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Molecular cloning and functional characterization of V_2 [8-lysine] vasopressin and oxytocin receptors from a pig kidney cell line

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[Arg^8]vasopressin and oxytocin are the two main members of the neurohypophysial hormone family found to be present in nearly all mammals. [Lys^8]vasopressin ([Lys^8]VP) has been identified as the antidiuretic hormone in pig and some marsupial families. The porcine-derived kidney epithelial cell line, LLC-PK$_1$, expresses both [Lys^8]VP receptors coupled to the activation of adenylate cyclase (V_2 receptors) and oxytocin receptors. Here we report the molecular cloning of the V_2 [Lys^8]VP receptor and the oxytocin receptor from LLC-PK$_1$ cells. The cloned V_2 [Lys^8]VP receptor differs from human and rat V_2 [Arg^8] receptors mainly in its N-terminal region, in residues located in the extracellular loops and in intracellular phosphorylation sites. When expressed in COS7 cells, the V_2 [Lys^8]VP receptor exhibits the relative order of ligand affinity [Lys^8]VP = [Arg^8]VP $\gg$ 1-deamino[D-Arg^8]VP $\geq$ oxytocin and adenylate-cyclase stimulation, expected for the porcine V_2 [Lys^8]VP receptor but different from V_2 [Arg^8]VP receptors. Adenylate-cyclase activation by [Lys^8]VP was inhibited in COS7 cells by a V_2 antagonist. The cloned oxytocin receptor exhibits in COS7 cells a ligand specificity typical of mammalian oxytocin receptors. mRNA-distribution analysis revealed a single 5.5-kb transcript in the uterus from pregnant guinea pig.

Vasopressin-oxytocin peptides were first characterized as neurophypophysial hormones in mammals. In recent years the phylogenetic distribution of this family of peptides has been greatly expanded. At present vasopressin-oxytocin peptides have been characterized in over 50 vertebrate species (Acher, 1984 and 1985; Acher et al., 1985). The hormone superfamily comprises more than a dozen variants, mostly nonapeptides with several highly conserved residues including two cysteine residues at position 1 and 6 which form a disulfide bridge. Each species has usually two neurohypophysial hormones: one belonging to the oxytocin family which is involved in reproduction and one belonging to the vasopressin (VP) family, which is involved in hydroosmotic regulation. Placental mammalian species (*Eutheria*) have oxytocin and [Arg^8]VP, except the family *Suidae* in which the pig has [Lys^8]VP instead of [Arg^8]VP (Popenoe et al., 1952). During evolution in *Methateria*, in some marsupial families two pressor hormones occurred: [Lys^8]VP together with either [Phe^2, Arg^8]VP in australian macropodials or with [Arg^8]VP in american opossums (Chauvet et al., 1983, 1985).

The molecular cloning of receptors for [Arg^8]VP (Morel et al., 1992; Birnbaumer et al., 1992b; Lolait et al., 1992) and oxytocin (Kimura et al., 1992) has been recently accomplished and has demonstrated that these receptors form a subfamily among the large number of guanine-nucleotide-binding-regulatory-protein (G protein)-coupled receptors with seven transmembrane domains. Cloning of receptors for other neurohypophysial hormones will provide an opportunity to study the relationship of species-specific hormone variants to variations in receptor sequences.

The porcine-kidney-derived epithelial cell line, LLC-PK$_1$ (Hull et al., 1976), expresses [Lys^8]VP receptors of the V_2 type coupled to the activation of adenylate cyclase (Ausiello et al., 1980). This established cell line has been extensively used as a cellular model system to study the V_2-receptor adenylate-cyclase system (Ausiello et al., 1980; Roy et al., 1981; Jans et al., 1989). A small number of oxytocin receptors has been detected on these cells (Stassen et al., 1988; Cantau et al., 1990). The low density of oxytocin receptors as compared to V_2 receptors and the overlapping ligand specificity of vasopressin and oxytocin receptors have limited a precise pharmacological and functional characterization of each receptor on LLC-PK$_1$cells.

The purpose of the present work was to clone and characterize the [Lys^8]VP V_2 receptor and the oxytocin receptor from LLC-PK$_1$ cells, and to find common and divergent structural domains in the sequence of the vasopressin-oxytocin-receptor family, which are related to their functional properties.

MATERIALS AND METHODS

Polymerase chain reaction (PCR)

Two degenerate oligonucleotides corresponding to transmembrane domains (TM) 2 and 6 were designed based on the cDNA sequences of the cloned vasopressin and oxytocin receptors (Morel et al., 1992; Birnbaumer et al., 1992b;

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Abbreviations. TM, transmembrane domain; PCR, polymerase chain reaction; G protein, guanine-nucleotide-binding regulatory protein.

Note. The novel nulceotide sequence data published here have been deposited with the EMBL sequence data banks and are available under accession numbers X71795 and X71796.

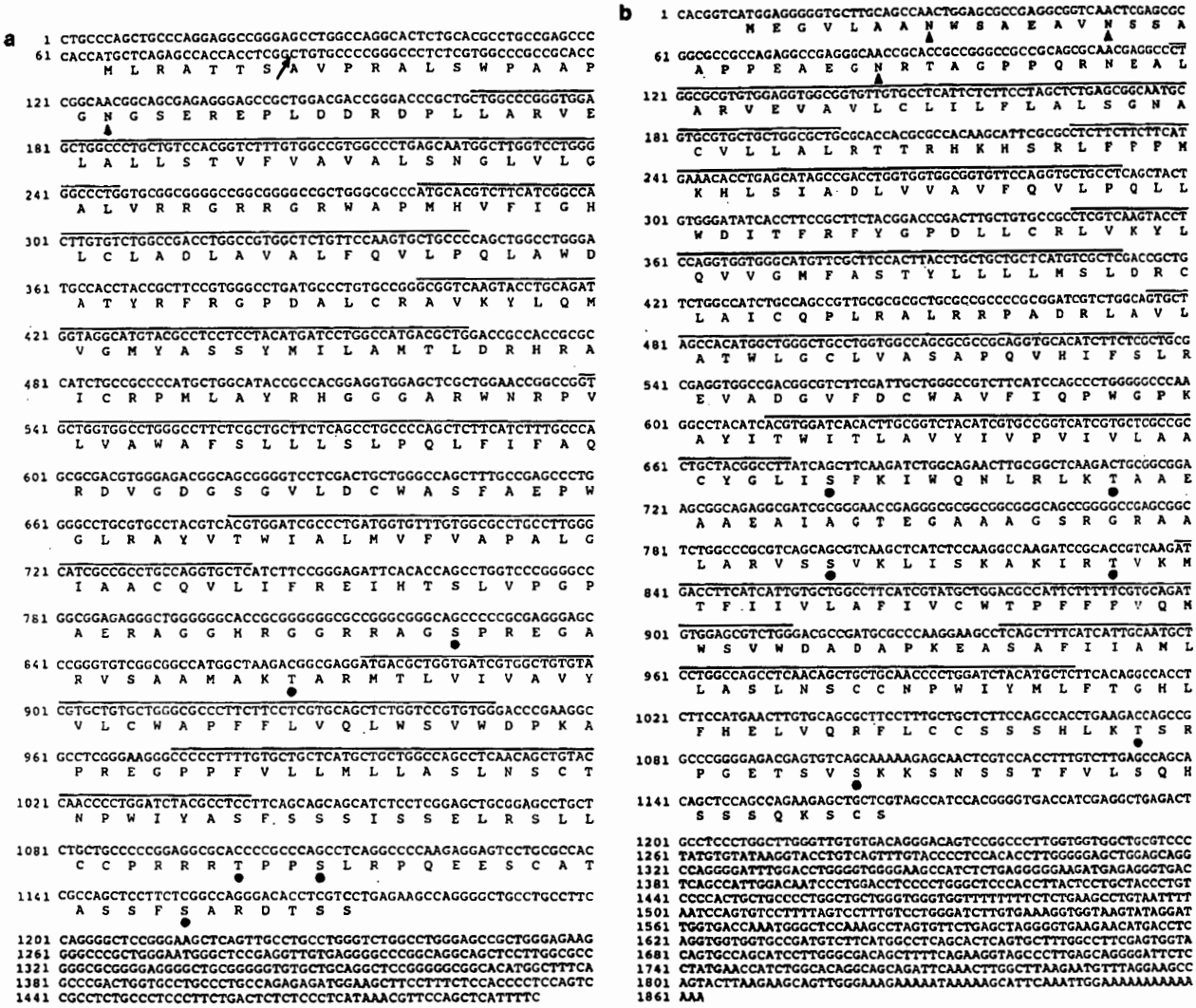

Fig. 1. Nucleotide and deduced amino acid sequence of the V_2-[Lys^8]VP receptor cDNA clone PK91 (a) and the oxytocin-receptor cDNA (b) from LLC-PK$_1$ cells. The putative transmembrane domains are overlined. (▲), Potential N-glycosylation sites; (●), possible phosphorylation sites. The position of the intron in PK12 cDNA is indicated by an arrow.

Lolait et al., 1992; Kimura et al., 1992). The 5′ primer was 5′-TTCCA (A/G) GT (G/A) (G/T) TGCC (G/G) GAGCT-3′ and the 3′ primer was 5′-GAAGAA (G/A) GG (T/C) GCCCAGCA (C/A)A-3′. PCR was performed using LLC-PK$_1$ cDNA as a template under the following conditions, 30 cycles 94°C 1 min; 55°C, 2 min; 72°C, 3 min. The PCR product was gel-purified, cloned into pGEM-7Zf(+) and partially sequenced.

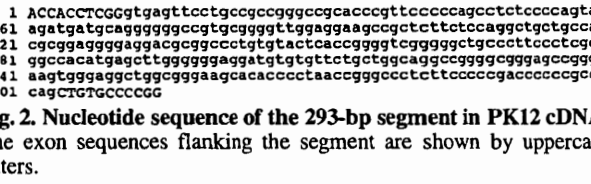

Fig. 2. Nucleotide sequence of the 293-bp segment in PK12 cDNA. The exon sequences flanking the segment are shown by uppercase letters.

Screening of cDNA library

mRNA was isolated from LLC-PK$_1$ cells grown to about 90% confluency by the standard guanidinium-thiocyanate method followed by oligo(dT)-cellulose chromatography. Oligo(dT)-primed cDNA was synthesized with Superscript reverse transcriptase (Gibco BRL), size-fractionated on an agarose gel and ligated to λgt10 *Eco*RI arms. To isolate V_2-VP-receptor cDNA clones, the λgt10 cDNA library was screened with the ^{32}P-labeled PCR fragment under highly stringent conditions: hybridization in 6×NaCl/Cit. (NaCl/Cit., 0.15 M NaCl, 15 mM sodium citrate, pH 7.0), 5×Denhardts' solution, 0.5% SDS at 65°C; final washing in 0.1×NaCl/Cit., 0.1% SDS at 65°C. To search for oxytocin-receptor cDNA clones, screening was done with two ^{32}P-labeled oligonucleotides 5′-ATCCA(G/G)GGGTTGCAGCAGC-3′ and CCA-CATCTG (G/G)ACGAAGAAGAA-3′, complementary to the human oxytocin-receptor mRNA (Kimura et al., 1992) within TM7 and the third extracellular loop, respectively. The conditions for hybridization and washing were as described previously (Gorbulev et al., 1992).

DNA sequencing

cDNA inserts from recombinant phages were recloned into pUC19 or pGEM-7Zf(+) and sequenced on both strands

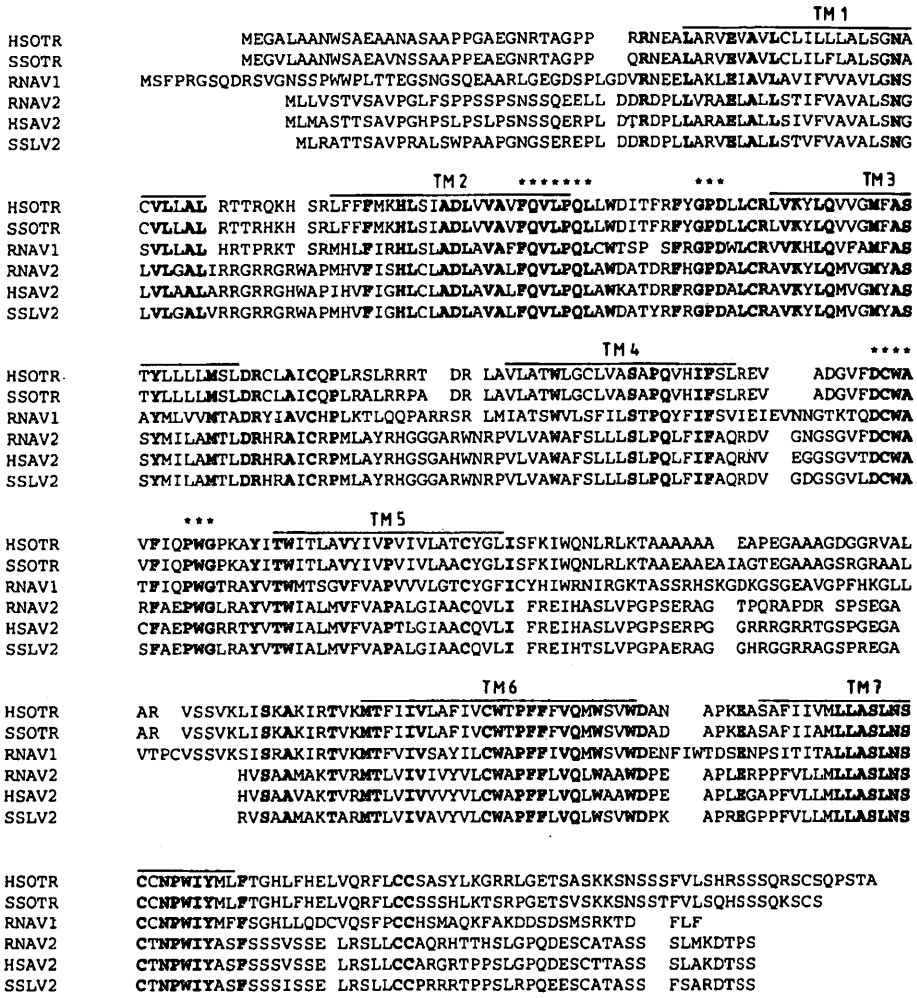

Fig. 3. Comparison of the amino-acid sequences of receptors from the oxytocin-vasopressin subfamily according to Feng and Doolittle (1990). Invariant residues are shown in boldface type. (*), Invariant amino acids conserved only within the oxytocin-vasopressin subfamily but not in other G-protein-coupled receptors. The putative transmembrane domains (TM1−TM7) are overlined. HSOTR, human oxytocin receptor; SSOTR, pig oxytocin receptor; RNAV1, rat V_1 receptor; RNAV2, rat V_2 AVP receptor; $HSAV_2$, human V_2 AVP receptor; SSLV2, pig V_2 LVP receptor.

by the dideoxynucleotide chain-termination method using a Sequenase version 2.0 kit (United States Biochemicals) and utilizing both the exonuclease III/mung bean nuclease approach (Henikoff, 1984) and appropriate oligonucleotide primers.

Northern hybridization

mRNA from frozen tissues or from cultured cells were isolated as described above. 1−5 μg of the mRNA preparations was resolved on a formaldehyde-agarose gel, transferred to Hybond-N membrane (Amersham) and hybridized with ^{32}P-labeled V_2 [Lys^8]VP-receptor cDNA or oxytocin-receptor cDNA under the conditions described previously (Gorbulev et al., 1992). The washing conditions are given in the figure legends.

Expression in COS cells and binding experiments

The entire cDNA inserts were subcloned into the expression vector, pCDM8 (Seed and Aruffo, 1987) using *Bst*XI adaptors. The resulting DNA was transfected into COS7 cells during 4.5 h using the DEAE-dextran method (Seed and Aruffo, 1987). After 70 h cells were harvested and treated as described previously (Gorbulev et al., 1992). The saturation analysis was performed by incubation of 200 μl cell suspension with varying concentrations of [^{3}H] [Arg^8]VP (26 Ci/mmol, NEN) or [^{3}H]oxytocin (36.6 Ci/mmol, NEN). Non-specific binding was determined in the presence of a 100-fold excess of non-radioactive hormone. The displacement experiments were performed with 10 nM [^{3}H] [Arg^8]VP (for V_2 receptor) or [^{3}H]oxytocin (for oxytocin receptor) in the presence of different concentrations of competitors. All incubations were performed for 30 min at 30°C in duplicate. Bound ligand was separated from free ligand by filtering the incubation mixture through Whatman GF/B filters soaked in 0.05% bovine serum albumin. The binding data were analysed using the Ligand program (Munson, 1983). The same conditions were used for binding experments with LLC-PK$_1$ cells grown to 90% confluency (Luzius et al., 1991).

cAMP assay

Transfected COS7 cells were harvested and disrupted to prepare cell homogenates as described (Birnbaumer et al.,

1992a). Cell homogenates were incubated with ligands in the presence of 0.2 mM isobutylmethylxanthine for 10 min at 30°C as described (Fahrenholz et al., 1980). Reactions were stopped with the addition of HCl to 0.2 M and the samples were frozen. After thawing and neutralization, cAMP accumulation was measured by using a cAMP assay kit (Amersham).

RESULTS

To amplify the cDNA sequences coding for the V_2 VP receptor in LLC-PK_1 cells, we utilized PCR with two degenerate oligonucleotides (see Materials and Methods). The most prominent PCR product was gel purified and cloned. Partial sequencing revealed significant similarity to V_2 [Arg^8]VP receptors. This PCR fragment was used to screen a LLC-PK_1 cDNA library. After two rounds of screening we isolated 18 strongly hybridizing clones from about 200000 bacteriophage plaques. Restriction analysis of DNA from four independent recombinant phages revealed that they represent two cDNA populations with different insert sizes, 1.5 kb and 1.8 kb. Two clones, PK91 (1.5-kb insert) and PK12 (1.8-kb insert), were chosen for further analysis.

The sequencing of these cDNA showed that they are related to each other, share the same nucleotide sequence, but that PK12 cDNA has in addition an extra G at the 5′ end, a poly(A) tail just after the 3′ terminal nucleotide of PK91 cDNA and a 293-bp segment between nucleotides 86 and 87 of the PK91 cDNA is shown in Fig. 1a. It contains 1494 bp and has an open reading frame for 370 amino acids. The deduced amino-acid sequence shows about 84% identity to human and rat V_2 [Arg^8] receptors (Birnbaumer et al., 1992b; Lolait et al., 1992) and only 40% identity to the rat V_1 [Arg^8]VP receptor (Morel et al., 1992), which is coupled to the activation of the phospholipase-C system. The position of the 293-bp segment in PK12 cDNA coincides with that of the first intron found in the human V_2 [Arg^8]VP-receptor gene (Seibold et al., 1992). The terminal sequences of the 293-bp segment and flanking sequences fit well to the consensus splicing signal on the exon-intron boundary (TCGG/GTGAGT...CCCAG/CT) (Breathnach and Chambon, 1981; Fig. 2). Thus, PK12 cDNA seems to represent an incompletely spliced transcript of the V_2-[Lys^8]-VP receptor gene.

When screening the same λgt10 cDNA library with two oxytocin-receptor-specific oligonucleotides (see Materials and Methods), we found one strongly hybridizing clone with an insert of about 1.9 kb. The complete sequencing of the cDNA insert from this clone revealed an open reading frame encoding a 386-amino-acid protein with 92% identity to the human oxytocin receptor (Fig. 1b). The oxytocin-receptor gene from LLC-PK_1 cells contains at the position corresponding to Gln385 of the human oxytocin receptor (Kimura et al., 1992) a substitution C→T resulting in a stop-codon five amino acids before the C-terminus of the human oxytocin receptor.

The cloned V_2 [Lys^8]VP and oxytocin receptors share most of the structural features common for this receptor subfamily (Fig. 3) including an unique stretch of seven amino acids (Phe-Gln-Val-Leu-Pro-Gln-Leu) at the end of TM2, one sequence in the first putative extracellular loop (Gly-Pro-Asp) and two sequences in the second extracellular loop (Asp-Cys-Trp-Ala and Pro-Trp-Gly). These sequences are conserved exclusively within this subfamily and may play an important role in ligand-receptor recognition. The V_2

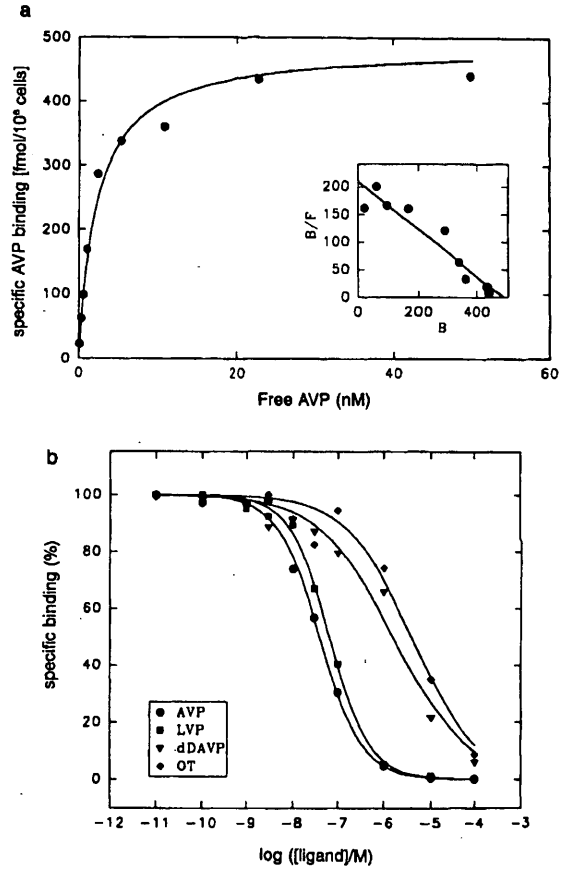

Fig. 4. Binding of [^{3}H] [Arg^8]VP to COS7 cells transfected with PK91 cDNA. (a) Saturation analysis. The results shown are the means of duplicate determinations and are representative of three independent experiments. Inset shows Scatchard plot of the binding data. (b) Displacement analysis. The data shown are the means of duplicate values and are representative of two [for 1-deamino[D-Arg^8]VP (dDAVP) and oxytocin] or three [for [Arg^8]VP (AVP) and [Lys^8]VP (LVP)] independent experiments.

[Lys^8]VP receptor has only one potential N-glycosylation site in its N-terminal segment, whereas the oxytocin receptor has three such sites. N-glycosylation has been shown to play a role in biosynthesis and internalization of the V_2 [Lys^8]VP receptor in LLC-PK_1 cells (Jans et al., 1992).

To functionally characterize the cloned V_2 [Lys^8]VP and oxytocin receptors, their cDNA were recloned into the eukaryotic expression vector pCDM8 and used to transfect COS7 cells. COS7 cells transiently express not only PK91 cDNA, but also PK12 cDNA indicating that the 293-bp intron of PK12 cDNA is properly spliced during the propagation of monkey kidney cells. Saturation analysis of COS7 cells transfected with PK91 cDNA (Fig. 4a) shows a dose-dependent binding of [^{3}H] [Arg^8]VP with an apparent dissociation constant (K_d) of 2.3 nM, which is similar to that determined for LLC-PK_1 cells (Jans et al., 1986). Competitive-binding studies with [^{3}H] [Arg^8]VP gave the inhibition constants (K_i) of 5.0 nM for [Arg^8]VP, 7.3 nM for [Lys^8]VP, 0.27 μM for 1-deamino [D-Arg^8]VP and 0.6 μM for oxytocin (Fig. 4b). The low affinity of the cloned V_2 [Lys^8]VP receptor for oxytocin and the specific V_2 agonist 1-deamino [D-Arg^8]VP is consistent with the values obtained on membranes derived from pig kidney (Roy et al., 1975b; Stassen et al., 1985). Our results confirm the observation that replacement of L-Arg in

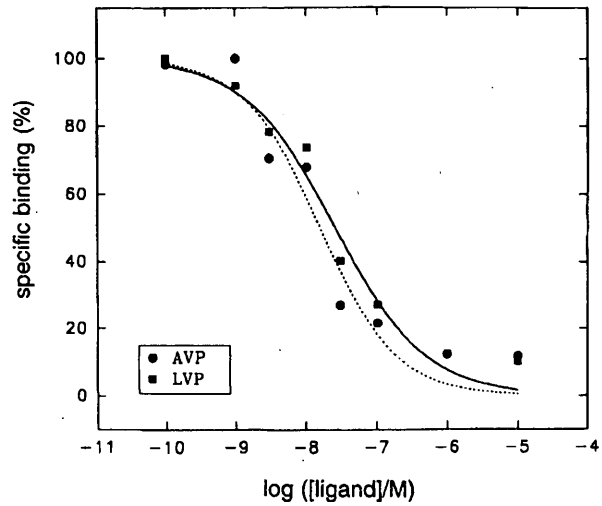

Fig. 5. Displacement of [^{3}H] [Arg^8]VP binding to LLC-PK$_1$ by [Lys^8]VP (LVP) (——) and [Arg^8]VP (AVP) (----). The data shown are the means of duplicate values and are representative of two independent experiments.

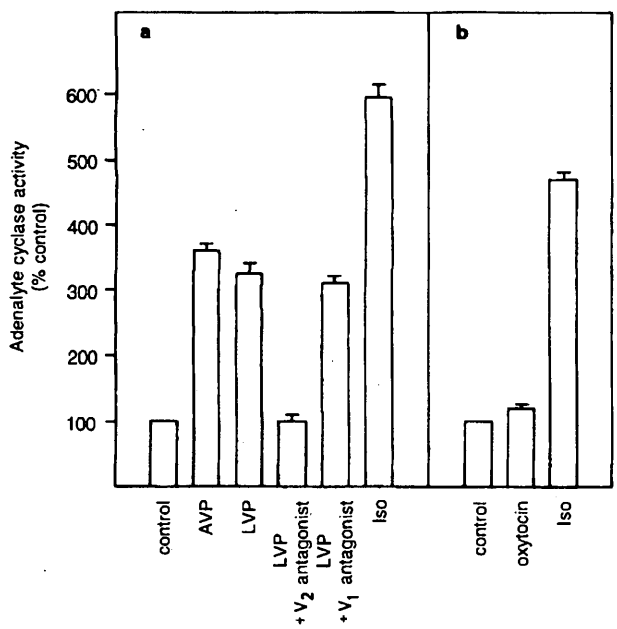

Fig. 6. Adenylate-cyclase stimulation assay for COS7 cells transfected with PK91 cDNA (a) and oxytocin-receptor cDNA (b). The ligands used are as follows; [Lys^8]VP (LVP) 100 nM; [Arg^8]VP (AVP), 100 nM; V_2 antagonist d(CH$_2$)$_5$[D-Phe^2, Val^4, Arg^8]VP (Manning et al., 1982), 50 μM; V_1 antagonist d(CH$_2$)$_5$[MeAla^7, Arg^8]VP (Fahrenholz et al., 1984), 50 μM; oxytocin, 100 nM. Control is cAMP accumulation without additives. Isoproterenol (Iso) was added to a final concentration of 1 μM. Incubations were performed in duplicates and the data shown are means ± SEM of two experiments.

position 8 by D-Arg, as in 1-deamino[D-Arg^8]VP, leads to a drastic decrease in affinity for the V_2 receptor in pig, whereas the human and rat V_2 receptors retain one to two orders higher affinity (Guillon et al., 1982; Butlen et al., 1987).

Contradictory data about the relative binding affinities of [Lys^8]VP and [Arg^8]VP in pig kidney have been reported (Roy et al., 1975a; Butlen et al., 1987). Therefore, we examined their affinity on LLC-PK$_1$ cells and obtained K_i values

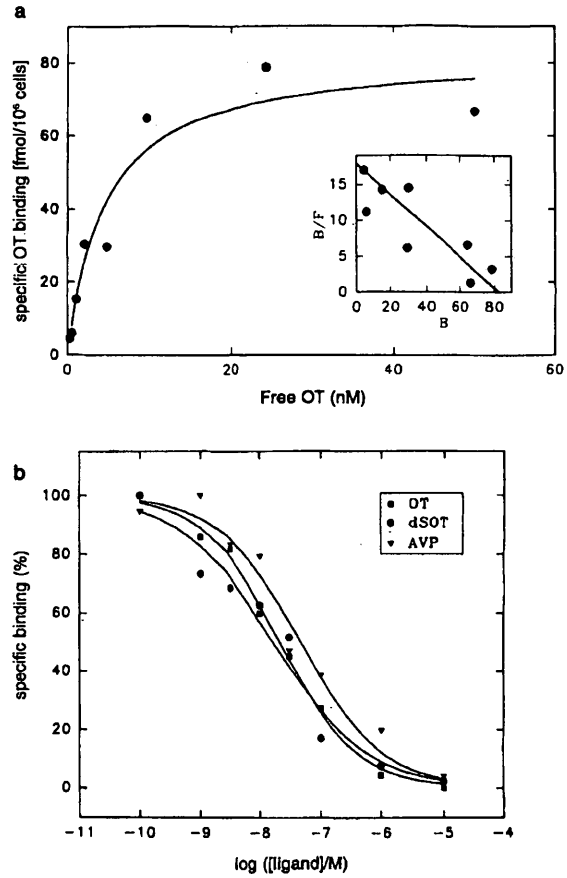

Fig. 7. Binding of [^{3}H]oxytocin (OT) to COS7 cells transfected with oxytocin-receptor cDNA. (a) Saturation analysis. The results shown are the means of duplicate determinations and are representative of two independent experiments. Inset shows Scatchard plot of the binding data. (b) Displacement analysis. The data shown are the means of duplicate values and are representative of two independent experiments. dSOT, 1-deamino[7-sarcosine]oxytocin; AVP, [Arg^8]VP.

of 1.0 nM for [Arg^8]VP and 1.7 nM for [Lys^8]VP (Fig. 5), i.e. the same order of affinities as for the cloned V_2 receptor.

The cloned [Lys^8]VP receptor stimulated cAMP accumulation in response to [Lys^8]VP or [Arg^8]VP (Fig. 6a). The adenylate-cyclase activity was inhibited in the presence of a V_2 specific antagonist and was not affected by a V_1-specific antagonist. Control experiments using COS7 cells transfected with oxytocin-receptor cDNA revealed no cAMP accumulation in response to oxytocin (Fig. 6b) as has been expected taking into account that oxytocin receptors couple to the phosphatidylinositol second-messenger system (Schrey et al., 1986). The saturation and displacement analysis of COS7 cells transiently expressing oxytocin receptors after transfection with oxytocin receptor cDNA gave a K_d value of 4.6 nM for [^{3}H]oxytocin (Fig. 7a) and the relative order of ligand affinities: oxytocin = 1-deamino[7-sarcosine]oxytocin (Grzonka et al., 1983) ≥ [Arg^8]VP with K_i of 4.6, 5.6 and 15.5 nM, respectively (Fig. 7b). These binding characteristics of the cloned oxytocin receptor are in accordance with those reported for the pig oxytocin receptor (Cantau et al., 1990; Jans et al., 1993).

Northern-blot analysis was performed to analyse the expression of V_2 [Lys^8]VP and oxytocin receptors in different tissues and cell lines. V_2 [Lys^8]VP-receptor mRNA was detected neither in liver, nor in brain (Fig. 8). Hybridization

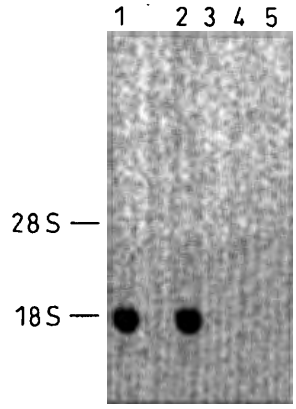

Fig. 8. Northern-blot analysis of V_2 VP mRNA expression. Lanes contain mRNA from VPR1 cells, 5 μg (lane 1), from LLC-PK_1 cells, 5 μg (lane 2) from pig brain, 4 μg (lane 3), from pig liver, 4 μg (lane 4) from pig kidney, 4 μg (lane 5). The filter was hybridized to ^{32}P-labeled V_2-[Lys^8]VP-receptor cDNA, washed to a final stringency 0.25 × buffer A (buffer A, 0.18 M NaCl, 10 mM sodium phosphate, pH 7.7, 1 mM EDTA), 0.1% SDS at 60 °C and exposed for 30 h.

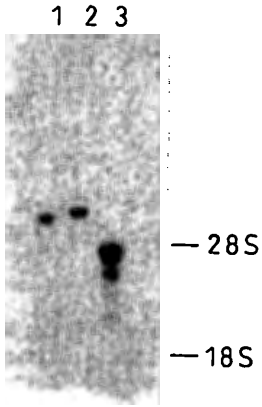

Fig. 9. Northern-blot analysis of oxytocin receptor mRNA expression. Lanes contain mRNA from LLC-PK_1 cells, 2 μg (lane 1), from guinea pig pregnant uterus, 4 μg (lane 2) and from human pregnant uterus, 2.5 μg (lane 3). The filter was hybridized to ^{32}P-labeled oxytocin-receptor cDNA, washed to a final stringency of 0.25 × buffer A, 0.1% SDS at 55 °C and exposed for 20 h.

with V_2-[Lys^8]VP-receptor cDNA revealed a transcript of 1.5 kb in LLC-PK_1 cells and 1.8 kb in kidney. The mRNA level of V_2 [Lys^8]VP receptor in kidney was significantly lower than in LLC-PK_1 cells. In VPR1 cells, a mutant of LLC-PK_1 cells defective in V_2 VP binding and function (Luzius et al., 1991), we found V_2-[Lys^8]VP-receptor mRNA at the same position as in LLC-PK_1 cells. This indicates that the mutation causing the V_2-receptor defect in VPR1 cells does not affect gene transcription. LLC-PK_1 cells have, however, an additional hybridizing band of about 3.4 kb, that is absent in VPR1 cells. Oxytocin receptor mRNA was not detected in pig kidney, pig liver and pig brain, but it was identified in LLC-PK_1 cells and in human and guinea pig pregnant uteri (Fig. 9). Human oxytocin-receptor mRNA is of 4.4 kb (Kimuara et al., 1992), whereas guinea pig uterus and LLC-PK_1 cells contain larger transcripts of 5.5 kb and 5.4 kb, respectively. As the cloned oxytocin-receptor cDNA has a polyadenlyation signal and a poly(A) tail, the results of Northern-blot analysis may suggest an unusually long 5′ non-translated region of pig oxytocin mRNA.

DISCUSSION

The cloned V_2 [Lys^8]VP receptor binds the two hormones [Lys^8]VP and [Arg^8]VP with roughly equal affinity. In this respect it is interesting to note that both [Lys^8]VP-like and [Arg^8]VP-like peptides have been detected in pig ovaries and testis (Nicholson et al., 1988). AVP synthesized in extrahypothalamic areas of this species might therefore bind to the V_2 receptor with the same high affinity as [Lys^8]VP. In contrast, V_2 receptors in mammalian species with [Arg^8]VP as the only VP show significantly higher binding for [Arg^8]VP compared to [Lys^8]VP (Hechter et al., 1978; Guillon et al., 1982; Butlen et al., 1987).

The V_2 [Lys^8]VP receptor and human V_2 [Arg^8]VP receptor demonstrate more than 90% identity within their putative transmembrane domains, 80% identity for their C-terminal regions and a relatively low identity of 62% for their N-terminal regions. This divergence cannot be attributed only to species differences, because oxytocin receptors, that belong to the same subfamily and have been cloned from the same species, have a similar identity for both N-terminal and C-terminal regions, of 86.5% and 81%, respectively. Studies primarily performed on adrenergic and muscarinic receptors suggest that the N-terminal part of G-protein-coupled receptors for catecholamines and acetylcholine is responsible for the transport of receptors to the cell membrane, but contains no determinants for ligand binding (Ostrowski et al., 1992). However, for receptors that recognize peptide hormones, the importance of the N-terminal region for ligand binding has been shown: for the lutropin-choriogonadotropin receptor (Xie et al., 1990; Nagayama et al., 1991) and recently for tachikinin and endothelin-receptor subfamilies (Yokota et al., 1992; Hashido et al., 1992). Thus, we suggest that the N-terminus of V_2 VP receptors may be involved in determining the relative affinity for [Lys^8]VP and [Arg^8]VP. Other differences between [Lys^8]VP and [Arg^8]VP V2 receptors are Tyr102 (in the first extracellular loop), Lys298 and Arg301 (in the third extracellular loop) in [Lys^8]VP V_2 receptor instead of Asp, Glu and Leu, respectively, located in the corresponding positions of [Arg^8]VP V_2 receptors. The importance of these substitutions for binding selectivity should be tested in future experiments.

Comparison of the three V_2 VP receptors also shows that only the porcine V_2 receptor contains three potential phosphorylation sites for protein kinase C (Woodget et al., 1986) and cAMP-dependent protein kinase (Kennelly and Krebs, 1991) in its C-terminal region. This difference in phosphorylation sites might explain the observation that cAMP-dependent protein-kinase activation induces down-regulation of the V_2 [Lys^8]VP receptor in LLC-PK_1 cells (Jans and Hemmings, 1991) but not of the human V_2 [Arg^8]VP receptor in stably transfected murine cells (Birnbaumer et al., 1992a).

Surprisingly, we could not detect oxytocin-receptor mRNA in lactating mammary glands from guinea pig and rat, although this tissue is known to express oxytocin receptors (Soloff, 1982). An explanation could be that mammary glands possess another subtype of oxytocin receptor and our non-homologous probe (pig oxytocin-receptor cDNA) was not able to detect it.

In conclusion, we cloned V_2 [Lys^8]VP and oxytocin receptors form LLC-PK_1 cells. The two receptors belong to the

same subfamily of G-protein-coupled receptors, bind related hormones and analogues, but couple to different second-messenger systems. One of them, the V_2 [Lys^8]VP receptor, discriminates well between VP and oxytocin, the other, the oxytocin receptor, binds both hormones with a similar high affinity (Figs 4b and 7b). These related receptors cloned from the same species, but having different selectivity for their ligands, and different G-protein-coupling specificity will provide suitable tools to determine their structural domains important for ligand binding and G-protein-mediated signal transduction.

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