From vasopressin receptor to water channel: intracellular traffic, constraint and by-pass

J F Laycock and J Hanoune

Department of Neuroendocrinology, Division of Neuroscience and Psychological Medicine, Imperial College School of Medicine, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK and 1Unité-99, INSERM, Hôpital Henri Mondor, F-94010 Créteil, France

(Requests for offprints should be addressed to J F Laycock)

Introduction

The antidiuretic hormone vasopressin (VP) is synthesised in the magnocellular neurone cell bodies located in the hypothalamic supraoptic and paraventricular nuclei, and released into the general circulation from the nerve terminals in the posterior lobe of the pituitary gland (the neurohypophysis). Physiological control of the synthesis and release of VP is related mainly to changes in plasma osmolarity which are detected by specialised osmoreceptors in regions of the anterior hypothalamus which lie outside the blood–brain barrier e.g. in the subfornical organ and the organum vasculosa laminae terminalis (e.g. Thrasher et al. 1982, Yang et al. 1994). Although the precise pathways connecting osmoreceptor activation to VP release remain to be determined, nitric oxide is one molecule recently shown to be an inhibitory modulator of the hypothalamic–neurohypophysial system in response to osmotic stimuli (Ota et al. 1993, Yasin et al. 1993, Kadokawa et al. 1994, Wang & Morris 1996). In addition, an inhibitory pathway relates blood pressure to the release of VP, such that an increase either in arterial blood pressure detected by baroreceptors or in central venous pressure detected by cardiac volume receptors inhibits release of the hormone via a neuronal projection from the ventrolateral medulla (Head et al. 1987, McAllen & Blessing 1987). Other central projections are also involved in overall VP regulation. Although VP has physiological actions at multiple sites including the vasculature and the central nervous system, its principal physiological effect is the well-described increase in water reabsorption which takes place in the renal collecting ducts in the presence of an osmotic gradient resulting in an antidiuresis, hence its synonym antidiuretic hormone (ADH).

Of the three principal receptors (V1a, V1b, and V2) associated with the various effects of vasopressin, it is the V2 receptor which mediates the antidiuretic action in the collecting ducts. This receptor is located in the basolateral membranes of the vasopressin-sensitive principal cells of the inner (initial and terminal segments) and outer medullary collecting duct as well as the cortical arcades. The V2 receptor, cloned in the rat (Lolait et al. 1992) and in the human (Birnbaumer et al. 1992a), is a member of a family of proteins having seven intramembranous segments and three extracellular and four intracellular domains. The receptor is linked to adenylyl cyclase (AC) enzymes via heterotrimeric G proteins in the membrane; thus, AC is activated as a consequence of VP binding to its V2 receptor resulting in the formation of the second messenger cyclic AMP. Subsequent phosphorylation of protein kinase A is associated with the final step of water reabsorption. Cyclic AMP is rapidly degraded to 5′-AMP by intracellular phosphodiesterase enzymes. While various intermediate stages in the signalling pathway remain unclear, an important final step has recently been elucidated: the insertion of water channels into the apical (or luminal) membrane of the target cell, mediating rapid cross-membrane water transport. There is a family of water channels called aquaporins (AQP) which have been identified in plants and animals. Five of these membrane integral proteins are distributed in mammalian tissues ranging from erythrocytes to the lens of the eye where they mediate water transport (see Nielsen & Agre 1995, Knepper 1997), but only one (AQP2) is vasopressin-sensitive. This recently cloned molecule (Fushimi et al. 1993) is located in the apical membranes of collecting duct cells, and within the cytoplasm, where they appear to be stored in vesicles called aggraphores (Harris et al. 1991, Nielsen et al. 1993). In the presence of VP there is an immediate increase in the presence of AQP2 in the apical membranes and an increased movement of aggraphores towards these membranes, followed by a longer-term increase in AQP2 production within the cells, as determined by immunofluorescence (Marple et al. 1995a). There is evidence that microtubules might be implicated in VP-stimulated water transport (Phillips & Taylor 1989), perhaps associated with the movement of aggraphores to the apical membrane. Furthermore, VP depolymerises the actin cytoskeleton of the apical membrane of rat inner medullary collecting duct cells, suggesting the possibility that the actin network might be associated with the fusion of aggraphores with the apical membrane (Simon et al. 1994).
1993). How water crosses the cytoplasm to leave the cell through other, VP-independent, water channels in the basolateral membrane (AQP3 and AQP4) remains unclear (Fig. 1).

**Regulation of vasopressin-mediated water reabsorption**

There are many common points at which a hormonal system can be regulated including the synthesis, storage, release and transport of the hormone, its inactivation and/or removal from the circulation, the prevailing feedback control loops, and mechanisms operating at target tissue level. The normal variation in circulating VP plasma concentrations lies between 1 and 12 pmol/l, with maximal urine concentration occurring at the latter concentration. Synthesis, storage and release of VP are controlled by changes in plasma osmolality and, to a lesser degree, blood volume/pressure, which activate central pathways with input to the hypothalamus (e.g. see Chowdrey & Lightman 1993, Yamashita et al. 1995). Little is known about any regulation of VP levels within the circulation,
although interestingly approximately 90% is reportedly transported in association with platelets in humans (Nussey et al. 1986), and levels of free hormone could thus be dependent on changes in platelet function and number. Certainly, changes in the metabolism and removal of VP from the blood can influence fluid balance status as seen, for example, in a number of clinical conditions such as liver cirrhosis and nephrosis where hepatic or renal function is compromised. The ultimate site for control is the target tissue such as the distal nephron where, while the precise molecular mechanisms induced by VP are still unclear, there have been recent advances in the unravelling of the renal intracellular elements involved. This has prompted the present brief review of current aspects of the regulation of the VP receptor-to-water channel pathway.

Regulation of vasopressin action in the collecting duct cell: cellular events

1. In the absence of vasopressin

V2 receptors For many hormonal systems the target cells can regulate the physiological end-point by various means. For example, the activation of different deiodinases in peripheral tissues dictates whether thyroxine (T4) is metabolised to a more biologically active molecule (triiodothyronine, T3) or to a biologically inactive molecule (reverse T3). More commonly, a change in receptor number and/or affinity is an important mechanism in regulating hormone function at the tissue level. Receptor regulation can occur acutely when the receptor may be uncoupled from its G protein, and/or more chronically when receptor numbers can be either increased or decreased (up- or down-regulation) e.g. by synthesis or endocytosis respectively. The stimuli for receptor alterations may be varied and can depend on the hormone concentration in the plasma or the pattern of activation. For example, the distinctive pulsatile release pattern of hypothalamic gonadotrophin releasing hormone (GnRH) is associated largely with an uncoupling of receptors in the gonadotroph cells, so that at peak GnRH levels there is a corresponding decrease in tissue sensitivity of the adeno-hypophysial gonadotrophs to the hormone (Clayton 1984). Catecholamine β-adrenoceptors have been shown to increase by 100% following long-term treatment with the β-blocker propranolol (Glaubiger & Lefkowitz 1977) while in the ob/ob mouse there is a 300-fold decrease in adipose tissue β3 receptor expression (Collins et al. 1994). Prolonged treatment of pregnant women with the other neurohypophysial hormone, oxytocin, is also associated with a pronounced decrease (by approximately 50-fold) of its receptor mRNA (Phaneuf et al. 1997). Thus up- and down-regulation of receptors is a common regulatory process for many hormone systems and occurs when the circulating hormone concentration is less or greater respectively than the normal range.

Whilst vasopressin receptor numbers and/or affinity also vary, there are certain features which make this regulatory process particularly interesting. Contrary to other hormonal systems, where an up-regulation of receptors is expected when the circulating concentration is reduced, for VP the reverse occurs. A useful experimental model is provided by the Brattleboro rat with hereditary hypothalamic diabetes insipidus (BDI) due to the absence of circulating VP. The receptor binding in the kidneys of these VP-deficient animals is reduced by 30% (Rajerison et al. 1977) and V2 receptor mRNA is also lower by approximately 30% (Shen et al. 1997) compared with normal rats of the parent Long Evans (LE) strain, which is a more appropriate control (Valtin 1982) than the heterozygotes which fail to show significant differences (Shewey & Dorsa 1986). Furthermore, treatment of BDI rats with the V2 receptor agonist dDAVP results in an approximate 30% increase in mRNA for the V2 receptor (Shen et al. 1998), restoring receptor levels to those seen in LE rats. Because of the wide-ranging effects of VP other defects are also found in this strain. For example, their renin–angiotensin–aldosterone system functions at a higher level (Mohring et al. 1974), they have a reduced corticotrophin–corticosterone responsiveness to stressors (Buckingham & Leach 1980) and they are hypokalaemic (Mohring et al. 1974, Gartside et al. 1981). Therefore, it is possible that the reduction in V2 receptor numbers and the increase seen in response to VP treatment may be associated with any of these, or other, associated defects. It would appear that this is unlikely, because normal Sprague–Dawley rats treated with a specific non-peptidergic V2 antagonist resulting in a urinary dilution also showed a small reduction in V2 receptor mRNA (Shen et al. 1998). Nevertheless, the possibility that the lack of VP during embryonic development may alter the adult phenotype needs to be borne in mind.

Thus, it appears that VP in the circulation directly influences the synthesis of V2 receptors on the collecting duct cells through a V2 receptor-mediated mechanism, and is the key determinant of normal receptor numbers. Consequently, in the absence of VP (or other V2 receptor agonist) – or in the presence of a V2 receptor antagonist – there is a decrease in V2 receptor number and message i.e. down-regulation and not up-regulation (Fig. 2).

G protein regulation Coupling of the V2 receptor to the adenylyl cyclase catalytic unit takes place through the G proteins (either stimulatory (Gs) or inhibitory (Gi) α subunits). BDI rats lacking circulating VP have similar Gaα mRNA to normal parent strain LE rats, but have reduced Gaα mRNA expression particularly Gaα1 and Gaα3 (Shen et al. 1997). Furthermore, treatment of BDI rats with the V2 agonist dDAVP is associated with increases in Gaα mRNA expression, with Gaα1 and Gaα3 being particularly affected, but again no effect on Gaα (Shen et al. 1998). In the same study administration of a V2 receptor...
antagonist to normal Sprague–Dawley rats produced small decreases in $G_{\alpha_i}^{-1}$ and $G_{\alpha_i}^{-2}$ mRNA expression only. Thus the inhibitory G proteins appear to be influenced by VP in the same way as the V$_2$ receptors, presumably as a consequence of the linkage between the two. Of interest is the related finding that the impairment of water balance regulation which occurs with aging is associated with impaired cAMP accumulation in the medullary thick limb of the loop of Henle but this is not associated with changes in circulating VP levels or with changes in V$_2$ receptor mRNA, again suggestive of a change in the coupling between receptor and second messenger (Klingler et al. 1997). In this situation also a change in G protein expression could account for the decrease in VP-stimulated water reabsorption.

**AC regulation** Another intracellular point of regulation involves the catalytic unit adenylyl cyclase, and here also the BDI rat has been a useful animal model to study. Whether the AC response to VP (i.e. cyclic AMP generation) is abnormal in the BDI rat is still controversial. Reduced responsiveness in kidney medulla homogenates has been reported (Dousa et al. 1975). An isolated nephron segment study indicated that the sensitivity of the medullary thick ascending limb of the loop of Henle to VP was reduced (Imbert-Teboul et al. 1978). Treatment of BDI rats with dDAVP is not associated with any clear increase in total kidney adenylyl cyclase activity, either basal or VP-stimulated (Bia et al. 1979, Shen et al. 1998), although *in situ* cyclic AMP content of the renal papilla is significantly higher in VP- or dDAVP-treated rats.

**Figure 2** The likely sequence of intracellular events associated with the renal V$_2$ receptor–adenyl cyclase–aquaporin system in the absence of VP (i.e. central diabetes insipidus), or in the presence of a V$_2$ receptor antagonist. $G_i$ and $G_s$ represent the stimulatory and inhibitory G proteins respectively, the four renal adenylyl cyclases are identified as AC4, AC5, AC6 and AC9, and PKA and AQP2 represent protein kinase A and aquaporin-2 respectively.
Vasopressin and renal intracellular pathways · J F LAYCOCK and J HANOUNE

(Bia et al. 1979). Interestingly, a number of reports suggest that the BDI rat is more, not less, sensitive to exogenously administered VP than normal rats with respect to physiological end-points such as urinary concentration (Jones & Lee 1967) and pressor sensitivity (Laycock & Lightman 1989). Although basal and maximal VP-stimulated cAMP generation are lower in the BDI rat, the sensitivity of the AC system does indeed appear to be greater in the vasopressin-deficient animal compared with controls (Shen et al. 1997).

Four isoforms of AC (AC4, AC5, AC6 and AC9) are expressed in adult mammalian kidneys, and of these AC6 is predominant (for review see Hanoune et al. 1997). In renal tissue of BDI rats mRNA expression of AC4, AC5, AC6 and AC9 was reduced compared with LE rats (Shen et al. 1997). Treatment of BDI rats with the V2 agonist dDAVP resulted in an increase in the mRNA for AC5, AC6 and AC9, although AC4 mRNA decreased further (Shen et al. 1998). Treatment of normal rats with a specific V2 receptor antagonist was associated with slight reductions in mRNA for AC5, AC6 and AC9, although AC4 mRNA actually increased slightly, compared with untreated controls (Shen et al. 1998). Thus, as for the V2 receptors, VP (or a V2 receptor agonist) appears to be necessary for the maintenance of a certain level of AC expression; in the absence of hormone binding to its V2 receptor, AC5, AC6 and AC9 expression is reduced and is generally restored following VP or appropriate agonist treatment. The relevance of the changes in AC4 mRNA requires further investigation. The regulation of the phosphodiesterases which degrade cAMP, about which relatively little is known, is another intracellular component of the overall control of VP activity.

Post-cAMP regulation (aquaporin-2) While little is known about intermediate stages between the cAMP-mediated phosphorylation of protein kinase A and the synthesis, intracellular aggregation and mobilization of the AQP2 water channels, these latter stages are an interesting focus for current research in various fluid balance disorders. The aquaporin-2 gene has a cAMP response element which probably mediates the signal originating from the AC system which is activated following the binding of VP to its V2 receptor, consequently promoting increased aquaporin-2 expression (Uchida et al. 1994). When VP secretion is inhibited e.g. by water loading, there is a decrease in renal AQP2 expression (Nielsen et al. 1993). The BDI rat lacking circulating VP also has a reduced expression of AQP2 in the collecting duct apical membranes; treatment with VP is associated with its increased expression (DiGiovanni et al. 1994).

During lithium treatment, either water restriction or the administration of the V2 agonist dDAVP results in partial restoration of AQP2 expression within the principal cells. Interestingly, the two treatments differ in the location of the increase in AQP2 labelling, with water restriction being associated mainly with an increase in vesicle AQP2 while dDAVP treatment causes an accumulation of AQP2 in the apical membranes. Furthermore, the two treatments differ in their efficacy in stimulating water reabsorption (Maroples et al. 1995b). This suggests that the increase in AQP2 expression and its translocation to the apical membrane may involve the induction of different pathways. Regulatory mechanisms, both short-term (within minutes) and long-term (hours to days) have been proposed. The short-term mechanism is associated with the increased traffic of aquaporin-2 to the apical membrane into which the water channel is inserted (Nielsen et al. 1995, Sabolic et al. 1995, Yamanoto et al. 1995) while the long-term mechanism involves additional increased aquaporin-2 expression in the cell cytoplasm, presumably indicating increased synthesis (Nielsen et al. 1993).

All the studies to date indicate that in the absence of circulating VP or when the V2 receptor–cAMP system is depressed, expression of aquaporin-2 is also generally decreased (down-regulated), in contrast with the more common increased responsiveness (up-regulation) one might expect in these circumstances (see Fig. 2). A basal concentration of circulating VP appears to be necessary in order to maintain a set-point level of V2 receptor–adenyl cyclase–aquaporin-2 in the collecting duct cells.

2. In the presence of high concentrations of circulating vasopressin

V2 receptors In common with many other hormones, higher than normal concentrations of VP are associated with a decrease in V2 receptors (Fig. 3). Work supporting this down-regulation of vasopressin V2 receptors includes cell culture studies. For example, both LLC-PK and Madine-Darby canine renal cell lines exhibit a decreased responsiveness to acute exposure to VP if they have previously been cultured in the presence of raised VP concentrations (Lester et al. 1985, Aiyer et al. 1990). Also, a mouse fibroblast cell line transfected with the human V2 receptor showed a similar loss in sensitivity (Birnbaumer et al. 1992b). In a more physiological situation, a decrease in V2 receptor message has also been associated with raised VP concentrations. Thus 24-h dehydration in rats increased the circulating VP concentration 13-fold and this was associated with a 33% decrease in V2 receptor mRNA (Terada et al. 1993). The chronic down-regulation of V2 receptors under these conditions could involve various mechanisms including enhanced endocytosis and subsequent degradation, or diminished receptor synthesis (for a recent review see Bohm et al. 1997) but, at present, little is known concerning the precise regulatory mechanisms involved. We have recently observed that mice with hereditary nephrogenic diabetes insipidus (NDI) due to enhanced rollipram-sensitive phosphodiesterase and a correspondingly reduced cAMP generation (Homma et al.

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also have a renal V₂ receptor mRNA expression 25% lower than that of normal control mice (T Shen, J F Laycock & J Hanoune, unpublished observation; see Fig. 4). Various human V₂ receptor mutations identified in NDI patients have been transfected into specific cell lines. Expression of the mutated receptor in such cells is often associated with a lack of adenylyl cyclase activity (Rosenthal et al. 1993, Birnbaumer et al. 1994). However, to our knowledge there is no information concerning actual AC activity in situ (i.e. in the affected kidneys), for instance from biopsy material obtained from human NDI patients. Another form of NDI, induced in rats by lithium treatment, is also associated with an inhibition of adenylyl cyclase (Christensen et al. 1985).

**G protein and AC regulation** When BDI rats are treated with a V₂ receptor agonist there is a restoration of mRNA expression for Gᵦ₁ protein and renal AC isoforms to levels found in control LE rats, with no effect on Gᵦ₄ mRNA detected (Shen et al. 1998). However, currently

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**Figure 3** A possible sequence of intracellular events associated with the renal V₂ receptor–adenylyl cyclase–aquaporin system in the presence of high circulating concentrations of VP, based on current knowledge. Gᵦ and Gᵢ represent the stimulatory and inhibitory G proteins respectively, the four renal adenylyl cyclases are identified as AC4, AC5, AC6 and AC9, and PKA and AQP2 represent protein kinase A and aquaporin-2 respectively.
there is little information concerning changes in G proteins or in AC activity in conditions when VP concentrations in the circulation are acutely or chronically raised to supra-physiological levels. It is likely that when the V₂ receptors are down-regulated, there might be a similar down-regulation of the AC system. Whether a decrease in Gᵢ proteins also occurs in this situation, comparable to the reduction which occurs when vasopressin is absent or a V₂ receptor antagonist is administered (see earlier), is unknown. Interestingly, of the four renal ACs we have examined in mice recently, only AC6 was detectable, both in normal mice and in mice with hereditary NDI. Furthermore, the mRNA for AC6 in the NDI mice shows the presence of additional bands indicating that alternative splicing occurs; whether these are transcribed to proteins is unknown (T Shen, J F Laycock & J Hanoune, unpublished observation; see Fig. 4). In addition, the same study shows that Gₛ and Gᵢ protein mRNA expression are also comparable in NDI and control mice, the only difference being a 29% lower expression in Gᵢ₋₁ mRNA.

Post cAMP regulation (aquaporin-2) In complete contrast to the decrease in V₂ receptor expression, increased aquaporin-2 expression occurs following chronic vasopressin administration to BDI rats (DiGiovanni et al. 1994) and in normal rats following dehydration (Nielsen et al. 1993), and this is associated with increased osmotic water reabsorption. Chronic heart failure is also associated with a raised VP concentration and increased water retention. Recent studies of animal models with chronic heart failure have shown an increase in renal AQP2 expression (Xu et al. 1997) which is mainly located in the apical membranes of collecting duct cells, indicating an increase in water channel traffic to the membrane (Nielsen et al. 1997). The increased diuresis and decreased AQP2 expression which follow V₂ receptor antagonist admin-

istration in this condition indicates that the increase in AQP2 expression mediating the enhanced water retention of heart failure is driven by the V₂ receptor (Xu et al. 1997). There is no information yet available about the AQP2 status in NDI mice which have raised VP levels, a modest reduction in V₂ receptor mRNA and decreased cAMP activity. Furthermore, NDI can result from AQP2 gene mutations in humans, which nevertheless represent a minority (approximately 10%) of cases (Van Lieburg et al. 1994). Other conditions of polyuria such as lithium treatment or hypokalaemia are also associated with a down-regulation in AQP2 expression (Marple et al. 1995b). Interestingly, it is not only the general increase in AQP2 expression which is associated with fluid balance status, but also its location within the cells. Thus, water loading is associated with an increased translocation of AQP2 from membrane to intracellular vesicle without any change in total cellular water channels, as indicated by gold particle labelling density (Marple et al. 1995a). In contrast, V₂ agonist treatment results in the shift of AQP2 molecules from the intracellular vesicles to the apical plasma membrane (Marple et al. 1995a).

These studies indicate that while there is a down-regulation in V₂ receptors (and perhaps consequently further down the intracellular regulatory pathway) there can be an increased expression of the end-point VP-sensitive AQP2 water channel in the apical membranes (Fig. 4). This may provide a physiological ‘escape’ mechanism in situations such as dehydration when V₂ receptors are down-regulated, but which can become pathophysiological in clinical conditions associated with increased circulating VP concentrations. The potential use of oral V₂ receptor antagonists in water retention conditions associated with raised circulating VP concentrations such as chronic heart failure, cirrhosis of the liver

Figure 4 Expression of V₂ receptor, AC6 and of the G protein subunits Gₛα, Gᵢ₋₁, Gᵢ₋₂ and Gᵢ₋₃ in kidneys of NDI and normal (C) mice: Northern blots showing mRNA levels and the band separation for AC6 mRNA for the NDI mice relative to those detected in control animals. The mRNA levels were normalised to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
Implications of the V₂ receptor–adenylyl cyclase–aquaporin-2 system

From the above review of our current knowledge of the regulation of the V₂ receptor–AC–AQP2 system there are certain preliminary implications that can be drawn.

1. Absence of V₂ receptor up-regulation above a physiological set-point

The V₂ receptor appears to be ligand-dependent so that in the absence of VP or a V₂ receptor agonist such as dDAVP the V₂ receptor mRNA and receptor numbers are reduced. Thus, treatment with replacement hormone or appropriate agonist merely restores V₂ receptor expression to the normal physiological set-point.

2. Relative stability of the V₂ receptor–adenylyl cyclase system

All the results presented to date appear to show a relatively stable V₂ receptor system. It can vary, but only to a relatively limited extent compared with other hormones such as the catecholamines. The implication is that there is a predetermined, narrow, physiological ‘window’ of receptor numbers, and that an intracellular self-regulatory mechanism links the V₂ receptor to the circulating hormone concentration. Very little information is available about the molecular mechanism, but one possibility is that the Gα₁ proteins may in some way be a part of the intracellular negative feedback pathway. Such an internal regulatory system would provide a relatively stable target cell responsiveness to physiological changes in circulating hormone concentrations, leaving primary control at the hormone synthesis–release level.

3. Two VP-AQP2 pathways?

Since down-regulation of V₂ receptor expression appears to occur at the same time as an up-regulation of AQP2 expression in the presence of high concentrations of circulating vasopressin, a second pathway linking vasopressin and AQP2 expression can be evoked. This could provide the ‘escape’ mechanism mentioned earlier. A similar conclusion can be reached from the examination of the differing effects of water restriction and dDAVP on AQP2 expression in the continued presence of lithium (also see earlier). Thus one pathway could be related primarily to the synthesis of AQP2 while the other could be directed more towards the insertion of the water channels into the apical membrane.

For some years the possibility that VP might affect renal target cells through more than one receptor and/or intracellular mechanism, has been entertained (e.g. Teitelbaum & Strasheim 1990), with vasopressin V₁ receptors (e.g. Barberis et al. 1995, Løkat et al. 1995) and the oxytocin receptor (Teitelbaum 1991, Han et al. 1993) being candidates as additional alternatives to the V₂ receptor. The location of V₁₆ receptors on cells of the inner medullary collecting duct remains controversial, although there is general agreement that these receptors can be identified in the cortical and outer medullary collecting duct (Terada et al. 1993, Serradeil-Le Gal et al. 1996). A V₁₆ receptor-mediated pathway in the inner medullary collecting duct has been suggested in a study using a specific V₁₆ receptor agonist (Champignuelle et al. 1993), but this receptor has been linked with increased renal sodium excretion and may not have a role in water reabsorption (Musubayane et al. 1997). Furthermore, there is indirect evidence to suggest that this receptor might also be located on the apical membranes of collecting duct cells (Burgess et al. 1994, Ikeda et al. 1994). The V₁₆ receptor-mediated action is associated with activation of phospholipase C and the inositol triphosphate and diacylglycerol pathways which influence the intracellular calcium ion concentration (Kirk et al. 1983). Certainly, VP-induced mobilization of intracellular calcium in the inner medullary collecting duct has been reported (Star et al. 1988, Champignuelle et al. 1993). Since the concentration of VP in the urine rises in proportion to the exogenous dose administered (and presumably its increase in concentration in the blood) (Laycock & Williams 1973), activation of this V₁₆ receptor pathway could feasibly occur as VP appears in the tubular fluid in increasing amounts. Vasopressin-induced (at relatively high, nanomolar, concentrations) activation of intracellular prostaglandin synthesis has also been suggested as a second intracellular mechanism of action, and this might be linked to V₁₆ receptors located on the basolateral membranes of collecting duct cells (see Breyer & Ando 1994). The prostaglandins could then have an intracellular inhibitory effect on the AC system (Noland et al. 1992), thus opposing VP-mediated water reabsorption. Oxytocin receptors have also been located in the inner medullary collecting ducts, and these are associated with phospholipase C activation and calcium mobilization (Maeda et al. 1993). Thus it is possible that VP could influence water reabsorption via oxytocin receptors, particularly when circulating VP concentrations are raised (see Fig. 5). However, evidence that oxytocin receptors are unlikely to be involved in any VP-induced water reabsorptive process has recently been advanced (see Ecelbarger et al. 1996).

Another alternative is that the second pathway, commonly activated in conditions when VP concentrations are raised, could actually be induced by some associated stimulus such as raised plasma (or interstitial fluid) osmolality and might be VP-independent. Certainly, prior water balance ‘history’ results in an alteration in the numbers of intramembrane particle clusters (related to
AQP2 expression) in response to vasopressin added to the bathing fluid of isolated nephrons (Lankford et al. 1991). However, there is little other evidence for such a vasopressin-independent mechanism for regulating AQP2 expression at present. Thus, all these alternative pathways for VP are controversial, and would either tend to oppose the V₂ receptor-mediated antidiuretic action, or may be involved in sodium reabsorption regulation, and/or are not yet serious contenders for a second intracellular mechanism enhancing the water reabsorptive process.

The existence of a second V₂ receptor-mediated intracellular messenger system regulating water transport has also been postulated (Star et al. 1988). This could involve the mobilization of intracellular calcium following activation of the phosphoinositol pathway in the terminal collecting ducts (Zhu et al. 1994). When VP binds to its V₂ receptor in the collecting duct cell both cAMP and calcium mobilization pathways can be activated (Ecelbarger et al. 1996). This latter mechanism could by-pass the AC system, perhaps explaining how dDAVP can increase AQP2 expression in the apical membrane during lithium treatment (Marple et al. 1995a). How this second postulated V₂ receptor mechanism might increase AQP2 expression when there is a reduction in receptor expression associated with increased circulating VP levels but not when that receptor expression is reduced in the absence of ligand stimulation, is unclear. However, possible interactions between different intracellular systems

Figure 5 Hypothetical alternative VP-activated pathways involved in distal nephron water reabsorption might involve additional receptors for VP, including the V₁ receptor and the oxytocin (OT) receptor (both of which are currently thought not to be involved in the water reabsorptive process) and could be associated with the stimulation of the inositol triphosphate/calcium and/or prostaglandin (PG) synthesis.
such as the cAMP and calcium mobilization pathways may be a key feature of hormone-regulatory mechanisms at the target cell level.

Conclusion

While it is important to appreciate the preliminary nature of some of the findings described in this review, nevertheless three points can be made concerning the VP-directed control of water reabsorption at the level of the target collecting duct cells of the kidney. (1) The V₂ receptor–AC system in collecting duct cells does not appear to be subject to up-regulation in the absence of circulating VP. Indeed a down-regulation seems to occur in this instance, indicating that basal circulating VP concentrations normally maintain V₂ receptor numbers at a set-point level through an unidentified mechanism. (2) The V₂ receptor–AC system appears to be finely regulated and consequently receptor and adenylyl cyclase expression are relatively stable. The possible contribution of the Gᵦᵣ proteins to the intracellular self-regulating process remains to be elucidated. (3) It is possible that a second VP-stimulated, adenylyl cyclase-independent mechanism of action contributes to the overall control of aquaporin-2 expression and traffic to (and insertion into) the apical membrane when the circulating VP concentration is raised above normal physiological levels. The prospect of future advances in our understanding of the intracellular mechanisms linking VP and water reabsorption is not only important for increasing our basic knowledge of the regulation of a vital physiological action but should also provide major improvements in the treatment of a variety of pathological conditions of fluid retention.

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