

REVIEW

Structural bases of vasopressin/oxytocin receptor function

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Introduction

The neurohypophyseal hormones vasopressin and oxytocin are two closely related nonapeptides, synthesized mainly by the magnocellular neurons of the hypothalamus. Their peptide sequences differ only in the amino acids at positions 3 and 8, but, for both hormones, the formation of a disulfide bond between Cys residues at the 1 and 6 positions results in a peptide constituted of a 6 amino acid cyclic part and a 3 amino acid C-terminal part. Both peptides exert various hormonal effects. Circulating oxytocin is mostly known for its ability to elicit the contraction of uterine smooth muscle at term and that of myoepithelial cells that surround the alveoli of the mammary gland during lactation. The main endocrine functions of arginine vasopressin (AVP) are the facilitation of water reabsorption by the kidney and the contraction of smooth muscle cells in arteries. AVP released in the portal blood in the median eminence acts as a potent secretagogue of adrenocorticotropin. In addition, it has become clear that vasopressin and oxytocin, besides mediating well-documented functions at the periphery, are also critically involved in numerous central processes including higher cognitive functions such as memory and learning (see Barberis & Tribollet 1996 for review).

Vasopressin and oxytocin were the first biologically active peptides to be synthesized. This achievement, by Du Vigneaud and coworkers, 40 years ago, ushered in the modern era of peptide chemistry. During the subsequent decades, many structural analogues of the neurohypophyseal hormones have been synthesized and pharmacologically characterized (Manning & Sawyer 1993).

Peripheral vasopressin and oxytocin receptors have been classified on the basis of both the second messenger system coupled to the receptors and the affinity of a series of vasopressin and oxytocin analogues with enhanced selectivity for a certain receptor type. These classification criteria have led to the distinction of V1a vasopressin (liver, smooth muscle cells from blood vessels, and most peripheral tissues expressing vasopressin receptors), V1b vasopressin (adenohypophysis), V2 vasopressin (kidney) and oxytocin (uterus, mammary gland) receptors (Jard *et al.* 1988). To date central receptors have been identified

as being of the vasopressin V1a and oxytocin subtypes. Moreover, a great number of molecular probes have been developed, including agonists and antagonists, and radio-labelled, fluorescent or photosensitive ligands. These make this receptor family a good model with which to study structure–function relationships.

Today, the four different receptor subtypes have been cloned in mammals, lower vertebrates and invertebrates. Molecular cloning studies have renewed interest in these neurohormone receptors. Vasopressin V1b receptor mRNA has been detected in peripheral tissues (kidney, thymus, heart, lung, spleen, uterus and breast) and numerous areas of the brain in the rat (Lolait *et al.* 1995), and this receptor subtype has also been characterized in rat adrenal (Grazzini *et al.* 1996). These studies suggest that the vasopressin V1b receptor may serve additional and unknown functions in the brain and at the periphery. The primary focus of this review is to summarize recent studies that have led to novel insights into the molecular bases of vasopressin and oxytocin receptor functions.

General structural features

Molecular cloning of this family has confirmed that vasopressin/oxytocin receptor subtypes are members of the G-protein-coupled receptor (GPCR) superfamily, consisting of seven hydrophobic transmembranes α -helices joined by alternating intracellular and extracellular loops, an extracellular N-terminal domain, and a cytoplasmic C-terminal domain (Fig. 1). They display a high degree of sequence identity, showing about 102 invariant amino acids among the 370–420 amino acids in the human receptors (Fig. 2). In addition, vasopressin/oxytocin receptors display the structural hallmarks characteristic of most GPCRs: glycosylation on Asn residues present in the extracellular domains, a disulfide bridge between two highly conserved Cys residues in the second and third extracellular domains, and two relatively well-conserved Cys residues within the C-terminal receptor domain, which have been shown to be palmitoylated in other GPCRs (Ovchinnikov *et al.* 1988, O'Dowd *et al.* 1989). Whereas the disulfide bond appears to be required for the

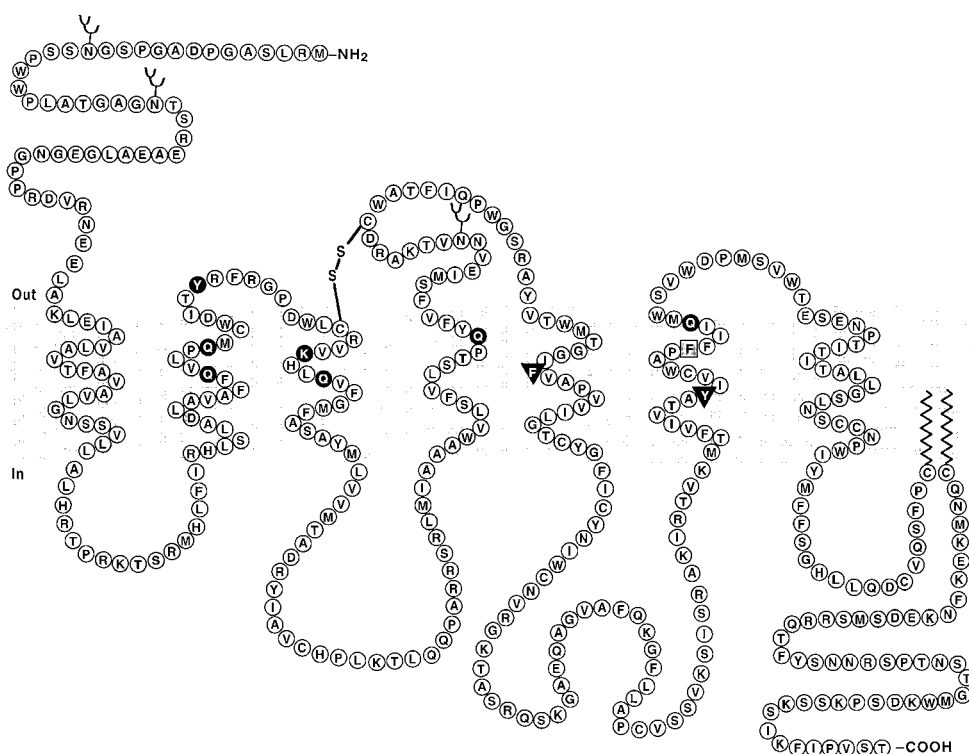


Figure 1 Transmembrane topology of the human vasopressin V1a receptor showing functionally important residues. Amino acids highlighted in black circles are critically involved in agonist binding (see text for details); that in the grey square is possibly involved in antagonist binding; that in the grey circle is a conserved amino acid involved in activation of the receptor; those in black triangles modulate the process of receptor activation in oxytocin receptor (see text for details). Potential glycosylation (on Asn 14, 27 and 196) and palmitoylation (on Cys 365 and 366) sites are also indicated.

correct folding of vasopressin/oxytocin receptors, the potential functional relevance of the glycosylation and palmitoylation of vasopressin/oxytocin receptors remains to be established. Cys¹²⁴ and Cys²⁰⁵ in the rat V1a receptor are possibly involved in the tertiary structure of the receptor, as suggested by the fact that [³H]AVP binding to V1a receptors of rat liver is altered by the presence of free sulfhydryl group alkylating agents such as *N*-ethylmaleimide (Gopalakrishnan *et al.* 1988). The same is true for human platelet V1a receptors and bovine and porcine V2 receptors (Pavo & Fahrenholz 1990, Thibonnier *et al.* 1993). In the pig V2 lysine vasopressin receptor expressed in the LLCPK1 cell line, it has been reported that glycosylation has a minor role in the transport and function of receptor (Jans *et al.* 1992). More recently, Innamorati *et al.* (1996) have shown that the functional properties (including hormone binding, coupling to the α subunit of the G proteins, desensitization and internalization) of the non-glycosylated human V2 vasopressin receptor are unaltered. Similarly, in the human V2 vasopressin receptor, palmitoylation does not seem to be important for receptor binding and signaling (Sadeghi *et al.* 1995, 1997).

Structural bases of ligand binding

Agonists

Consistent with findings obtained with the adrenoceptors (Dohlmán *et al.* 1991) and muscarinic receptors (Wess 1996), ligand binding to vasopressin receptors is predicted to occur in a pocket formed by the ring-like arrangement of the seven transmembrane domains (Mouillac *et al.* 1995). In view of the great number of residues that appear to be involved in vasopressin binding, it may be speculated that the hormone–receptor complex is characterized by an intricate network of hydrogen bond interactions, rather than by a few well-defined points of contact (Fig. 3). The conclusions drawn from the mutagenesis experiments are consistent with three-dimensional molecular modeling studies of vasopressin and vasotocin receptors, which suggest that the agonist-binding site is located in a narrow cleft delimited by several transmembrane (TM) domains (primarily TM II–VII), about 15 Å away from the extracellular surface.

Mutagenesis studies have shown that replacement of the conserved Gln residues in the rat V1a receptor situated in TM II, III, IV and VI and a Lys residue localized in TM

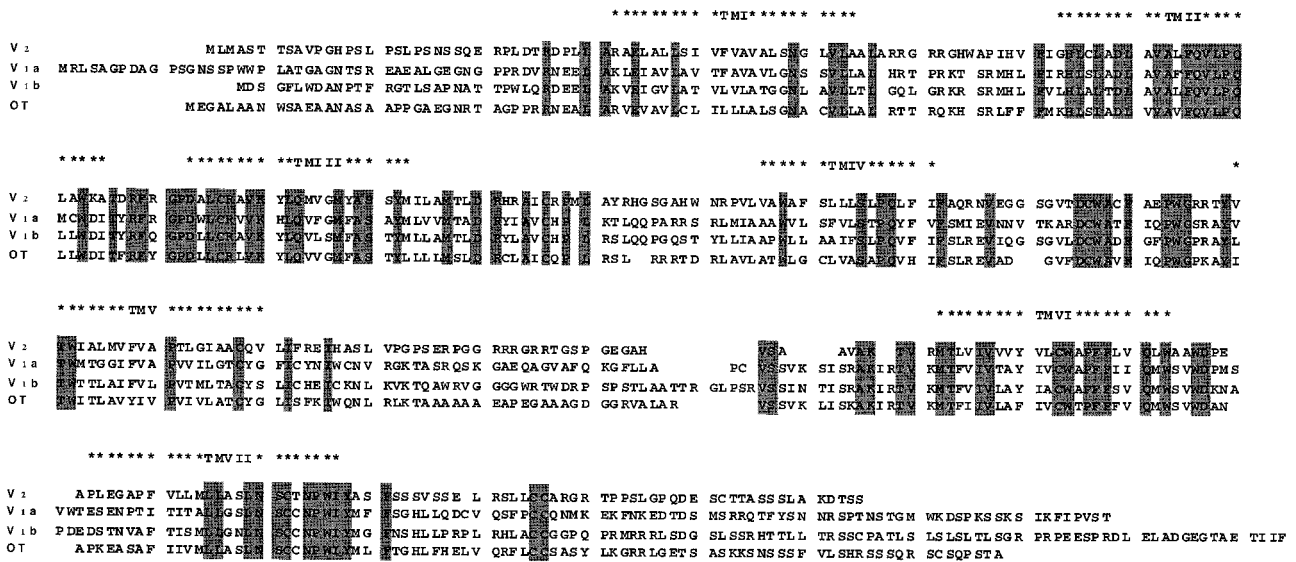


Figure 2 Primary sequence comparisons between human vasopressin/oxytocin receptors. Gaps have been introduced to maximize sequence identities. Asterisks denote predicted transmembrane regions I–VII. V2, amino acid sequence of the human V2 receptor (Birnbauer *et al.* 1992); V1a, amino acid sequence of the human V1a receptor (Thibonnier *et al.* 1994); V1b, amino acid sequence of the human V1b receptor (de Keyser *et al.* 1994, Sugimoto *et al.* 1994); OT, amino acid sequence of the human oxytocin receptor (Kimura *et al.* 1992).

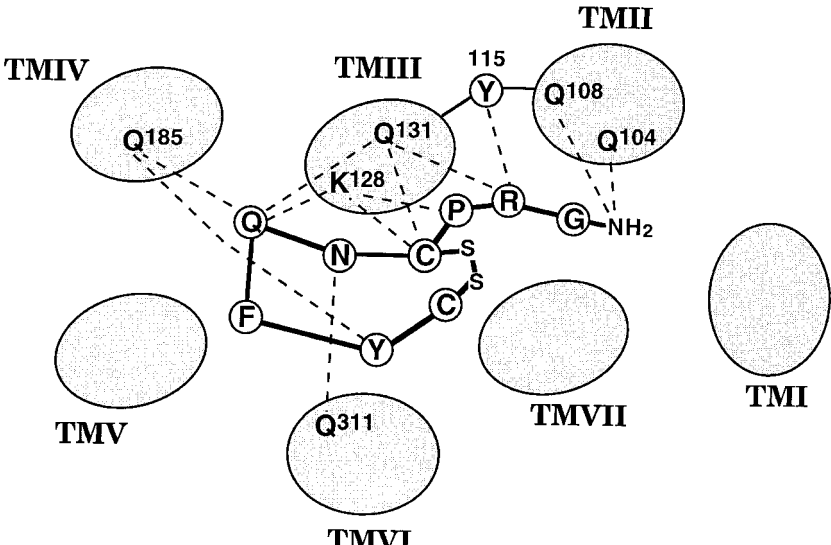


Figure 3 Schematic representation of the interaction of vasopressin with the membrane-spanning domains of the vasopressin V1a receptor, viewed from the surface of the plasma membrane. Amino acids shown to be involved in this interaction are indicated (see text).

III (Figs 1 and 2) by Ala residues results in receptors that have a decreased affinity for the vasopressin receptor agonists, vasopressin, oxytocin and [Phe²,Orn⁸]-vasotocin (Mouillac *et al.* 1995). Interestingly, these residues are highly conserved in all the vasopressin and oxytocin receptors. It was therefore proposed that the agonist-binding pocket is common to all the different subtypes of

this receptor family (Mouillac *et al.* 1995). Indeed, more recently, mutational analyses of the fish [Arg⁸]-vasotocin receptor have shown that substitution of Lys¹⁰¹ (the residue homologous to Lys¹²⁸ in TM III of the rat V1a receptor) by Met or Ala resulted in a reduction in vasotocin binding. Substitution of Gln¹⁰⁴ (the residue homologous to Gln¹³¹ in rat V1a receptor) by a Leu

residue resulted in a loss of vasotocin binding (Hausmann *et al.* 1996). It should be noted that this Gln in AVP/oxytocin receptors corresponds, in sequence alignment, to the Asp on TM III, which is highly conserved in cationic neurotransmitter receptors and has a key role in their binding. Interestingly, replacement of Thr²²³ in the rat V1a receptor with Ala residue had no effect on vasopressin binding, suggesting that this Thr residue, which corresponds to a Ser residue that is conserved in catecholamine receptors and contributes to catecholamine binding, is not important for correct recognition of vasopressin.

In addition to residues situated in the transmembrane regions, residues located in the extracellular domains also interact with the hormones. This notion is strongly supported by photoaffinity labeling of the first extracellular loop of bovine V2, using an analogue of lysine vasopressin that has a photoactivatable group on the lateral chain of residue 8 (Kojro *et al.* 1993). In addition, mutagenesis studies have shown that replacement of Tyr¹¹⁵ (in the first extracellular loop of the rat V1a receptor; Figs 1 and 3) with Asp or Phe, the amino acids naturally occurring in the human V2 and oxytocin receptors, results in a potent increase in V2 or oxytocin agonist-binding affinities (Chini *et al.* 1995). Similar results have also been obtained with the bovine V2 receptor (Ufer *et al.* 1995). On the basis of these studies, primary sequence analyses and studies of the structure–activity relationships of other neuropeptides and their receptors, the corresponding amino acid in the first extracellular loop was proposed to have a homologous role in conferring affinity and selectivity. This would seem to be particularly true in the case of angiotensin, cholecystokinin, neuropeptide Y and neurokinin receptors (Trumpp-Kallmeyer *et al.* 1995).

Other strategies have been used to locate the agonist-binding domains. Chimeric constructs have been made. On the basis of the exchange of extracellular domains between the pig V2 receptor and oxytocin receptor, it has been proposed that the three extracellular loops are important (Postina *et al.* 1996). Similarly, chimeric constructs encoding parts of the white sucker fish [Arg⁸]-vasotocin receptor and parts of the isotocin receptor have shown that the N-terminus and a region spanning the second extracellular loop and its flanking transmembrane segments contribute to the affinity of the [Arg⁸]-vasotocin receptor (Hausmann *et al.* 1996).

Naturally occurring mutations of the human V2 receptors that are responsible for the X-linked nephrogenic diabetes insipidus have also been used to analyze further the structural bases of these receptors. In the human V2 receptor, the natural mutation of Arg¹¹³, situated next to the Cys that is involved in a disulfide bridge, to Trp¹¹³ significantly reduces receptor expression in transfected cells, receptor–ligand binding affinity and Gs coupling, to such an extent that kidneys challenged either by dehydration or by infusion of 1-deamino[D-Arg⁸]VP were unable to produce concentrated urine, thus

displaying a complete nephrogenic diabetes insipidus (Bichet *et al.* 1993, Birnbaumer *et al.* 1994). A similar reduction in binding affinity and inability to concentrate the urine is found in association with another mutation: deletion of Arg²⁰² situated in the second extracellular loop of the human V2 receptor (Ala *et al.* 1998).

Another approach to the study of hormone–receptor interactions involves the use of small synthetic peptides that mimic the sequence of the supposed active region of the receptor. This approach has been used in the case of the vasopressin receptor: a 12 amino acid synthetic peptide of the first extracellular loop of the V1a vasopressin receptor inhibited both the binding of agonist and antagonist radioligands to this receptor and the vasopressin-stimulated glycogen phosphorylase activity in isolated rat hepatocytes (Howl & Wheatley 1996). One 25 amino acid synthetic peptide of the second extracellular loop of the V1a vasopressin receptor, with an additional Cys residue at the C-terminal end, also inhibited radioligand binding and AVP-induced accumulation of inositol phosphate (Mendre *et al.* 1997). These studies may indicate that the extracellular receptor surface facilitates the initial ‘capture’ of both peptide and non-peptide ligands.

Antagonists

Whereas some progress has been made in elucidating the structural determinants underlying vasopressin/oxytocin receptor agonist binding, the receptor domains involved in the binding of vasopressin/oxytocin receptor antagonists are only poorly defined. In particular, it remains unclear which vasopressin/oxytocin receptor subsites are recognized by the bulky hydrophobic ring systems or side chains present in virtually all potent peptide cyclic or linear vasopressin/oxytocin receptor antagonists (Manning *et al.* 1995).

Mutagenesis studies have shown that those mutations affecting agonist-binding affinities have little effect on antagonist-binding affinities, indicating that the receptor subsites involved in the binding of vasopressin/oxytocin receptor antagonists are not those binding the receptor agonists (Mouillac *et al.* 1995, Postina *et al.* 1996). However, the Lys¹²⁸ residue in TM III of the rat V1a receptor seems to be involved not only in recognition of vasopressin and arginine vasotocin, but also in the binding of the competitive non-peptide V1a antagonist, SR 49059. Another residue that could be critical for the binding of both agonists and peptide and non-peptide antagonists to the V1a receptor is the conserved Gln¹⁸⁵ situated in TM IV.

In order to delineate the receptor domains that contribute to the binding of vasopressin V1a receptor antagonists, radioiodinated photosensitive ligands have been prepared and used to label covalently the rat V1a receptor (Carnazzi *et al.* 1994, 1997). Using this ligand, it

was possible to identify the photolabelled domain of the human V1a receptor also. Combining the photolabelling results with the predictions of molecular modelling studies, it has been suggested that a hydrophobic cluster of aromatic residues situated in transmembrane VI (Fig. 1) may be involved in the binding of peptide antagonists (Phalipou *et al.* 1997). Another study, using chimeric oxytocin/V2 receptor in which the third extracellular loop plus the upper part of the TM VII were exchanged between both wild-type receptors, has shown that the upper part of the TM VII contributes to the binding of an oxytocin antagonist (Postina *et al.* 1996).

Domains conferring G protein-coupling selectivity

Kidney vasopressin V2 receptors have long been known to interact with adenylyl cyclase to generate cAMP intracellularly and to cause the antidiuretic effects of AVP (Orloff & Handler 1967). This interaction is made possible through the coupling of the receptor with the α s subunit of the G protein. The receptors of the vasopressin V1a type were initially defined as those receptors present in the hepatocytes that mediate the hydrolysis of phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate and diacylglycerol and cause an increase in cytosolic calcium (Michell *et al.* 1979). This vasopressin V1a receptor activation involves G proteins of the Gq/G11 family, the α -subunits of which, upon receptor activation, regulate the activity of the β -isoforms of phospholipase C. Several studies have demonstrated a variety of signaling pathways associated with the V1a receptor endogenously expressed in tissues or cell lines. These include activation of phospholipases A2, C and D, increase in intracellular calcium and cell acidification through activation of the Na^+/H^+ exchange (Briley *et al.* 1994).

Adenohypophysis V1b receptors also stimulate phospholipase C. Oxytocin receptors activate phospholipase C and induce an increase in cytosolic Ca^{2+} concentration, which results in strong contractions of the uterus at term (Mironneau 1976, Marc *et al.* 1986).

Single amino acids have been found to be important for the activation of this family of receptor. In the rat V1a vasopressin receptor, the functional importance of the conserved Asp⁹⁷ residue in TM II has been confirmed by mutational analysis (Figs 1 and 2) (Mouillac *et al.* 1995); this has also been observed for many other GPCRs (Savarese & Fraser 1992). In human oxytocin receptors, it was found that AVP may act either as a complete agonist, as demonstrated by analysis of the electrophysiological response in *Xenopus* oocytes (Kimura *et al.* 1994), or as a partial agonist, as demonstrated by measurement of the release of inositol phosphate in various cell lines (CHO, Ltk⁻, Cos 7; Chini *et al.* 1996). This latter property was utilized to identify amino acids that have a key role in regulating agonist–oxytocin receptor interactions. It was

found that the Tyr²⁰⁹ in the TM V and the Phe²⁸⁴ in the TM VI of the human oxytocin receptor have such a role. Replacement of these two amino acids with the corresponding residues of the V1a receptor (Figs 1 and 2) resulted in mutant receptors, for which the intrinsic activity of oxytocin itself was not significantly altered, whereas that of AVP was dramatically increased (Chini *et al.* 1996). As aromatic residues are present at these positions in most rhodopsin-like GPCRs, it was proposed that these residues may modulate the process of receptor activation in various members of the GPCR family.

Another highly conserved residue has also been found to have a key role in the vasopressin/oxytocin receptor activation process. As has been shown for the muscarinic M3 receptor (Wess *et al.* 1993), the conserved Pro³²² situated on TM VII in the human V2 receptor is probably necessary to allow the relative movements within the helical bundle that are required for receptor activation. Two different mutations of the amino acid have been identified in different families suffering from nephrogenic diabetes insipidus, which resulted in a mutant receptor that was severely impaired in its ability to mediate adenylyl cyclase activation (Ala *et al.* 1998). The mutation Pro³²² to Ser³²² yielded a mutant receptor that had a decreased binding affinity for the hormone but was still able to stimulate adenylyl cyclase (although to a lesser degree); this mutation gave a mild clinical phenotype. In contrast, the mutated Pro³²² to His³²² receptor is completely uncoupled in terms of cellular signaling, which is consistent with the phenotype observed in a patient having a complete clinical expression of the disease (Tajima *et al.* 1996).

As has been shown for many other receptors, the highly conserved triplet Asp–Arg–Tyr (Asp–Arg–His or Asp–Arg–Cys in human V2 or human oxytocin receptors respectively) located at the N-terminal of the i2 loop is also required for efficient G protein activation (Savarese & Fraser 1992). In the human V2 receptor, an Arg¹³⁷ to His¹³⁷ mutation was found to abolish coupling to G proteins and cause a complete phenotype of nephrogenic diabetes insipidus (Rosenthal *et al.* 1993).

To study the structural elements interacting with the G proteins responsible for the functional diversity found within the vasopressin receptor family, V1a–V2 hybrid receptors have been created in which distinct intracellular domains were exchanged between both wild-type receptors (Liu & Wess 1996). Substitution of the V2 receptor i2 loop for the homologous V1a receptor sequence resulted in a mutant receptor that gained the ability to couple efficiently to Gq/G11, but retained the ability to couple to Gs. Analogously, replacement of the i3 loop in the V1a receptor with the homologous V2 receptor sequence yielded a hybrid construct that gained efficient coupling to Gs, but was still able to activate Gq/G11 in a fashion similar to the wild-type V1a receptor. These data strongly suggest that the i3 loop of the V2 receptor has a

key role in correct recognition and activation of Gs, whereas the i2 loop of the V1a receptor is critically involved in selective activation of Gq/G11.

Conclusions

In the past 5 years, there has been considerable progress in knowledge of the structures of the vasopressin and oxytocin receptors, and in the development of molecule agonists and peptide and non-peptide antagonists. Initial studies have already provided some information on the structural requirement for ligand binding and selectivity. However, many aspects of vasopressin and oxytocin receptor function remain only poorly understood. Specifically, the nature of the dynamic changes in the receptor proteins that take place in ligand binding and G protein activation are unknown. Undoubtedly, the availability of a high-resolution structure of vasopressin and oxytocin receptors will greatly facilitate the refinement of three-dimensional models of these receptors and hence the acquisition of new information on the receptor domains responsible for agonist or antagonist binding and for G protein activation. This understanding of receptor function, at a molecular level, should be of great help in the development of new molecules with high selectivity for the different subtypes of receptors, and thus in the development of new molecules of potential therapeutic interest. The current clinical implications of AVP and oxytocin receptors lie in oxytocin itself, used in the promotion of labor and delivery, in atosiban, an oxytocin antagonist used in the treatment of preterm labour, and in desmopressin, a selective AVP-V2 agonist used in the treatment of diabetes insipidus, in which there is a deficiency of circulating AVP (see Freidinger & Pettibone 1997 for review). Alternatively, the knowledge of ligand structure-activity and receptor structure-function relationships may help in the future design of specific agonists able to activate mutated V2 receptors responsible for the X-linked nephrogenic diabetes insipidus.

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