will be used in some instances in this review. (2) Extracts from pig neurohypophysis have long been used as a source of VP before the synthetic hormone became available. Because a genetic shift had occurred in the suborder of Suina (to which the pig belongs), replacing an arginine in position 8 by a lysine, it was necessary to distinguish between ‘lysine–vasopressin’ and ‘arginine–vasopressin’ found in all other mammals. Now that pig neurohypophyseal extracts are no longer used, it does not seem useful to keep adding ‘arginine’ to the term VP. This is why the abbreviation VP will be used in this review, rather than AVP.

2. Vasopressin secretion and its concentration in body fluids

2.1. Stimuli for vasopressin secretion

VP is synthesized in specific neurons of the supraoptic and paraventricular nuclei and stored in the neurohypophysis. It is released in the blood under two main stimuli (Fig. 1) (numbers 1–4 in Fig. 1 refer to different levels of VP secretion which will be discussed in subsequent sections of this review). The most important stimulus under physiologic conditions is the ‘effective’ osmotic pressure of the plasma (i.e. that generated by solutes that do not cross freely cell membranes). Changes in osmotic pressure are detected by special ‘osmoreceptor’ neurons located in the hypothalamus. Vasopressin secretion is also stimulated by changes in blood volume and pressure, detected by pressure-sensitive receptors in the cardiac atria, aorta and carotid sinus, and carried to the central nervous system by neurons of the vagus and glossopharyngeal nerves [4].

It is important to note that VP secretion is far more sensitive to small changes in plasma osmolality ($P_{osm}$) than to changes in blood volume, as illustrated in Fig. 1. VP is said to be ‘suppressed’ below a certain $P_{osm}$ threshold (which is probably due to our inability to measure very low concentrations of VP). Above this threshold, plasma VP concentration ($P_{VP}$) rises steeply in direct proportion with $P_{osm}$. An increase in osmolality of only 1% will change $P_{VP}$ by an average of 1 pg/ml, an amount sufficient to significantly alter urine concentration and flow, and VP may reach 15–20 pg/ml under a strong osmotic stress (Fig. 1). This extraordinary sensitivity confers on the osmoreceptor the primary role in mediating the antidiuretic response to changes in water balance. In contrast, the response to pressure–volume changes is exponential. Reductions in plasma volume by 5–10% usually have little effect on $P_{VP}$ whereas falls of 20–30% result in intense hormone secretion bringing $P_{VP}$ to a level many times higher than that required to produce maximum antidiuresis (up to 50–100 pg/ml) (Fig. 1) [5]. This is observed for example in the case of hemorrhage, during which the vasoconstrictive action of VP is crucial in attempting to limit the fall in blood pressure. When $P_{VP}$ needs to be measured in small animals, blood withdrawal may induce a prompt vasopressin secretion. It is thus necessary, in most cases, to sample trunk blood after rapid decapitation. However, body compression has been shown to induce intense VP release (VP can rise to several hundreds of pg/ml in a minute), possibly by eliciting stimuli associated with volume contraction [6]. In very small animals like
mice, this could lead to artefactly high $P_{VP}$ if blood collection is not quick enough and/or the animal is handled too tightly. Anesthesia and surgery also increase $P_{VP}$ significantly [7].

The osmoregulatory mechanism is not equally sensitive to all plasma solutes. Sodium chloride, which contributes for more than 80% of total $P_{osm}$, is the solute that most potently stimulates VP release [4]. Mannitol is also very effective. On the other hand, a rise in $P_{osm}$ due to urea or glucose causes little or no increase in $P_{VP}$. These different potencies probably originate in the rate at which the solutes move from plasma to inside the osmoreceptor neurons [4].

Nicotine is a potent stimulus of VP secretion [8–10]. Thus, in studies showing that smoking is a risk factor for several cardiovascular and renal diseases, a possible contribution of VP to these adverse effects of tobacco should be considered [11].

VP and thirst are jointly involved in maintaining body fluid balance. The threshold value of $P_{osm}$ that stimulates thirst is a little higher than that of VP release so that during most of usual life, VP is constantly present in the blood whereas thirst is perceived only discontinuously [12]. Body fluid balance can be severely compromised only if both limbs of this regulatory mechanism are perturbed simultaneously (or if free access to water is not provided). Alterations in only one of these two limbs will result in a different set point for plasma osmolality. Excess drinking (potomania) will tend to lower plasma osmolality which will then reduce VP secretion and allow an abundant urine output matching the excessive intake. On the opposite, a primary defect in hormone secretion or in its action on renal target cells will lead to a rise in plasma osmolality which will stimulate thirst and enable sufficient fluid intake to compensate for the marked increase in urine flow-rate.

2.2. Vasopressin in plasma and urine

The usual $P_{VP}$ in healthy, normally hydrated subjects is about 1 pg/ml ($10^{-12}$ M) and exhibits significant diurnal rhythmcy. Many (but not all) studies report values during the night about twofold higher than those during the day [13–15,4]. It is important to keep in mind that the usual range of $P_{osm}$ in healthy humans and most experimental animals is close to, or even distinctly lower than the detection limit of most present assays (which is around 0.3–0.5 pg/ml), and that small but functionally significant changes in $P_{VP}$ within the physiological range, may remain undetected. In water diuretic rats [16] and humans [17,18], infusion of very low amounts of VP can profoundly reduce urine flow-rate and rise urine osmolality ($U_{osm}$), as displayed in Fig. 2. Infusion of VP at 1 and 5 pg/min×kg BW, although resulting in very distinct antidiuretic effects, induced no detectable changes in $P_{VP}$ concentration. Only after infusion 25 pg/min×kg BW did a detectable increase in $P_{VP}$ occur (Fig. 2). Similarly, infusion of VP at a rate of 2.5 pg/min in Brattleboro rats with hereditary central DI (due to a genetic defect in the VP gene [19]) doubled their $U_{osm}$ and halved their urine flow-rate ($P<0.001$), but $P_{VP}$ remained below the minimum detectable level. Infusion rates of 25 and 100 pg/min induced greater antidiuretic effects and $P_{VP}$ reached 2.3 and 8.0 pg/ml, respectively [16]. Thus, much of the variation of $P_{VP}$ occurring in usual life in both humans and rats probably lies in the region below or close to 1 pg/ml [20].

VP is found in significant amounts in urine. After its filtration in the glomerulus, VP undergoes less reabsorption
and/or degradation in the renal tubule than many other peptidic hormones because of its disulfur bond. Moreover, because VP is concentrated in urine along with other solutes, urinary VP (and thus VP concentration in medullary collecting duct) may be 50–100 times higher than in plasma (120 pg/ml urine in rats [21], 50 pg/ml in humans [D.G. Bichet, J.L. Bresson, L. Bankir, personal observation]). Because of countercurrent shunting of water in medullary vasa recta, VP is also 10–20-fold more concentrated in papillary blood and interstitium than in peripheral plasma [22].

In some studies, it may be tempting to measure urinary VP excretion rather than plasma VP. However, changes in VP excretion may not reflect equivalent changes in $P_{vp}$. The amount of VP excreted depends on the amount filtered (which will vary with GFR) and on the amount reabsorbed by the nephron or degraded in the lumen (mainly in the proximal tubule). Now, VP clearance is dramatically influenced by osmolar clearance [23] and salt intake/output [24]. Measurements of VP excretion thus do not provide a constantly reliable index of changes in $P_{vp}$ and must be interpreted cautiously when GFR or solute clearance is inconstant or abnormal.

2.3. Experimental protocols used to modify the vasopressin/fluid axis

Many protocols used to modify endogenous VP level in vivo, such as oral water loading or dehydration, are often inadequate because they induce acute perturbations in water balance that greatly exceed the physiologic range of regulations seen in normal life, and thus induce a number of other neuro–humoral changes that may obscure the interpretation of the results. Water loads start to be excreted only after some delay and thus induce some degree of volume expansion and a fall in $P_{osm}$. Administration of exogenous VP in this condition does not mimic the spontaneous release which usually occurs in a setting of volume contraction and rise in $P_{osm}$. Several results obtained after water loading or massive i.v. infusion of hypotonic solutions (intended to ‘suppress’ endogenous VP secretion) revealed natriuretic effects of VP [25,26,17] which are not observed when $P_{vp}$ is risen in more physiological conditions, and which can probably be attributed to ANP release [27,28,20]. With variations of exogenous or endogenous VP within a normal range, without prior water loading, VP is actually antinatriuretic [16,29–32].

Most investigations of human renal function (not addressing the VP/body fluid axis) are carried out in a situation of high water diuresis intended to ensure easy voiding and thus more reliable urine collections. However, this procedure has been shown to represent a confounding factor in the understanding of several hormonal regulations related to the control of GFR and solute excretion [31,33–37].

Total water deprivation in experimental animals also provides an inadequate view of the influence of VP in the normal range of regulation. Too severe dehydration (e.g. 24 or 48 h in rats) results in significant volume contraction, loss in body weight, reduction in food intake (and thus in solute load to excrete and to concentrate). A better model of high endogenous VP is achieved by partial water restriction (e.g. to one third of spontaneous intake) for several days [35,36]. Administration of exogenous VP or of a V2 receptor agonist like dDAVP (desmopressin, a peptidic analogue of VP devoid of pressor action [40]) does not reproduce the same physiologic situation as that seen with the progressive rise in endogenous VP induced by water restriction [38].

In the first case, water is initially retained in the body and $P_{osm}$ tends to decrease. This reduces thirst, and a steady state can be reached with a lower urine flow-rate and a lower fluid intake. In the second case, water is initially lost from the body without possible replacement, and VP secretion and thirst are increased in response to a rise in $P_{osm}$. A steady state is not reached if water is totally suppressed. Volume contraction increases progressively together with body weight loss due not only to water loss but also to reduction in food intake. Thus a number of neurologic and hormonal regulations must be triggered in different ways with the two protocols. These differences may explain, at least in part, why higher maximum urinary concentrating capacity has been observed with endogenous rise in AVP than with exogenous administration [41]. Sodium and protein intake also influence the responsiveness of the kidney to vasopressin and maximum urinary concentrating activity [1,42].

A final difference must be taken into account if the infused hormone is a selective V2 agonist like dDAVP. In this case, the initial water retention will suppress endogenous VP release. As a result, all V1 receptor-mediated effects will be suppressed. In contrast, with a rise in endogenous VP, both V1 and V2 receptor-mediated actions will be triggered simultaneously. Symmetrically, infusion of a V2R antagonist will suppress V2R-mediated effects but will result in an increase in endogenous VP secretion [43–45] which will reinforce V1R mediated effects, along with V2 antagonism.

2.4. Gender, interindividual, interstrain, and interspecies differences

In several human studies, $P_{vp}$ was found to be about two-fold higher in males than in females and to be higher in African Americans than in Caucasians [24,46]. Less often mentioned is the fact that urine osmolality ($U_{osm}$) is also higher in males than in females (Fig. 3) [47,48]. A significant sexual dimorphism in the secretion and antidiuretic action of VP (and also in its vascular action, a topic not addressed in this review) has been well documented in rats and humans by Share and coworkers [49,50]. These authors showed that the antidiuretic response to i.v. infusion of VP (after an initial large water...
load suppressing endogenous VP secretion) is stronger in male than in female rats during the non-oestrus phases of their ovarian cycle, over a large range of VP infusion rates [51]. This higher response in males can be explained by a 60% higher density of V2 receptors in the papillary collecting duct of males (other segments of the collecting duct were not studied) resulting in a stronger ability of VP to stimulate cAMP production in papillary collecting duct cells [51]. Experiments involving gonadectomy and selective hormone replacement showed that these gender differences are due to estradiol which attenuates the antidiuretic action of VP, whereas progesterone and male steroids do not seem to influence this action [52,53]. Additional studies in rats in which prostaglandin production was inhibited by indomethacin revealed that renal prostaglandins, more abundantly produced in the female than male kidney, increase blood flow to the renal medulla and blunt the antidiuretic action of VP [47,54,55]. This action could depend on a V1a-mediated stimulation of prostaglandin production (see below).

Systematic studies in healthy subjects have established that the levels of VP secretion and thirst (thresholds and slopes of the relation with $P_{\text{osm}}$) exhibit large inter-individual variability, but high reproducibility in a given individual over several months or years [56]. Similarly, we have observed that $U_{\text{osm}}$ (on 24 h average) is widely variable among subjects (Fig. 3), but is quite reproducible in the same subjects [M.M. Trinh-Trang-tan and Bankir, unpublished results]. The same is true for rats even when they are all of the same strain, age, and sex and are fed the same diet with free access to food and water. In 42 male Sprague–Dawley rats (Iffa Credo, France), adapted to metabolic cages for 1 week, individual differences in mean daily $U_{\text{osm}}$ and flow-rate extended over an almost three-fold range, from 1100 to 3200 mosm/kg H$_2$O and from 6 to 17 ml/day, respectively [S. Fernandes, N. Bouby, L. Bankir, unpublished results]. Thus, in both rats and humans, VP secretion, thirst, and the level of urinary concentrating activity exhibit large inter-individual variability and are most probably genetically determined [56]. In line with this genetic influence, we have observed strain differences in rats. Sprague–Dawley rats exhibit higher VP levels and higher $U_{\text{osm}}$ than do Wistar rats [57].

Normal $P_{\text{vp}}$ in rats and dogs is in the same range as that reported in humans. In some rodents adapted to life in arid environments (gerboa, gerbil), much higher $P_{\text{vp}}$ values have been reported [58]. Mice, which possess a relatively high urine concentrating ability and exhibit a complex and well developed medulla, also seem to have high $P_{\text{vp}}$ [59] (however, artefactual high $P_{\text{vp}}$ could also result from blood sampling problems, as explained above).

3. Different sites of action of vasopressin in the kidney, liver and respiratory tract, related to water conservation

3.1. Vasopressin receptors, vasopressin agonists and antagonists

Three VP receptors have been identified and cloned, V2 (with cyclic AMP as a second messenger), and V1a and V1b (also named V3) (with calcium as second messenger) [60] (they will be referred to here as V1aR, V2R, and V1bR). They are expressed in many tissues and mediate a number of different cellular responses, many of which are part of a general integrated function linked to water conservation. The antidiuretic action of VP mainly depends on V2R-mediated effects in the renal collecting duct (CD). However, other actions mediated by renal V1aR probably also contribute, either to reinforce, or to blunt this antidiuretic action. They are listed in Table 1 and described in more detail below. Not shown in Table 1 is the presence of adenylate cyclase responsiveness to VP (suggesting the presence of V2R) in thin ascending limbs of Henle’s loops (with no identified function yet) [61] and the (suspected) presence of V1bR in the kidney. A calcium signal induced by dDAVP in the terminal CD has been considered to result from VP binding to ‘atypical’ V2R. It was recently identified as the V1bR [62].

A number of structural analogues of VP have been synthesized and their antidiuretic potency evaluated. Among them, deamino-8d-arginine vasopressin (dDAVP), considered to be a selective V2R agonist with negligible vasopressor activity, has been widely used in a number of animal and human studies [40]. However, recent studies have shown that this drug exhibits a similarly high affinity for V1b as for V2 receptors [63,64]. Thus, it is conceivable that some of the effects attributed to V2R are actually due, at least in part, to V1bR-mediated effects. Novel selective non-peptide V2R agonists have been recently produced.
Table 1
Target cells to vasopressin in kidney, liver, and lung, and their physiologic response to vasopressin

<table>
<thead>
<tr>
<th>Target cells</th>
<th>Receptor type</th>
<th>Physiologic actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal vessels and interstitial tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Glomerular mesangial cells</td>
<td>V1a (or OT?)</td>
<td>Contraction (in vitro), reduction in ultrafiltration coefficient (in vivo)</td>
</tr>
<tr>
<td>• Vasa recta (vascular smooth muscle cells and pericytes)</td>
<td>V1a</td>
<td>Decrease in blood flow to inner medulla (with preservation of blood flow to outer medulla)</td>
</tr>
<tr>
<td>• Medullary interstitial cells</td>
<td>V1a</td>
<td>Stimulation of prostaglandin synthesis</td>
</tr>
<tr>
<td>Nephron and collecting duct</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Henle’s loop</td>
<td>V2</td>
<td>Stimulation of sodium reabsorption</td>
</tr>
<tr>
<td>• Collecting duct (CD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Thick ascending limb</td>
<td>V2 (basolateral)</td>
<td>Increase in permeability to water (due to an effect on AQP2)</td>
</tr>
<tr>
<td>• In cortical and outer medullary CD</td>
<td>V2 (basolateral)</td>
<td>Stimulation of prostaglandin synthesis</td>
</tr>
<tr>
<td>• In terminal inner medullary CD</td>
<td>V2 (basolateral)</td>
<td>Stimulation of potassium secretion</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Hepatocytes</td>
<td>V1a</td>
<td>Stimulation of ureagenesis (along with gluconeogenesis)</td>
</tr>
<tr>
<td>Respiratory tract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Pneumocytes, type 2</td>
<td>V2</td>
<td>Stimulation of sodium reabsorption (through activation of ENaC)</td>
</tr>
</tbody>
</table>

[64,65] but differences between their integrated effects in vivo and those of dDAVP have not yet been evaluated.

A number of peptidic V2 antagonists have been designed but they retained significant agonist activity, especially in humans [66] and could not be administered orally. New selective, non peptidic, orally active V2R antagonists (called ‘aquaretics’) have been produced recently [44,67,68]. They offer a novel therapeutic strategy and represent useful research tools in the field of VP.

3.2. Multiple effects of vasopressin in the connecting tubule and collecting duct

Three different actions of VP on the CD, mediated by V2R and subsequent activation of adenyl cyclase, contribute to the urinary concentrating process by influencing (1) water permeability, (2) urea permeability, and (3) sodium transport (see Fig. 4A). These different effects are at least partially dissociated along the CD and may require different levels of VP to be recruited, as explained below.

First, VP increases the permeability of the CNT and CD to water, an effect depending on the insertion, in the luminal membrane of the cells, of preformed vesicles containing aquaporin 2 (AQP2). The basolateral membrane of these cells is not a limiting factor for water permeability of the CD because it constitutively expresses other aquaporins. Second, VP increases the permeability to urea of the terminal CD (tIMCD) located in the deep part of the inner medulla (IM) [69], an effect involving an activation of preexisting urea transporter UT-A1 molecules [70–72]. tIMCD cells express both UT-A1 and AQP2 but the two proteins are not located in the same subcellular fraction. Third, VP stimulates sodium reabsorption in most of the CD (except in the portion located in the inner stripe of outer medulla and in the upper IM). This effect is amiloride-inhibitable and involves an increase in the apical membrane Na⁺ conductance which is achieved through an activation of the epithelial sodium channel (ENaC) [73–76].

The three combined actions of VP on the CD all contribute to increase $U_{osm}$ in different and complementary ways, provided prior accumulation of solutes in the medulla has been achieved via countercurrent multiplication (mainly originating from the osmotic work of the thick ascending limbs) in order to generate an osmotic driving force for water. Water reabsorption progressively concentrates the luminal fluid in the CD. Because permeability of the CD to urea is relatively low, urea gets concentrated by this water removal until it reaches the tip of the papilla where VP increases urea permeability. This allows concentrated urea to diffuse into the medullary interstitium. A significant fraction of this urea is ‘trapped’ in the IM (or actually, continuously returned to the IM via a recycling process [70–72]), thus enhancing further the osmotic driving force for water reabsorption. This intra-renal urea recycling process increases the flow of urea in the loops of Henle (where urea enters through the urea transporter UT-A2 [77]) and enhances the ratio of urea concentration in urine relative to plasma in proportion to the rise in urine concentration. However, it also results in a lower ef-
Fig. 4. Effects of VP on the mammalian CD. (A) Diagram of the nephron showing the three V2 receptor-dependent effects of VP on CD. 1 = Increase in water permeability along the whole CD (effect on AQP2) which enables osmotic equilibration between CD lumen and surrounding interstitium. 2 = Increase in urea permeability (effect on UT-A1), occurring only in the terminal part of the IMCD, and enabling urea diffusion in the inner medullary interstitium, where it contributes to papillary hyperosmolality and thus to more intense driving force for water reabsorption. 3 = Stimulation of sodium reabsorption (through ENaC) which secondarily drives additional water reabsorption. This takes place most intensely (but not exclusively) in the cortical and outer medullary CD. (B) Diagram of a typical collecting duct principal cell showing the dual influence of V2 and V1a receptor-mediated effects on AQP2 and ENaC. Prostaglandins (PGs) produced upon V1a stimulation (probably luminal) reduce cAMP accumulation in the cell by stimulating specific phosphodiesterases, and thus blunt all V2-dependent actions. This is also probably true for the effect of VP on UT-A1 (not shown in this diagram). Non-steroid anti-inflammatory drugs (NSAID) reinforce the antidiuretic action of VP by inhibiting this pathway.

Besides these three V2R-mediated actions concurring to improve urine concentration, VP exhibits V1aR-mediated actions which tend to limit the V2 effects. V1aR are probably located in the luminal face of the cells [79–81]. Other studies have shown that V1a stimulation in CD activates prostaglandin synthesis which in turn reduce the V2R-dependent stimulation of adenylyl cyclase, thus reducing the intensity of V2R-mediated cellular effects [82,83] (Fig. 4B). These V1a–V2 antagonistic actions have been demonstrated only in rabbit CD but were not observed in rat CD, in vitro. However, we hypothesize that this V1a–V2 antagonism should also occur in the rat, and that some unidentified factor(s) may have impeded their disclosure in this species, because, in several experiments, we observed larger changes in various parameters related to the urinary concentrating mechanism when dDAVP (V2 stimulation only) than when VP was infused (V1 and V2 stimulation) or than when endogenous VP was increased by dehydration [38,84,85]. The attenuation of V2 effects by prostaglandins is well illustrated in vivo in dogs and humans by the fact that indomethacin (a prostaglandin synthesis inhibitor), as well as other non-steroidal anti-inflammatory drugs, significantly enhance $U_{osm}$ and/or the antidiuretic action of exogenously administered VP [86,87]. Similar results have also been observed in isolated or in situ rat kidney [88,29]. The reason why V1aR-mediated effects partially blunt the antidiuretic action of VP may be to prevent excessive antidiuresis when VP secretion rises to relatively high values [89]. α2-Adrenergic agonists also blunt the V2-mediated antidiuretic action of VP by reducing cAMP accumulation in CD cells [90], resulting in a decrease in their apical sodium conductance [91]. In vivo in rats, infusion of clonidine (an α2-adrenergic receptor agonist) in the renal artery induces the same diuretic and natriuretic influence as a V2 antagonist [30]. Finally, dopamine has also been shown to inhibit both VP-dependent cAMP production, water permeability and sodium transport in the rat CCD [92]. Thus, three different mediators are susceptible to attenuate V2 antidiuretic and antinatriuretic effects, prostaglandins, α2-adrenergic agonists, and dopamine (but only the first one is produced in response to VP itself).
Are the levels of vasopressin required to elicit the different actions of VP on the CD the same, or are the different actions recruited successively (and in which order) with progressively rising levels of VP? In vivo, extremely low infusion rates of VP have been shown to reduce urine flow-rate in water diuretic humans and rats (Fig. 2) [16,18]. This suggests that the effect on water permeability is very rapid and occurs for very low concentrations of VP. The effect on urea permeability is possibly also elicited for low VP concentrations, but the resulting increase in $U_{\text{osm}}$ will take more time to become apparent in vivo because urea accumulation in the renal medulla is a slow process, due to significant escape of urea through venous medullary blood (note that this escape is minimized in some species by unique anatomical adaptations [93–95]). In vitro, the effects of VP on water permeability of the CD and on the density of membrane particle clusters in the luminal membrane of CD cells (which are now known to represent AQP2-rich vesicles) are dose-dependent, as elegantly demonstrated by Harmanci et al. [96]. Star et al. established dose–response curves to VP in the rat CD in vitro. They observed parallel increases in the responses of both water and urea permeability (expressed as percentage of maximal response), for concentrations of VP ranging from $10^{-11}$ to $10^{-8}$ M [97], suggesting a coordinated simultaneous regulation of the two permeabilities (although UT-A1, the vasopressin-regulated urea transporter, and AQP2 are not localized in the same subcellular compartment). Note however that, for technical reasons, this study explored urea movements in the terminal CD in an unphysiological direction (from bath to lumen, i.e. opposite to what occurs in vivo) and that other experiments suggest that urea transport in the terminal collecting duct may not be symmetrical, as also reported for red cells [71].

In what concerns the effects of VP mediated by V1R, it is interesting to note that a 100-fold higher VP concentration is required to activate a calcium signal in rat IMCD than to induce an increase in cyclic AMP accumulation [97]. Because V1Rs seem to be located luminally (see above), these different sensitivities are in good agreement with the fact that VP is much more concentrated in urine than in circulating blood (see above).

With respect to the V2R-mediated effect of VP on sodium reabsorption, although no study has directly addressed this point, we postulate that it probably requires a significantly higher VP concentration than does the effect on water permeability (as for sodium transport in the thick ascending limb, see below). This different sensitivity of VP-dependent water and sodium transport in CD is apparent when reinvestigating the data of Hawk et al. who performed a dose–response study of VP effects on isolated rat cortical CD (CCD), as shown in Fig. 5A [98]. Moreover, this different sensitivity is also suggested by the fact that effects of very low infusion rates of VP to water-diuretic subjects in vivo reduces only urine flow-rate whereas larger infusion rates (still within physiological limits) reduce both urine flow-rate and sodium excretion [18]. Similarly, in healthy humans, we have observed that hourly sodium excretion is reduced along with urine flow-rate, in sequential urine collections throughout the day.
only when $U_{\text{osm}}$ rises above 600 mosm/kg H$_2$O [32] [L. Bankir and J.P. Mallié, unpublished results].

Another action of VP in the CD is to stimulate K$^+$ secretion [99]. This effect was shown to depend on cAMP and is thus likely mediated by V2R [100]. A recent study suggests that it could also be activated by luminal V1aR [81]. This effect on K$^+$ transport does not contribute to the urine concentrating activity but prevents one of its possible adverse consequences, i.e. K$^+$ retention that would otherwise result from the VP-induced reduction in urine flow-rate and the greater passive K$^+$ backflux occurring when transit time of urine in CD is lengthened [101,75,81].

3.3. Unimportance of vasopressin effect in the thick ascending limb of Henle's loop

After the discovery of a VP-sensitive adenylate cyclase activity in the rabbit and rat thick ascending limb (TAL) of Henle's loop by Morel [61], a number of studies have been devoted to various aspects of vasopressin action on this nephron segment [102]. In brief, VP stimulates sodium reabsorption in both the medullary and the cortical parts of the TAL, by activation of the basolateral Cl$^-$ conductance [103], as do other peptidic hormones, glucagon, calcitonin, and parathyroid hormone (the latter only in the cortical portion) [104]. The expected consequence of this effect is a more efficient accumulation of NaCl in the medulla by countercurrent multiplication, and thus a better driving force for water reabsorption in CD. Glucagon, calcitonin, and parathyroid hormone do indeed improve urine concentrating activity in vivo [104–108]. However, in the case of vasopressin, its effect on the TAL seems unimportant for the following reasons. (1) This effect requires a significantly higher $P_{\text{vp}}$ than that inducing an increase in water permeability in CD as illustrated in Fig. 5B [109]. (2) In a strain of rats which lack this VP-sensitivity of the TAL, urinary concentrating activity was normal even after 24 h water deprivation, and only a modest defect was noted after 48 h [110]. (3) This effect of VP on TAL is observed only in some rodents. It is absent in the human kidney and weak in the rabbit (species with relatively poor concentrating ability), significant in the rat, and strongest in the mouse and hamster (species with relatively high concentrating ability). Accordingly, this effect is regarded as an adaptation related to the improvement in urinary concentrating ability and is probably restricted to rodents [111].

The TAL is the site of significant reabsorption of Ca$^{2+}$ and Mg$^{2+}$, and VP has been shown to stimulate this reabsorption in isolated cortical but not medullary mouse TAL in vitro [112]. This effect of VP is also very intense in vivo, as shown by the unusually high fractional excretion of these two ions in Brattleboro rats, and its dramatic reduction by chronic dDAVP treatment [113]. This VP effect on divalent cations is not assumed to improve the urinary concentrating mechanism. However, because the salts of these cations have a relatively low solubility, a reduction in their abundance, as urine gets more concentrated under the influence of VP, will reduce the risk of stone formation and thus will prevent a potential adverse effect of VP.

3.4. Different effects of vasopressin on outer and inner medullary blood flow

A number of studies in the 1950–1990 period have established that VP reduces the so-called 'medullary' blood flow. This effect of VP makes sense because a low blood flow in the medulla should minimize the escape of solutes from the medullary interstitium via ascending vasa recta, thus favoring the maintenance of a high osmotic pressure which is crucial for inducing water reabsorption from the CD. However, the renal medulla contains several different zones which not only contain different nephron segments but also exhibit with very different vascular architecture and pattern of blood supply [93–95]. Recent studies have shown that the reduction of blood flow seen after physiological elevation in $P_{\text{vp}}$ is restricted to the inner medulla (IM), and that blood flow in the outer medulla (OM) is not reduced by VP [114]. A reduction of blood flow would be counterproductive in this area in which oxygen supply is not overdimensioned (as it is in the cortex) [115], given the intense metabolic activity of the TALs, related to their active sodium reabsorption, a crucial step in the concentrating process [116]. That VP is able to selectively decrease blood flow to IM without affecting that in OM is probably due to the combination of two features. First, a direct V1aR-mediated vasoconstriction probably occurs selectively or predominantly in the most central vasa recta of the vascular bundles, equipped with a thicker layer of muscle cells than more peripheral vasa recta, and extending down to the deepest regions of the medulla [117]. Second, stimulation of V2R (presumably in CD because no V2R has been found in intrarenal vessels [118]) results in the release of nitric oxide which attenuates the vasoconstrictor effects mediated by vascular V1aR [114]. Prostaglandins have also been shown to modulate the effects of VP on IM blood flow [82,83]. Indomethacin administration results in a significant increase in medullary blood flow both in vivo and in vitro, and more so in females (which have a more intense renal prostaglandin production) than in males [54].

3.5. Vasopressin action in non-renal tissues, likely also contributing to water conservation

In addition to its effects on the kidney, it is worth mentioning that VP stimulates urea synthesis, together with gluconeogenesis, in the liver (as do glucagon and epinephrine), an effect mediated through V1aR (see review in [70,123]). This could contribute to providing more urea to the kidney for improving urinary concentrating capacity.
This interpretation is supported by the fact that V1aR in the liver are much less abundant in humans and rabbits than in rats, and absent in sheep, a pattern that parallels the degree of adaptation to water conservation in these species (see review in [70]).

The rat and human fetal and adult lung possess V2 receptors [119], most probably co-localized with ENaC in type II pneumocytes. VP has been shown to play a major role in the clearance of alveolar fluid after birth [120,121]. In addition, recent studies suggest that VP upregulates ENaC in the lung, as it does in the kidney [76]. This VP effect appears to reduce respiratory water losses (indirectly approximated by calculation of ‘non-renal’ water losses) as shown in Fig. 6 [76,122]. VP has been shown to be antipyretic, i.e. to limit the rise in body temperature induced by bacterial infection. However, no data is available regarding a possible influence of VP on body temperature in normal conditions. No study, to our knowledge, has sought for a possible elevation in body temperature in Brattleboro rats, lacking VP, but indirect arguments suggest that it might be the case, as discussed by Bardoux et al. [123]. Because water loss through the respiratory tract depends on the temperature difference between alveoli and outside air, a VP-dependent reduction in body temperature could contribute, although modestly, to further limit water losses through airways.

4. Vasopressin-dependent water reabsorption: quantitative aspects

The most immediate criteria for evaluating an antidiuretic response is to look at the reduction in urine flow-rate (urine volume per unit time) and/or increase in $U_{\text{osm}}$. However, these two parameters provide a reliable index of the urinary diluting/concentrating activity only if the amount of solutes excreted per unit time is unchanged (or is equal between groups, if groups have to be compared). In case osmolar excretion changes (or is different between groups), the best index for evaluating and/or

Fig. 6. Urine flow-rate and extrarenal water losses (calculated as fluid intake minus urine flow-rate) in two different series of experiments in rats (means±S.E.M.). Mean urine osmolality (±S.E.M.) of the groups is shown below the graphs. (A) Urinary concentrating activity was chronically (1 week) either lowered by addition of large (HWI) or moderate (MWI) amounts of water added to the food, or enhanced by infusion of dDAVP (dDAVP) (Cont=normal rats) (three rats per group, each studied on two occasions). Data from [134] and from N. Bouby and L. Bankir, unpublished observation. (B) Two groups of six rats each were studied, one of which received a chronic infusion of the V2 receptor antagonist SR121463A (means±S.E.M.). Data from [122]. In both experiments, extrarenal water losses varied in parallel with urine flow-rate, suggesting that VP influences water reabsorption not only in the kidney but also in non-renal tissues (probably the respiratory tract). Asterisks indicate significant differences from respective control groups: *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$. 

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comparing the intensity of urinary diluting/concentrating activity is to calculate the ‘solute-free water clearance’ \( C_{H_2O} \) (\( C_{H_2O} = \text{urine flow-rate} - \text{osmolar clearance} \)). When urine is hypotonic to plasma (dilute urine), \( C_{H_2O} \) is positive, meaning that the kidney excretes ‘solute-free water’. When urine is hypertonic to plasma, \( C_{H_2O} \) is negative. In this case, the ‘solute-free water reabsorption’ \( (T_{H_2O}^c = -C_{H_2O}) \) represents the amount of water reabsorbed by the kidney to concentrate the solutes excreted at the observed osmolality above that of plasma [95].

### 4.1. Urinary dilution always precedes concentration

The capacity of the kidney to dilute urine appeared in lower vertebrates and was crucial for conserving solutes in species living in fresh water such as amphibians. The distal part of their nephron includes a ‘diluting segment’ which is able to reabsorb solutes in excess to water. The capacity to concentrate urine appeared only later with the folding of the nephron in a hairpin-shaped loop and the lengthening of the thin limb, running in parallel and countercurrent with the diluting segment (now called thick ascending limb), and thus permitting the countercurrent multiplication process which makes use of the ‘osmotic work’ of the diluting segment to accumulate solutes in the medulla [95].

ADH (vasotocin in lower vertebrates and VP in mammals) increases the permeability to water of the terminal part of the excretory organs (bladder in lower vertebrates and CD in mammals) and thus enables water to be reabsorbed when a favorable osmotic driving force exists. In lower vertebrates, \( U_{osm} \) can, at best, be re-equilibrated with \( P_{osm} \) under the influence of ADH. In mammals, urine can be re-equilibrated with \( P_{osm} \) in nephron segments located in the cortex (and expressing AQP2 and V2R), i.e. connecting tubule (CNT) and CCD. Only when urine flows in the medulla, in which solutes have been accumulated to generate a hyperosmotic environment, can additional water be reabsorbed and lead to production of hyperosmotic urine.

Thus, because urine is first inevitably diluted (irrelevant to the needs for water excretion or conservation), ADH action on the mammalian kidney is required, not only to concentrate urine above plasma osmolality, but also, in a first step, to raise \( U_{osm} \) from a very low value to the level of plasma (= re-equilibration). Dilution and re-equilibration (taking place in the cortex) and concentration (taking place in the medulla) are always successive steps in the formation of a concentrated urine. Dilution can occur without subsequent concentration, but concentration never occurs without prior dilution. Dilution is not achieved only in the TAL, because they can actively reabsorb sodium, the distal convoluted tubule (DCT), the connecting tubule (CNT), and the CD also contribute to dilute urine. This dilution can be observed only in the absence of ADH, when the reabsorbed solutes cannot drive an equivalent flow of water because of the too low basal water permeability of these nephron segments. This is why urine produced after large water loads (or prolonged water diuresis) exhibits an osmolality significantly lower than fluid collected in the early DCT, just close to the TAL exit (about 75 mosm/kg \( H_2O \) for urine versus 150 mosm/kg \( H_2O \) in the early DCT, as measured in rats). When ADH is present, this active solute reabsorption in post-TAL segments will drive additional isoosmotic amounts of water. Such ‘isoosmotic’ water reabsorption probably takes place in the late part of the cortical CD (CCD), beyond a site at which re-equilibration has been reached, and probably more intensely so with increasing levels of VP [75, 76].

### 4.2. Relative amounts of water reabsorbed in the different segments of the CD

As illustrated in Table 2 and explained below, a large fraction of the VP-dependent water reabsorption occurs in the renal cortex and lesser amounts of water are further reabsorbed in the medulla. This situation is functionally appropriate for improving the efficiency of the urinary concentrating process because it limits the transit of water in the medulla and thus the risk of dissipation of the cortico–papillary osmotic gradient. When tubular fluid exits from the diluting segment in the cortex, its osmolality is well below that of plasma and surrounding interstitium.

### Table 2

<table>
<thead>
<tr>
<th>Neuronal segment</th>
<th>Permeability to water</th>
<th>Ambient osmolality</th>
<th>Osmolality of tubular fluid</th>
<th>Volume of fluid remaining</th>
<th>Amount of water reabsorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>TAL, then DCT</td>
<td>Impermeable</td>
<td>300</td>
<td>100</td>
<td>12 ml</td>
</tr>
<tr>
<td></td>
<td>CNT, then CCD</td>
<td>Permeable (VP)</td>
<td>300</td>
<td>300</td>
<td>4 ml</td>
</tr>
<tr>
<td>Outer Medulla</td>
<td>OMCD</td>
<td>Permeable (VP)</td>
<td>600</td>
<td>600</td>
<td>2 ml</td>
</tr>
<tr>
<td>Inner Medulla</td>
<td>Upper IMCD</td>
<td>Permeable (VP)</td>
<td>1200</td>
<td>1200</td>
<td>1 ml</td>
</tr>
<tr>
<td></td>
<td>Terminal IMCD</td>
<td>Permeable (VP)</td>
<td>2400</td>
<td>2400</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>

Calculations in this table are made assuming that the solute load remains constant along the nephron (no solutes reabsorbed). If some solutes were reabsorbed, additional water would be reabsorbed isoosmotically to the solutes, but this would represent only small amounts compared to the figures shown here. For example, if 10% of the solutes were reabsorbed in the upper IMCD, it would lead to an additional 0.084 ml water reabsorbed to maintain tubular fluid isoosmotic to the interstitium.

For convenience, doubling of ambient osmolality was assumed to take place in OM, upper IM, and deep IM, successively.

For abbreviations of the neophron segments (TAL, DCT, CNT, CCD, OMCD, IMCD), see text.
If ADH is normally present and increases water permeability in the CD up to a point allowing full equilibration of osmotic pressure through CD epithelium, water reabsorption in CCD will bring tubular fluid to iso-osmolality with plasma. Assuming no simultaneous solute transport, this will halve the amount of water flowing in the nephron. Subsequent fluid reabsorption along the medullary CD will concern lesser and lesser amounts of water while $U_{\text{osm}}$ will progressively rise. The total amount of water reabsorbed to concentrate luminal solutes from 300 to 2400 mosm/kg $H_2O$ (assuming no simultaneous solute reabsorption) is 3.5 ml, i.e. less than the 8.0 ml reabsorbed in the cortex to concentrate urine from 100 to 300 mosm/kg $H_2O$. If 2/3 of the solutes present in the late CCD are actually reabsorbed in the medullary CD, urine volume will be 2/3 of 0.5 ml, and the total volume of water reabsorbed in the medulla will still be lower than that reabsorbed in the cortex (3.5+2/3 of 0.5 ml=3.83 ml).

CDs do not merge in the cortex and outer medulla [93]. Successive mergings of CD in IM result in a progressive reduction in the total cross-sectional area of CD luminal epithelium towards the tip of the papilla, and thus in less surface area of water-permeable membrane available for water reabsorption. The pattern of CD merging differs among species (less rapid in rats than in rabbits) [124]. Besides the area available for exchanges, the abundance of AQP2 molecules in the luminal membrane is also important. Kishore et al. quantified AQP2 protein per unit tubular length in different subsegments of the rat CD [125]. AQP2 abundance was about two-fold higher in outer medulla than in cortex, and decreased gradually along the course of CD in IM. Arcades, i.e. CNT (also expressing AQP2 and V2R [126]) were unfortunately not studied. It may be assumed that their AQP2 abundance and their capacity to reabsorb water are high since they are the first VP-responsive nephron segment situated just after the diluting segments, TAL and DCT.

The terminal portion of the inner medullary CD (IMCD) is a special segment, and its (t)IMCD cells are not identical to principal cells of earlier portions of the CD, including the early IMCD. In addition to exhibiting specific anatomical features [3,127], tIMCD cells express proteins that are not present in upstream parts of the CD, including the facilitated urea transporter UT-A1 and membrane receptors for atrial natriuretic peptide (ANP). They exhibit some (modest) active urea secretion [128]. Moreover, these cells exhibit, in unstimulated conditions, a higher basal permeability to water and to urea than cells of the more superficial IMCD [69]. As a result, the rise in water permeability induced by VP is less intense in this segment than in earlier portions of the CD. Note also that AQP2 abundance is lower in this late IMCD than in earlier portions of this structure [125]. Altogether, these observations suggest that the effect of VP on water transport is less intense in the terminal IMCD. In any case, very little water remains to be reabsorbed in this region (see above).

4.3. Amounts of water reabsorbed to concentrate urine up to different levels

Producing hypoosmotic urine does not mean that VP is not present and active. $U_{\text{osm}}$ in patients with nephrogenic diabetes insipidus or in rats with maximum diuresis is currently in the 75–100 mosm/kg $H_2O$ range [129,130,4,122]. Amounts of water involved in progressive VP-dependent urine re-equilibration and concentration are shown in Fig. 7A. The curve depicts the relationship between urine flow-rate and $U_{\text{osm}}$ in normal human urine, for a constant osmolar clearance of 1.7 ml/min (corresponding to an osmolar excretion of 510 μosm/min if plasma osmolality is 300 mosm/kg $H_2O$). The ordinate also shows the solute-free water clearance, $C_{\text{w,0}}$. Raising $U_{\text{osm}}$ from 75 to only 150 mosm/kg $H_2O$ is achieved by reabsorbing half of the initial water amount (bringing $V$ from 6.8 to 3.4 ml/min). At each subsequent doubling of $U_{\text{osm}}$, the amount of water reabsorbed by virtue of VP action is divided by two (see insert) and equals only 0.85 ml/min when $U_{\text{osm}}$ raises from 600 to 1200 mosm/kg $H_2O$.

These non-linear effects of VP explain why small doses of VP will have very intense effects on $V$ in the lower range of $U_{\text{osm}}$ whereas in the upper range of $U_{\text{osm}}$ when VP is already high, additional effects on urine flow-rate will be much less intense, even when large amounts of VP or V2 agonist are infused (Fig. 7B, and see also Fig. 2). Conversely, diuretics acting on the distal nephron (TAL, DCT and CD) or V2 receptor antagonists will seem very potent when endogenous VP levels are low, because they will lead to marked increase in urine flow-rate, whereas their effect will seem less spectacular in the upper range of $U_{\text{osm}}$, and higher doses will be needed to significantly inhibit the effects of endogenous VP.

4.4. Maximum water diuresis: species comparison

Rodents, in which a number of physiological studies are performed, differ from humans in several respects. First, one should keep in mind that their diurnal rhythm is inverse from that in humans, with most of the food intake and a greater fluid and solute excretion during the night than during the day. Thus, short-term studies in rodents, most frequently taking place during the day, fall in the resting period of the animals. Second, a number of functions including food intake and osmolar excretion are not scaled directly in proportion of body weight. With a markedly lower body weight and a higher food intake per unit body weight leading to higher relative excretory needs, rats and mice exhibit an overdimensioned kidney, compared to humans. Finally, these two species exhibit special morphological and functional adaptations of their kidney that result in a much more powerful urinary concentrating capacity than in humans [94] (Table 3). However, all three species are omnivorous and the composition of the diet given to rats and mice in modern...
Fig. 7. Non-linearity of VP effects. (A) Relationship between urinary flow-rate and osmolality for a constant osmolar clearance of 1.7 ml/min. Solute-free water clearance is also shown on the left side of the graph. The amount of water (per unit time) that needs to be reabsorbed under the influence of ADH for successive doubling of urine osmolality in the 150–1200 mosm/kg H2O range are shown by vertical double arrows and indicated in the insert. Very large in the range of hypoosmotic urine, the amount of water ‘saved’ by the effects of ADH declines sharply when urine reaches a range of higher osmolality. (B) Relationship between VP concentration and the intensity of its antidiuretic effects. In the low range of VP concentration, small increases in hormone level result in large effects (I), whereas much larger changes in VP concentration are required to induce only modest additional effects in the higher range of VP concentration (II). Conversely, relatively large amounts of antagonist are necessary to lower urine osmolality in the upper range, whereas small doses of antagonist will markedly increase diuresis in the lower range of VP concentration.

Laboratories is such that the proportions of the different osmoles in the urine (mainly sodium, potassium and urea) are relatively similar among these species (at variance with carnivores and herbivores) [70]. Because osmolar excretion per unit body weight as well as usual Uosm are far higher in rats and mice than in man, the loss of urine concentrating ability due to the absence of VP results in a much more severe diuretic state in rodents, as displayed in Table 3. Mice with inactivation of the V2 receptor or of the VP-dependent water channel AQP2 exhibit an extremely
severe DI phenotype and die shortly after birth, probably because of severe renal damage due to hydronephrosis [131,132].

At variance with these mice, Brattleboro rats [19], do not exhibit a too severe form of DI (Table 3). Their current $U_{osm}$ (180–250 mosm/kg H$_2$O) is distinctly above the minimum achievable in rats. Administration of a V2R antagonist in these rats revealed that a significant V2R-mediated concentrating activity takes place in their kidney. This is possibly due to modest (and unregulated) VP synthesis in peripheral organs, to spontaneous activation of V2 receptors, and/or to occupancy of V2R by oxytocin [122].

When an ADH treatment (VP or V2 agonist) is initiated in subjects or animals with DI or intense water diuresis, the resulting rise in $U_{osm}$ comprises two different steps. For example, in Brattleboro DI rats, $U_{osm}$ raises up to about 800–1000 mosm/kg H$_2$O in less than an hour, but full recovery of normal $U_{osm}$ requires about 24 h with a continuous infusion of VP or dDAVP. The rapid and initial rise results from osmotic equilibration of CD luminal fluid with surrounding interstitium permitted by the rapid VP-dependent increase in water permeability of the CD (note that, even in the absence of VP, sodium chloride — but not urea — is accumulated in the inner medullary interstitium [129,130,95]). The subsequent rise in $U_{osm}$ probably results from VP-dependent effects on urea permeability and sodium reabsorption in CD, and reduction in inner medullary blood flow. In contrast to sodium, urea accumulation in the interstitium requires ADH action on the CD, first, to concentrate urea in CCD and upper IMCD lumen by water reabsorption, and second, to enhance the permeability of urea in the terminal IMCD so as to allow urea diffusion in the interstitium [70–72].

5. Integrated and graded actions of vasopressin

5.1. Progressive recruitment of the different vasopressin effects with increasing plasma vasopressin concentration

In each VP-sensitive tissue, the intensity of VP effects depends on the level of VP to which receptors are exposed. The urinary concentrating process is a complex phenomenon which depends on a combination of factors in which the direct VP effects on the CD are only one aspect. Moreover, it is likely that the effects of VP which contribute to limit its antidiuretic action do not exhibit the same sensitivity to the hormone as those which induce its antidiuretic action. These different effects of VP are thus most probably recruited progressively and successively. No direct evaluation of this progressive recruitment has been established. Thus, the picture displayed in Fig. 8 is a proposed sequence of events that remains hypothetic, but which is based on reasonable assumptions (most of which are documented in preceding sections of this review).

In a normal situation, with normal access to fluid intake, VP varies within a relatively narrow range of plasma concentration and its main action on the concentrating activity of the kidney is probably exerted mainly through its effects on water and urea permeability of the CD (level 1 in Fig. 8). When VP level rises a little more (level 2), these effects become more intense and the influence of VP on sodium reabsorption in CD (and in TAL in rodents) may become significant. This VP level probably also activates V1aR which will reduce inner medullary blood flow (without affecting blood flow to the outer medulla) by its vasoconstrictive action on some vasa recta, and also possibly increase liver ureagenesis.

With higher needs to conserve water (longer period without drinking, or increased water loss due to heat or physical activity), $P_{VP}$ increases to a higher level (level 3 in Fig. 8) and this will result in an intensification of the effects induced by levels 1 and 2 of the hormone. Too intense antidiuretic effects will however be prevented by the progressive influence of VP on prostaglandin synthesis in both the CD (through V1a luminal receptors) and medullary interstitial cells. Prostaglandins will attenuate both the V2-mediated effects of VP on the CD and the V1a-mediated effects of VP on vasa recta. These combined effects prevent a too strong reduction in urine flow-rate (which would otherwise compromise solute excretion) and medullary ischemia. This attenuation of V2 effects with increasing level of VP above a certain threshold may explain why, when studying the dose–response of Brattleboro rats to chronic VP infusion, Cheng et al. noted that...
Fig. 8. Diagram showing the different effects of VP recruited successively as VP plasma level increases with increasing needs to conserve water. Levels 1–4 may approximately correspond to levels of hormone secretion shown in Fig. 1. With increasing levels, previously recruited effects are intensified, while other effects are added. *, VP stimulation of sodium reabsorption in TAL occurs only in rodents.

the highest rate of infusion induced less intense effects than the preceding infusion rate [133]. It may also explain why chronic AVP infusion in rats induces less dramatic effects than dDAVP on urea transporter expression in normal rats [84], or on the decline of renal function in rats with 5/6 nephrectomy [85]. This prostaglandin-mediated attenuation of antidiuretic VP effects is not a feedback control of the hormonal action because it is not triggered by the disappearance or reduction of the stimuli responsible for VP secretion. It is rather a local modulatory element preventing excessive reduction in the kidney’s excretory function.

With severe dehydration, all effects of VP are intensified, but they are less easy to identify because of the confounding influence of hemodynamic and neuro-humoral changes resulting from severe volume contraction. Moreover, GFR declines, thus bringing smaller amounts of tubular fluid to VP-sensitive nephron segments. Level 4 of VP secretion is not (or not only) triggered by hyperosmolality with variable degree of volume contraction, but (also) by isosmotic reduction in blood volume. In this case, the high level of VP is sufficient to induce general V1a mediated vasoconstriction and thus preserve adequate blood pressure control.

5.2. Vasopressin increases GFR in the normal range of urinary osmolality

In contrast to the fall in kidney perfusion and GFR observed when high VP secretion is due to severe dehydration and volume contraction, increases in VP concentration within a more physiological range, as seen in normal life, seem to enhance renal hemodynamics. Several studies have shown that sustained increase in VP level and the resulting high urinary concentrating activity result in an increased renal plasma flow and GFR [16,134] associated with an increase in kidney weight [1]. Acute changes in $P_{VP}$ also influence GFR if water loads are avoided. GFR was observed to vary in parallel with experimentally induced changes in $U_{osm}$, but only when the kidney produces hyperosmotic urine [135,136]. Moreover, as shown in Fig. 9, among different individuals (rats or humans), a significant correlation is observed between GFR and $U_{osm}$ or $T_{H,O}^{-1}$, only for values of $U_{osm} > P_{osm}$ [116,37]. The mechanism which may positively link GFR to the kidney’s concentrating activity is not yet elucidated. It seems to be indirect, as discussed elsewhere [78,134,70]. Its physiological significance could be (as for the rise in GFR occurring after a protein meal of a sustained high protein intake) to limit the rise in plasma urea concentration that results from the decreased efficiency of urea excretion in a concentrated urine [78]. That GFR is not sensitive to urinary concentrating activity in the hypotonic range is actually appropriate, because a reduction in GFR in this situation would reduce the amount of fluid delivered to the diluting segments of the nephron and thus limit the capacity of the kidney to excrete solute-free water when needed.
several actions of VP on the collecting duct and medullary circulation involving both V2 and V1a receptors. With more intense rise in VP secretion, a subtle balance is achieved between the antidiuretic effects of the hormone and additional modulatory effects which tend to prevent an excessive reduction in urine flow-rate and to maintain normal potassium and urea excretion in the face of an increased reabsorption.

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I want to thank two close collaborators, Nadine Bouby and Marie-Marcelle Trinh-Trang-Tan, as well as several Ph.D. students and other collaborators, Mina Ahloulay, Brigitte Pouzet, Carole Nicco, and Pascale Bardoux, who, by their experimental studies and by our frequent scientific discussions, contributed significantly to the ideas developed in this review. I also want to thank James A. Schafer (Birmingham, Alabama) for making available to me detailed experimental data from one of his previously published studies, thus enabling a more precise reanalysis of the dose--response curves shown in Fig. 5A.

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A large part of the ideas and figures presented in this review were presented at the 2nd Congress of the Federation of European Physiological Societies (FEPS, Prague, 1999).

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[3] Kriz W, Bankir L. A standard nomenclature for structures of the kidney. Published simultaneously in several journals. Am J Physiol 1988;254:F1–F7; Kidney Int 1988;33:1–7; Pflügers Arch 1988;411:113–120; Anat Embryol 1988;178:N1–N8. This relation was however not found in the 17 rats of the same study in which urine osmolality was lower than that of plasma, and which thus were excreting (not reabsorbing) free water. Adapted after [116]. (B) The same pattern was observed in six healthy humans undergoing GFR measurements during either high fluid intake leading to water diuresis (open circles, NS), or normal fluid intake (closed circles, \( P=0.011 \)) (two clearance periods per subject in each condition). Reproduced from [37].

6. Conclusion

In summary, the antidiuretic actions of VP observed in normal life depend on very modest changes in VP concentration which can induce relatively large changes in water excretion. This effect results from a combination of several actions of VP on the collecting duct and medullary circulation involving both V2 and V1a receptors. With more intense rise in VP secretion, a subtle balance is achieved between the antidiuretic effects of the hormone and additional modulatory effects which tend to prevent an excessive reduction in urine flow-rate and to maintain normal potassium and urea excretion in the face of an increased reabsorption.

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In summary, the antidiuretic actions of VP observed in normal life depend on very modest changes in VP concentration which can induce relatively large changes in water excretion. This effect results from a combination of


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