

## Central vasopressin: dendritic and axonal secretion and renal actions

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**Keywords:** dehydration; renal sympathetic stimulation; social aspects of vasopressin and oxytocin secretion; TRPVs; vasopressin receptors

### Introduction

On a daily basis, practising nephrologists evaluate hyponatremic or, on the opposite side, polyuric states using physio-pathological concepts related to central vasopressin secretion and water conservation by the kidneys. The purpose of this review is to highlight new basic and clinical developments describing the hypothalamic release and peripheral renal actions of the antidiuretic hormone, arginine-vasopressin. The classical vasopressin neuroendocrine pathway involves the axonal release of vasopressin from the neurohypophysis directly into the circulation. From there, vasopressin acts on V1 and V2 receptors to regulate blood volume and peripheral resistance. In the second neuronal pathway, vasopressin is released from axonal projections of paraventricular parvocellular neurons into the rostral ventrolateral medulla, nucleus tractus solitarius and intermediolateral column of the spinal cord, areas responsible for the integration of peripheral sympathetic and vagal outflow [1, 2] (Figure 1). A third source of vasopressin is the neuronal cell bodies and dendrites of vasopressinergic neurons [3].

I will dwell here on three new aspects of vasopressin as a central and peripheral hormone:

- The central 'dendritic' release of vasopressin from magnocellular cells of paraventricular nuclei stimulates neighboring preautonomic parvocellular neurons of the same nuclei bearing vasopressin V1a receptors and contributes to an increased renal sympathetic outflow: as a consequence, a dehydrated patient will have increased water reabsorption and renal vasoconstriction; both actions contribute to restore volume homeostasis [4] (Figure 2).
- The central, 'axonal' release of vasopressin and oxytocin targeting the brain amygdala expressing vasopressin and oxytocin receptors. Here, oxytocin decreases anxiety and stress and facilitates social encounters, maternal care and the extinction of conditioned avoidance behavior in rodents. At the same anatomical level, activation of vasopressin receptors will have opposite effects: vasopressin enhances aggressiveness, anxiety, stress levels and the consolidation of memory [5, 6]. Other central projections of vasopressinergic

neurons towards the ventral pallidum, lateral septum, retrosplenial cortex and anterior hypothalamus participate in pair bonding in monogamous voles and possibly in humans [7, 8].

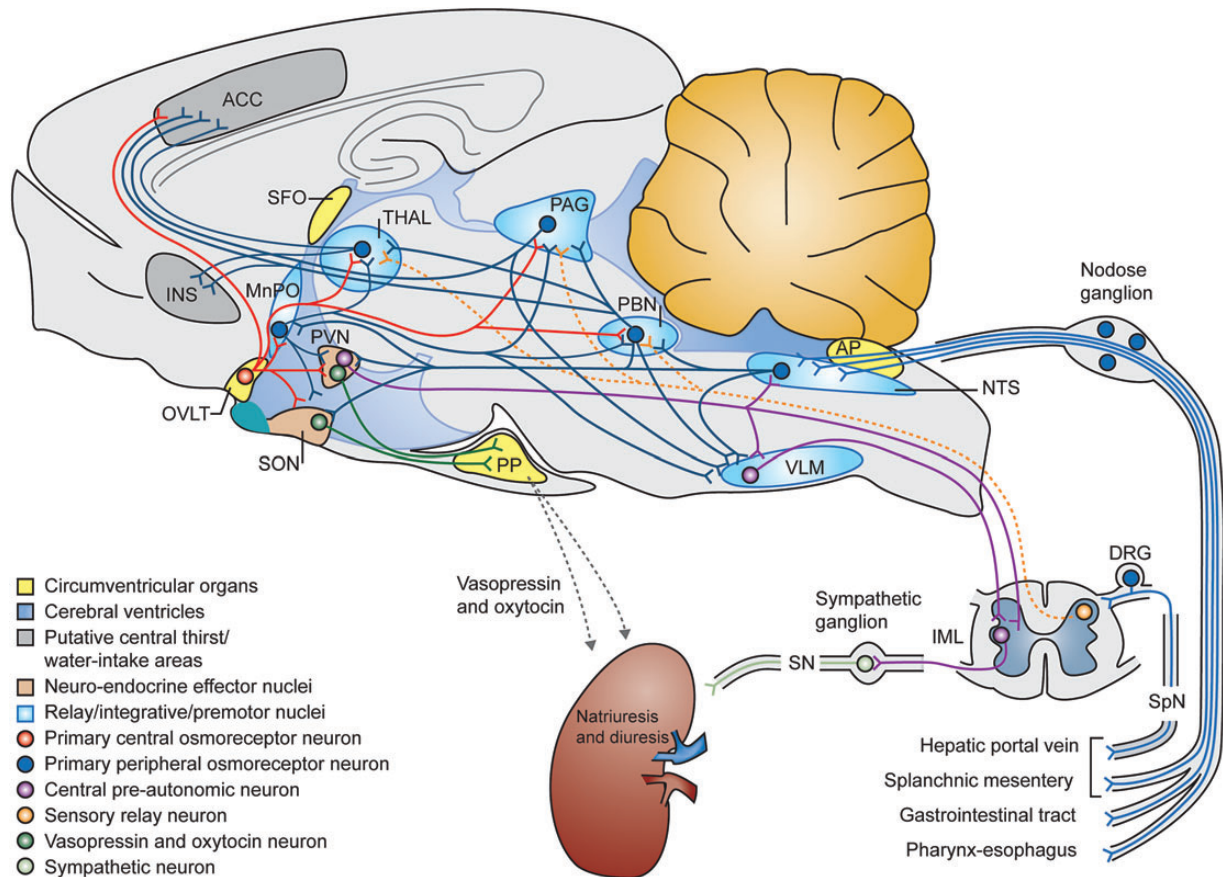
- After release from the axonal nerve terminals, at the posterior pituitary level, circulating vasopressin will rapidly engage vasopressin V2 receptors in the principal cells of the collecting duct and lead to the rapid insertion of aquaporin 2 water channels: as a consequence, an adult patient with central diabetes insipidus treated with vasopressin will benefit from the transcellular water reabsorption of 12–14 L of final urine presented normally at the beginning of the collecting duct, his urine excretion will only be <2 L/day [9].

The neuropeptide vasopressin primary signals thus include not only plasma vasopressin but also concentration of vasopressin in the extracellular fluid of the brain [3].

### Axonal vasopressin release from nerve terminals in the posterior pituitary gland into the general circulation

#### *Anatomy of vasopressin secretion from axon terminals*

Circulating plasma vasopressin is synthesized by a few (around 10 000 in the rat, 100 000 in humans) large (magnocellular) neurons whose cell bodies are located in the supraoptic and paraventricular nuclei of the hypothalamus [3]. Each magnocellular neuron has one axon that projects to the posterior pituitary, where it gives rise to ~10 000 neurosecretory endings that are packed with large dense-core vesicles (LDCV) containing the products of the vasopressin (AVP) gene: vasopressin itself, neurophysin II and copeptin. Vasopressin is small, only nine amino acids, and difficult to measure by radioimmunoassay; copeptin is slightly larger, amenable to a sandwich immunoluminometric assay by clinical biologists [10] and appreciated by neuroradiologists since it is responsible for the pituitary 'bright spot', an imperfect measure of the vasopressin content of the posterior pituitary: this hyper-signal could be lost in both central and nephrogenic



**Fig. 1.** Osmoregulatory circuits in the mammalian brain and the periphery. Neurons and pathways are color-coded to distinguish osmosensory, integrative and effector areas. Afferent pathways from the OVLT to ACC are responsible for thirst perception. Central preautonomic neurons in the PVN are responsible for the increased renal sympathetic activity mediated by perception of dehydration by magnocellular cells in closed proximity (see Figure 1). ACC, anterior cingulate cortex; AP, area postrema; DRG, dorsal root ganglion; IML, intermediolateral nucleus; INS, insula; MnPO, median preoptic nucleus; NTS, nucleus tractus solitarius; OVLT, organum vasculosum laminae terminalis; PAG, periaqueductal grey; PBN, parabrachial nucleus; PP, posterior pituitary; PVN, paraventricular nucleus; SFO, subfornical organ; SN, sympathetic nerve; SON, supraoptic nucleus; SpN, splanchnic nerve; THAL, thalamus; VLM, ventrolateral medulla. Reproduced from ref [1] with permission.

diabetes insipidus (NDI) with either low vasopressin secretion or, in NDI, with vasopressin release exhaustion [11]. Do not rely on the absence of the posterior pituitary 'bright spot' to confirm the diagnosis of central diabetes insipidus, but rather use it to describe the hypothalamic and posterior pituitary anatomy, look for secondary causes of central diabetes insipidus, in all patients with central unexplained polyuria.

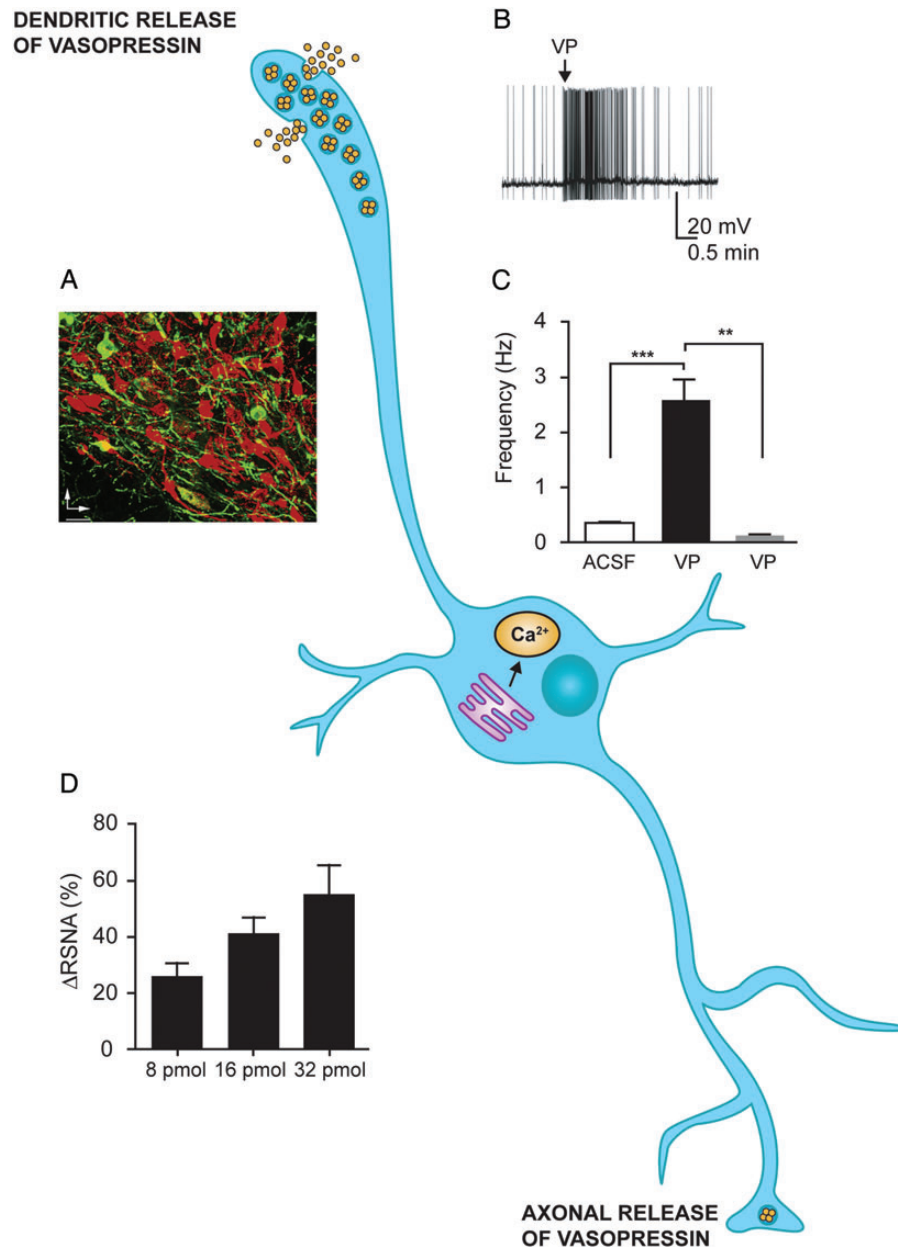
#### Stimuli of vasopressin secretion and thirst perception

The two main stimuli of vasopressin secretion and thirst perception are dehydration and volume depletion [12]. Dehydration is sensed by transient receptor vanilloid-type (TRPV1) channels inserted at the plasma membrane of magnocellular cells but also in osmoreceptive cells of the organum vasculosum of the lamina terminalis (OVLT) [1] (Figure 3). Cells of the OVLT are perfect osmoreceptors [14]. Cell shrinking induced by dehydration generates action potentials at the cell bodies of OVLT and magnocellular neurons, which propagate down their axons to invade nerve terminals. Depolarization of the nerve terminals triggers the exocytosis of LDCVs which release their contents into the general circulation. Neurons of the OVLT project to the anterior cingulate and insular cortex directly

and through thalamic relays and are responsible for the thirst sensation arousal and its behavioral effects [1]. Thirst and vasopressin release are also stimulated by afferent inputs generated through a decrease in effective blood volume perceived by high- and low-pressure receptors and ascending through the IX and X nerves (Figure 1). This central thirst perception originating from stretch receptors at the membrane surface of 'perfect' osmoreceptor cells in the OVLT, SON and PVN is fascinating: the five basic tastes (sour, sweet, umami, salty, bitter) are recognized by specific receptors on taste bud cells of papillae of the tongue [15], but water is not recognized by a specific receptor or channel there. Rather, dehydration signals are transferred to conscious brain areas in the anterior cingulate cortex and insula (Figure 1) [1].

#### The last millisecond in the life of a large dense-core vesicle containing vasopressin for synaptic or dendritic release

Regulated exocytosis of LDCVs to the nerve terminal membrane involves at least two membrane proteins synaptogamin and synaptobrevin/vesicle-associated membrane protein 2 (VAMP-2) of LDCVs and two proteins of the axon membrane, syntaxin-1 and soluble N-ethylmaleimide attachment protein of 25 kDa (SNAP-25). The general mechanism of membrane



**Fig. 2.** Schematic representation of dendritic and axonal central secretion of vasopressin from a magnocellular neuron of the paraventricular nucleus. (A) Vasopressin release from dendrites of magnocellular cells (green) is in close proximity to somato dendritic elements of presympathetic neurons (red). (B) 1 mM of vasopressin delivered on a presympathetic paraventricular neuron evokes a high-frequency burst of action potentials blocked by a vasopressin V1a receptor antagonist (C). (D) Dose-dependent increase in renal sympathetic nerve activity (RSNA) after microinjections of vasopressin (8, 16 and 32 pmol) onto the paraventricular nucleus (data and drawings from refs [3, 4]).

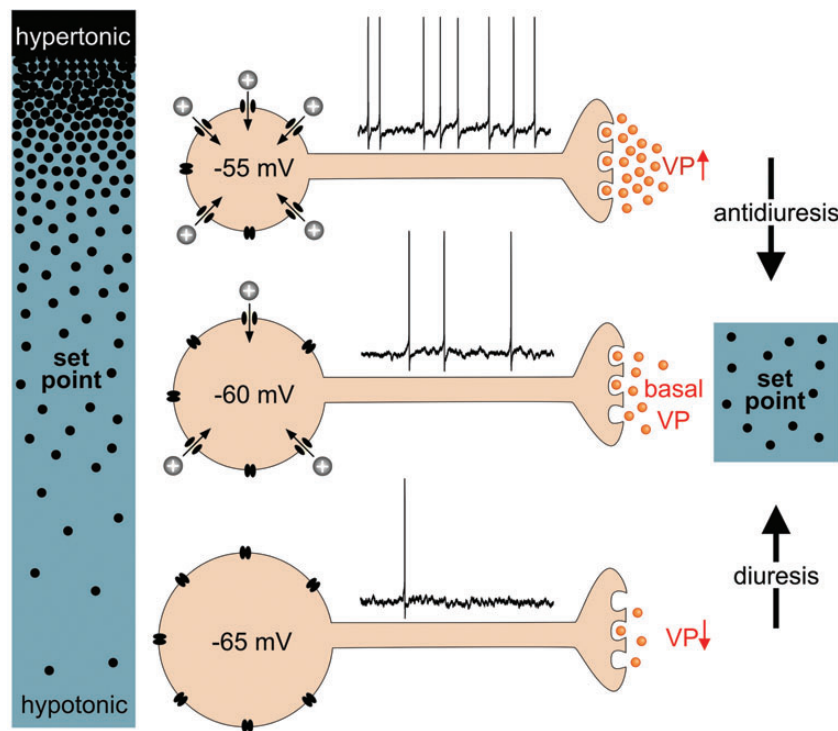
fusion has been described by Thomas Südhof, James Rothman and Randy Sheckman, recipient of the Nobel Prize in Medicine 2013 [16]. The release of oxytocin, and by analogy vasopressin, within the supraoptic nucleus can occur in a millisecond time scale [17].

There are differences in the expression and localization of exocytosis proteins between the axon terminals and somato dendritic compartments, since the exocytosis of large dense-core vesicles (LDCVs) from somata/dendrites do not use VAMP-2 and SNAP-25, the classical proteins mediating exocytosis at the nerve terminals [18]. The absence of VAMP-2 and SNAP-25 immunoreactivity suggests that different soluble N-ethylmaleimide sensitive fusion protein attachment protein receptor (SNARE)

protein isoforms might be expressed on LDCVs directed to the somata and dendrites. Synaptogamin, the proposed calcium sensor of exocytosis was not clearly seen, so we do not know the ‘turbocharger’ responsible for dendritic vasopressin secretion [19].

### Dendritic, hormone-like, intracerebral release of vasopressin: homeostatic responses and social recognition

Dendrites account for up to 90% of a neuron’s surface area, and dendrites from magnocellular cells are the



**Fig. 3.** Cell autonomous osmoreception in vasopressin neurons. Changes in osmolality cause inversely proportional changes in soma volume. Shrinkage activates nonselective cation channels (NSCCs) and the ensuing depolarization increases action potential firing rate and vasopressin (VP) release from axon terminals in the neurohypophysis. Increased VP levels in blood enhance water reabsorption by the kidney (antidiuresis) to restore extracellular fluid osmolality toward the set point. Hypotonic stimuli inhibit NSCCs. The resulting hyperpolarization and inhibition of firing reduces VP release and promotes diuresis. Reproduced from reference [13] with permission.

predominant source of vasopressin released in the brain (Figure 2) [3].

The release of vasopressin from dendrites and not the synaptic/axonal release of vasopressin explain the mismatches between vasopressin (peptidergic) innervation and V1a/b receptor distribution in the brain: there are no projections of vasopressin neurons in many areas of the brain, yet they respond to vasopressin stimulation and are endowed with V1a/b receptors. Mismatches for many other brain peptides seem to be more the norm than the exception [3]. Dendritic release of peptides can generate a prolonged hormone-like signal in the brain that can be self-sustaining. Vasopressin will be released in the extracellular brain compartment, its concentration, as measured by microdialysis experiments, has been shown to be as high as 1000 pg/mL, compared with usual plasma concentrations <10 pg/mL.

At least three populations of vasopressin-producing neurons demonstrate physiological responses through their dendritic release of vasopressin:

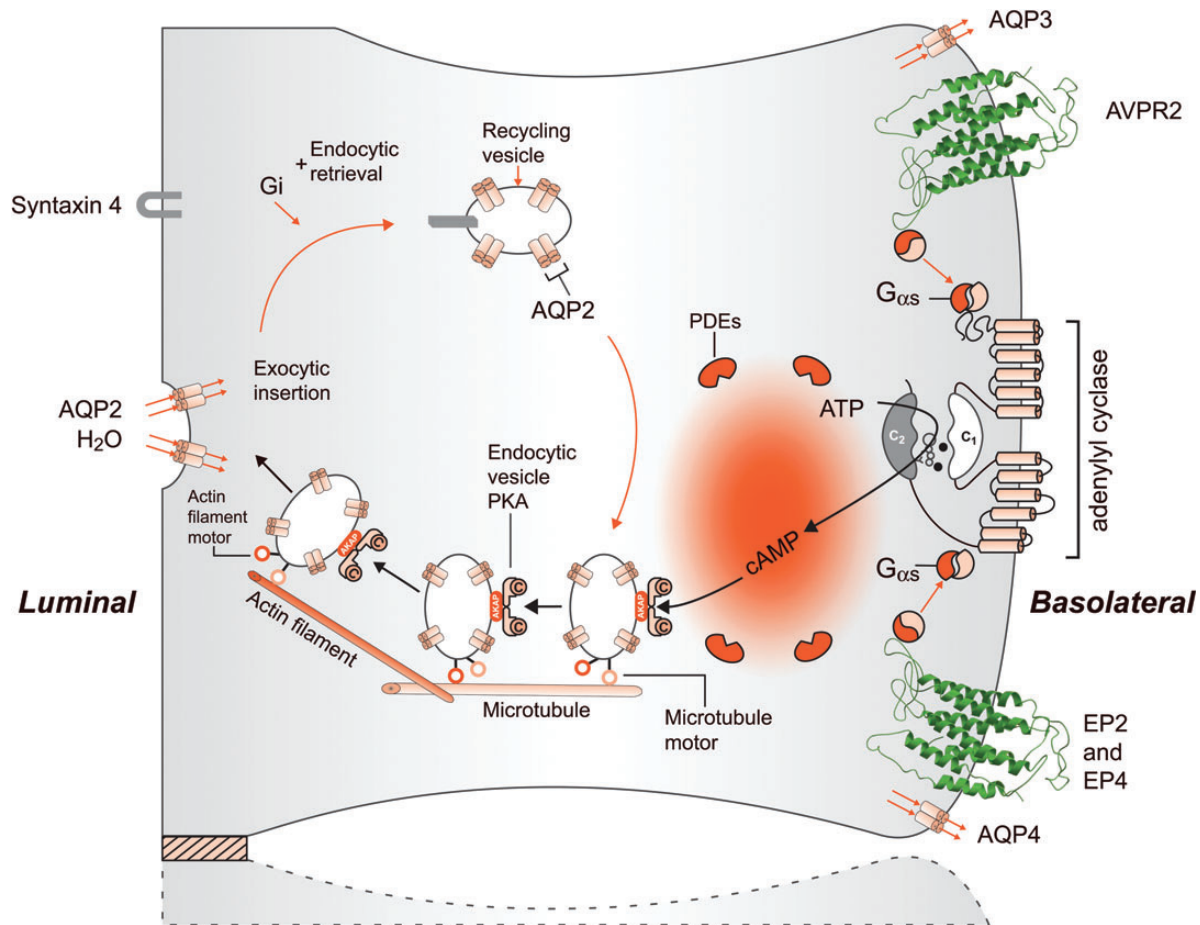
- (a) vasopressin neurons of the paraventricular magnocellular nuclei, responding to graded hypertonic intracarotid infusions of hypertonic solutions, release dendritic vasopressin stimulating neighboring parvocellular preautonomic neurons of the same nucleus bearing vasopressin V1a receptors and contribute to an increased renal sympathetic outflow [4]. This is an orchestrated homeostatic response within paraventricular nuclei aimed to respond to dehydration by both release of vasopressin and maintenance of blood pressure by the activation of the sympathetic nervous system (Figure 2).
- (b) the vasopressin neurons of the supra-chiasmatic nuclei release vasopressin from their dendrites as well as from

their nerve terminals and this release contributes to the CSF concentration of vasopressin which shows a circadian rhythmicity implying widespread diffusion of vasopressin from these supra-chiasmatic nuclei as well from the close-by magnocellular supra-optic neurons [20].

- (c) interneurons of the rat olfactory bulb express vasopressin and these vasopressin producing cells do not project outside the olfactory bulb, yet, they are an essential component of social recognition in rodents [21]. Vasopressin receptors, mainly V1a, are widespread in the main and accessory olfactory bulbs. Blocking the actions of vasopressin by a non-peptide V1 receptor antagonist into the olfactory bulb impairs the social recognition abilities of rats. Vasopressin is here a retrograde signal that filters activation of the mitral cells. Somato dendritic priming by vasopressin and oxytocin in the main olfactory regions may facilitate the formation of short-term social memories [22]. This is an experience-dependent release of vasopressin that could facilitate social recognition. Wacker and Ludwig are not suggesting that social recognition in humans depends on olfactory signals since vasopressin affects social behavior at many other sites (amygdala, hippocampus, striatum) as well as at the olfactory bulb, and in humans, olfactory recognition probably has a small role.

We are here entering the fascinating subject of social recognition mediated by neuropeptides including vasopressin and oxytocin. As described by Wacker and Ludwig, for social recognition to occur, an animal must recognize another animal as something it has encountered before. This involves an initial sensing of the subject, by sight, smell, touch, hearing, etc., the formation and potential





**Fig. 4.** Schematic representation of the effect of vasopressin (AVP) to increase water permeability in the principal cells of the collecting duct. AVP is bound to the V2 receptor (a G-protein-linked receptor) on the basolateral membrane. The basic process of G-protein-coupled receptor signaling consists of three steps: a hepta-helical receptor that detects a ligand (in this case, AVP) in the extracellular milieu; a G-protein ( $G_{\alpha s}$ ) that dissociates into a subunit bound to GTP and beta-gamma subunits after interaction with the ligand-bound receptor; and an effector (in this case, adenylyl cyclase) that interacts with dissociated G-protein subunits to generate small-molecule second messengers. AVP activates adenylyl cyclase, increasing the intracellular concentration of cAMP. The topology of adenylyl cyclase is characterized by two tandem repeats of six hydrophobic transmembrane domains separated by a large cytoplasmic loop, and terminates in a large intracellular tail. The dimeric structure (C1 and C2) of the catalytic domains is represented. Conversion of ATP to cAMP takes place at the dimer interface. Two aspartate residues (in C1) coordinate two metal co-factors (Mg21 or Mn21, represented here as two small black circles), which enable the catalytic function of the enzyme. Adenosine is shown as an open circle and the three phosphate groups (ATP) are shown as smaller open circles. Protein kinase A (PKA) is the target of the generated cAMP. The binding of cAMP to the regulatory subunits of PKA induces a conformational change, causing these subunits to dissociate from the catalytic subunits. These activated subunits (C) as shown here are anchored to an aquaporin-2 (AQP2)-containing endocytic vesicle via an A-kinase anchoring protein. The local concentration and distribution of the cAMP gradient is limited by phosphodiesterases (PDEs). Cytoplasmic vesicles carrying the water channels (represented as homotetrameric complexes) are fused to the luminal membrane in response to AVP, thereby increasing the water permeability of this membrane. The dissociation of the A-kinase anchoring protein from the endocytic vesicle is not represented. Microtubules and actin filaments are necessary for vesicle movement toward the membrane. When AVP is not available, AQP2 water channels are retrieved by an endocytic process, and water permeability returns to its original low rate. Aquaporin-3 (AQP3) and aquaporin-4 (AQP4) water channels are expressed constitutively at the basolateral membrane. Other G-protein coupled receptor, such as EP2, EP4 and the secretin receptor may also contribute to intracellular c-AMP increase (see text).

long-term consolidation of a memory, and the eventual remembrance of the subject in a subsequent encounter. Upon this recognition, an appropriate response may be aggression or affiliation depending of previous encounter and memory [22]. For example, female sheep can recognize their own offspring, allowing them, but not strange lambs, access to their milk.

#### 'Non-canonical' signaling of the vasopressin V2 receptor and biased g-protein coupled receptors (GPCRs) signaling from inside

The four different vasopressin/oxytocin receptor subtypes, respectively V1a, V1b, V2 and oxytocin (OT), have been

cloned in mammals, lower vertebrates and invertebrates. These are four members of the rhodopsin family within the more than 800 members of the superfamily of guanine-nucleotide (G)-protein-coupled receptors [23]. The V1a, V1b, V2, and OT receptors are strikingly similar in both size and amino acid sequence. However, the V1a, V1b, and OT receptors are selectively coupled to G-proteins of the Gq/11 family which mediate the activation of distinct isoforms of phospholipase C resulting in the breakdown of phosphoinositide lipids. The V2 receptor, on the other hand, preferentially activates the G-protein, Gs, resulting in the activation of adenylyl cyclase. The classical vascular smooth muscle contraction, platelet aggregation, and hepatic glycogenolysis actions of AVP are mediated by the V1a receptor that increases cytosolic calcium. V1a receptor mRNA was found to be extensively distributed

throughout the brain where AVP may act as a neurotransmitter or a neuromodulator in addition to its classical role on vascular tone [24]. Brain AVP receptors have been proposed to mediate the effect of AVP on memory and learning, antipyresis, brain development, selective aggression and partner preference in rodents, cardiovascular responsiveness, blood flow to the choroid plexus and cerebrospinal fluid production, regulation of smooth muscle tone in superficial brain vasculature and analgesia. V1b receptors are not only expressed in the anterior pituitary and kidney as originally reported, but also in the brain, uterus, thymus, heart, breast, lung and liver [25]. V2 transcripts are heavily expressed in cells of the renal collecting ducts (in humans and rodents) and in cells of the thick ascending limbs of the loops of Henle (in rodents only) [9]. The transfer of water across the principal cells of the collecting ducts is described in Figure 4.

In the current 'canonical' model of G-protein-coupled receptor signaling, arrestin terminates receptor signaling by impairing receptor-G-protein coupling and promoting receptor internalization. The vasopressin V2 receptor does not follow this conventional desensitization paradigm since  $\beta$ -arrestins prolong G protein (GS)-mediated cAMP generation triggered by vasopressin [26]. This recent observation is related to NDI since there was previous evidence that a mutant vasopressin V2 receptor associated with NDI remains in the endoplasmic reticulum yet generates cAMP when challenged with a membrane-permeant agonist [27]. To increase urine osmolality in NDI patients bearing loss-of-function of the vasopressin V2 receptor, some new avenues exist, including ligands [28] that could trigger a specific stimulation of intracellular cyclic-AMP through other GPCRs, like prostanoid receptors, also expressed in principal cells of the collecting duct [29].

*Conflict of interest statement.* None declared.

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Received for publication: 5.5.14; Accepted in revised form: 6.5.14