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Evidence that atypical vasopressin V_2 receptor in inner medulla of kidney is V_{1B} receptor

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Abstract

Vasopressin V_2 receptors at high-density and V_{1B} receptors are candidates for the V_2-like receptor, which evokes an increase in $[Ca^{2+}]_i$ when stimulated by the vasopressin V_2 receptor agonist 1-desamino-8-D-arginine vasopressin (dDAVP) in kidney inner medullary collecting duct. We compared the pharmacological characteristics of vasopressin V_2 and V_{1B} receptors in Chinese hamster ovary (CHO) cells to those of vasopressin V_2-like receptors in rat inner medullary collecting duct cells. The vasopressin V_{1B} receptor-selective agonist [deamino-Cys[1], D-3-(Pyridyl)-Ala[2], Arg[8]]vasopressin (D3PVP) did not stimulate the $[Ca^{2+}]_i$ increase in high-density vasopressin V_2 receptor-expressing CHO cells, but did in inner medullary collecting duct cells. Moreover, the vasopressin V_{1A}/V_2 receptor dual antagonist 4′-[(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-*d*][1] benzazepin-6-yl)carbonyl] 2-phenylbenzanilide (YM087), which has no effect on vasopressin V_{1B} receptors, did not block the $[Ca^{2+}]_i$ increase in inner medullary collecting duct cells when stimulated by dDAVP and D3PVP. On reverse transcription-polymerase chain reaction (RT-PCR) analysis of kidney, vasopressin V_{1B} receptor mRNA was detected only in the medulla. We propose that the true nature of the vasopressin V_2-like receptor in the inner medullary collecting duct is the vasopressin V_{1B} receptor, rather than the vasopressin V_2 receptor expressed at high-density.

Keywords: Vasopressin; Vasopressin receptor; Vasopressin V_{1B} receptor; Vasopressin V_2 receptor, CHO (Chinese hamster ovary) cell; Medullary collecting duct, inner

1. Introduction

Arginine vasopressin plays an important role in mediating antidiuresis, vasoconstriction and the modulation of adrenocorticotropic hormone (ACTH) release (Lázló et al., 1991). Vasopressin receptors belong to the G-protein-coupled receptor family and are classified into two major types, V_1 and V_2, according to their secondary messenger systems (Michell et al., 1979; Barberis and Tribollet, 1996). The vasopressin V_1 receptor, which has been molecularly subdivided into V_{1A} (Morel et al., 1992) and V_{1B} (or V_3) receptors (Sugimoto et al., 1994), stimulates heterotrimeric G-protein subtype G_q, resulting in secondary messenger responses such as the formation of inositol phosphates and an increase in intracellular calcium concentration ($[Ca^{2+}]_i$) (Thibonnier et al., 1997; Tahara et al., 1998a,b). The vasopressin V_2 receptor was molecularly identified as a single receptor subtype which causes the formation of intracellular cyclic adenosine 3′,5′-monophosphate (cAMP) (Birnbaumer et al., 1992; Lolait et al., 1992; Tahara et al., 1998b) through the G-protein subtype G_s.

Another type of vasopressin receptor, termed ''V_2-like'', has been identified in tissues such as inner medullary collecting duct of kidney (Star et al., 1988; Ishikawa et al., 1988; Champigneulle et al., 1993; Han et al., 1993; Maeda et al., 1993), pancreas (Richardson et al., 1990) and hypothalamus (Jurzak et al., 1995). This receptor is stimulated by the vasopressin V_2 receptor agonist 1-desamino-8-D-arginine vasopressin (dDAVP), resulting in an $[Ca^{2+}]_i$ increase without cAMP accumulation. This stimulation is not inhibited by vasopressin V_{1A} receptor antagonists. Recently, it was reported that high-density G-protein-coupled receptors which couple to G-protein subtype G_s cause an increase in $[Ca^{2+}]_i$ levels when stimulated by their native ligands (Zhu et al., 1994). Ecelbarger et al. (1996)

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suggested that the vasopressin V_2 receptor in inner medullary collecting duct behave as a vasopressin V_2-like receptor because it is expressed at a high-density. However, it was also reported that the vasopressin V_{1B} receptor-selective agonist [deamino-Cys[1], D-3-(Pyridyl)-Ala[2], Arg[8]]vasopressin (D3PVP) acts on vasopressin V_2-like receptors to evoke G_q-mediated effects in inner medullary collecting duct cells (Han et al., 1993; Maeda et al., 1993). The inner medullary collecting duct is an important site for vasopressin to regulate urea and water permeability. In this region, it was reported that supranomolar concentrations of vasopressin decrease osmotic water permeability via vasopressin V_2-like receptors accompanied by an increase in intracellular Ca^{2+} and production of inositol tri-phosphate, while subnanomolar concentrations of vasopressin increase water permeability via vasopressin V_2 receptors (Han et al., 1993). To clarify which of the receptors is the actual vasopressin V_2-like receptor in inner medullary collecting duct, we used a panel of agonists/antagonists to distinguish the vasopressin receptor subtypes, namely, the vasopressin V_{1B}/V_2 receptor agonist dDAVP, the vasopressin V_{1B} receptor-selective agonist D3PVP, the vasopressin V_{1A} receptor-selective agonist [Phe[2], Ile[3], Orn[8]]vasopressin (PO-VT), the oxytocin receptor agonist [Thr[4],Gly[7]]oxytocin (TG-OT), and the V_{1A}/V_2 receptor dual antagonist 4′-[(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-*d*][1]benzazepin-6-yl)carbonyl]2-phenylbenzanilide (YM087). Using these agents, we investigated the pharmacological characteristics of human vasopressin V_2 receptors expressed at high-density and human V_{1B} receptors, both permanently expressed on Chinese hamster ovary (CHO) cell lines, and compared them to those of V_2-like receptors in cultured inner medullary collecting duct cells prepared from rat kidney.

2. Materials and methods

2.1. Materials

Materials were obtained from the following sources: vasopressin and oxytocin from Peptide Research (Osaka, Japan); D3PVP, TG-OT and amethopterin from Sigma (St. Louis, MO, USA); dDAVP from Bachem California (Torrance, CA, USA); PO-VT from Peninsula Laboratories (Belmont, CA, USA); myo-[^{3}H]inositol and [α-^{32}P]deoxyATP AMP from New England Nuclear (Boston, MA, USA); Dulbecco's modified eagle's medium (DMEM) and minimum essential alfa medium (MEM-α) from Gibco-BRL (Grand Island, NY, USA); and Fura 2-acetoxymethyl ester (AM) from Dojindo Laboratories (Kumamoto, Japan).

YM087, (2*S*) 1-[(2*R* 3*S*)-(5-chloro-3-(2-chlorophenyl)-1-(3,4,-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]pyrrolidine-2-carboxamide (SR 49059) and 1-[4-(*N*-*tert*-butyl-carbamoyl)-2-metoxybenzene sulfonyl]5-etoxy-3-spiro-[4-(2-morpholinoethoxy)-cyclohexane]indol-2-one; equatorial isomer (SR 121463A) were synthesized at Yamanouchi Pharmaceutical (Tsukuba, Japan).

2.2. Cell culture

CHO cells expressing human vasopressin V_2 receptors (B_{max} = 7020 fmol/mg protein) and human V_{1B} receptors (B_{max} = 5230 fmol/mg protein) were established as described previously (Tahara et al., 1998b). Cells were diluted twice weekly and cultured in 5% CO_2 in MEM-α without nucleosides, supplemented with 1 μM amethopterin, 10% fetal calf serum, 50 units/ml penicillin and 50 μg/ml streptomycin. The procedure for isolating inner medullary collecting duct cells was modified from the studies of Ishikawa et al. (1988) and Han et al. (1993). Male Wistar rats weighing 150 to 200 g were decapitated, and both kidneys were removed and placed in 20 ml chilled sterile Hanks' balanced salt solution (HBSS). Coronal sections were cut from the kidneys and transferred to a chilled dissection dish. Inner medullary collecting ducts were dissected using a sharp blade from 50–75% of the inner medulla, away from the inner–outer medullary junction to the papillary tip. The outer medullary collecting duct was dissected from the outer medulla, away from both the inner–outer medullary junction and cortex. The minced renal tissues were transferred to 50-ml tubes containing 10 ml collagenase (1 mg/ml) and kept at 37°C in a water bath for 60 min. At 20-min intervals, the tubes were gently shaken to resuspend the tissues. The suspension was drawn up and down a pipette to break up the tissue clump and then centrifuged at $500 \times g$ for 4 min. The pellets were washed three times with HBSS and further incubated with 10 ml collagenase (1 mg/ml) at 37°C for 30 min. After vigorous pipetting and centrifugation, the pellets were resuspended in DMEM supplemented with 10% bovine fetal serum, penicillin (100 U/ml) and streptomycin (100 μg/ml), and cultured on cover glasses (13.5 mm diameter) in a humidified incubator under 5% CO_2. The cultured cells were subjected to experiments on days 5 to 7 of culture.

2.3. Measurements of inositol phosphate production

Inositol phosphate concentrations were measured as previously described (Saito et al., 1997). In brief, human vasopressin V_2 receptor-expressing CHO cells and human V_{1B} receptor-expressing CHO cells were plated on 24 well plates (1×10^5 cells/well) at 37°C for 24 h. After a 16 to 22 h labeling period with 2 μCi/ml myo-[^{3}H]inositol in inositol-free DMEM, cells were incubated in DMEM containing 20 mM LiCl at 37°C for 15 min with various concentrations of vasopressin receptor agonists. Incubation was stopped by addition of perchloric acid (5% v/v), followed by 4 M $KHCO_3$. The supernatant was applied to an AG1-X8 resin column, washed with 0.1 M formic acid

and ^{3}H-labeled inositol phosphates were eluted by a 1 M ammonium formate/0.1 M formic acid mixture.

2.4. Measurement of intracellular Ca^{2+} concentration

$[Ca^{2+}]_i$ was measured as described previously (Tahara et al., 1998b). In brief, CHO cells expressing human vasopressin V_2 receptors and inner medullary collecting duct cells were plated on cover glasses (13.5 mm diameter) and serum-starved for 24 h. After Fura 2-AM (2 μM/cover glass) was loaded for 30 min at 37°C, the cover glass with cell monolayer was placed in a quartz cuvette and fluorescence was recorded with a CAF-110 spectrofluorometer (Japan Spectrometer, Tokyo, Japan). $[Ca^{2+}]_i$ was calculated as described by Grynkiewicz et al. (1985) from the ratio of emission at 340 and 380 nm.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR) Southern hybridization analysis of rat vasopressin V_{1B} receptor mRNA

Extraction of poly(A)$^+$ RNAs from rat whole kidney, renal cortex and medullary segments was performed using the guanidine thiocyanate method, followed by application to an oligo dT column, as previously described (Sugimoto et al., 1992). Each poly(A)$^+$ RNA (1 μg) was converted into first-strand cDNA using a random hexamer. The efficiency of poly(A)$^+$ RNA isolation and cDNA synthesis was estimated by PCR (25 cycles) with the primers 5′-CCATCACCATCTTCCAGGAG-3′ and 5′-CCTGCTTCACCACCTTCTTG-3′ based on rat glyceraldehyde-3-phosphate dehydrogenase cDNA which was validated to amplify the 576-bp fragment by nucleotide sequencing. A RT-PCR analysis of rat vasopressin V_{1B} receptor transcript was performed using primers 5′-AGCATCAGTACCATCTCCAGG-3′ and 5′-TGGTCTCCATAGTGGCTTCC-3′. PCR primers for RT-PCR were designed on the basis of the genomic sequence corresponding to both sides of the splicing junction in the amino acid coding region (Saito et al., 1995). This primer set has been validated to only amplify the 463-bp fragment of rat vasopressin V_{1B} receptor cDNA by nucleotide sequencing and not to amplify any other fragments of vasopressin V_{1A} and V_2 receptor cDNA. The cDNA (1/25 volume) was used for PCR as follows: initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 1 min and extension at 72°C for 2 min. Aliquots (15 μl) of PCR products were separated by electrophoresis on 1% agarose gel and blotted onto Hybond-N nylon membrane. The membrane was hybridized overnight at 42°C in 50% formamide, 5× standard saline citrate (SSC, 1× SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.4), 10× Denhart's solution, 2% sodium dodecyl sulfate (SDS), and 100 μg/ml sheared and denatured salmon sperm DNA, with the ^{32}P-labelled probe corresponding to the whole amino acid sequence of rat vasopressin V_{1B} receptor. This hybridized probe has already been validated to be selective for the amplified fragment of rat vasopressin V_{1B} receptor and does not hybridize fragments amplified by rat V_{1A} and V_2 receptor cDNA fragment under the hybridization and wash conditions described above. The membrane was washed twice for 30 min in 1× SSC, 0.1% SDS at room temperature. It was then further washed twice for 30 min in 1× SSC, 0.1% SDS at 57°C, followed by detection of radioactive signals using a BAS 2000 Bioimaging Analyzer (Fuji Photo Film).

2.6. Data analysis

The EC_{50} and IC_{50} values were estimated from the concentration–response curves with the non-linear regression program, Graphpad PRISM. Experimental results are expressed as the means ± S.E.M. or the means with 95% confidence limits. Statistical comparisons were made by

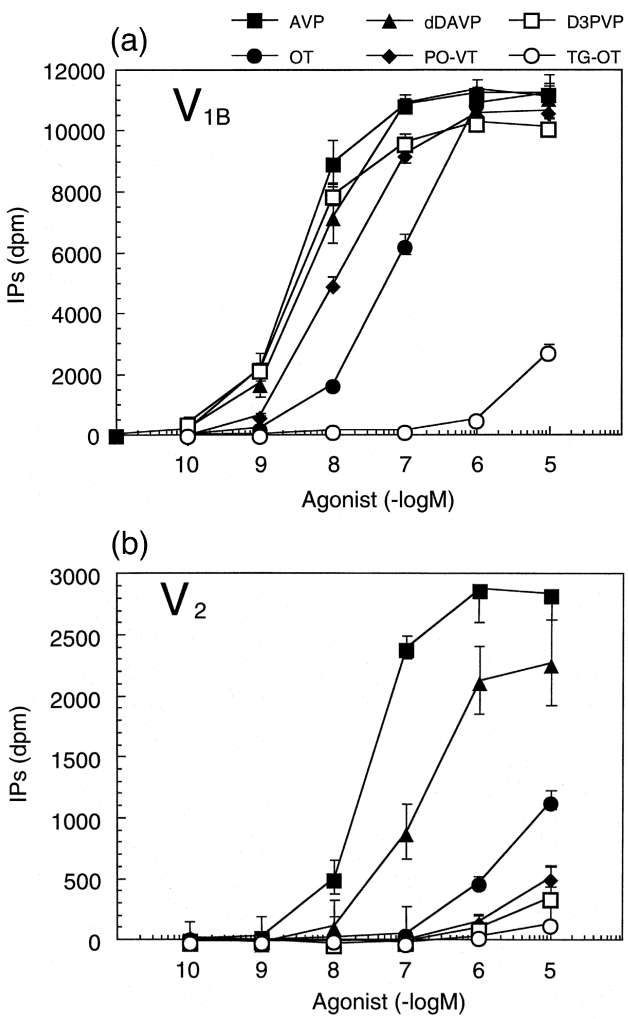

Fig. 1. Agonistic effects of vasopressin receptor agonists, vasopressin, dDAVP, oxytocin, PO-VT, D3PVP and TG-OT, on inositol phosphate production in human vasopressin V_{1B} receptor-expressing CHO cells (a), and in human vasopressin V_2 receptor-expressing CHO cells (b). Values are means ± S.E.M. from six to eight independent duplicate experiments.

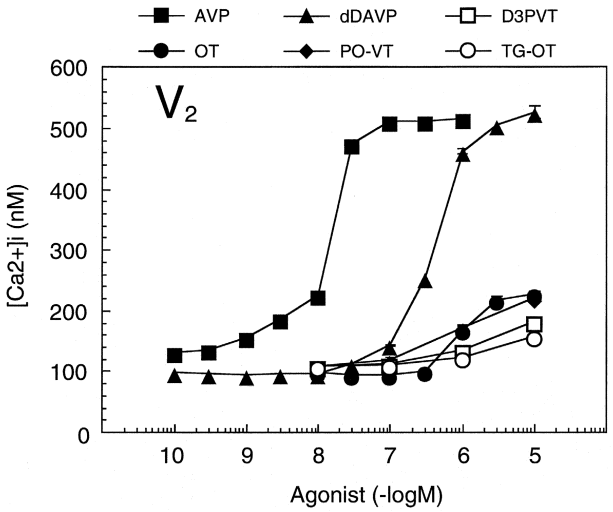

Fig. 2. Agonistic effects of vasopressin receptor agonists, vasopressin, dDAVP, oxytocin, PO-VT, D3PVP and TG-OT, on $[Ca^{2+}]_i$ in human vasopressin V_2 receptor-expressing CHO cells. Values are mean ± S.E.M. from six to eight independent duplicate experiments.

the Dunnett multiple test using the SAS program (SAS Institute, Japan).

3. Results

3.1. Effect of agonists on inositol phosphate production through human vasopressin V_{1B} and V_2 receptors

In vasopressin V_2 receptor-expressing CHO cells (B_{max} = 2250, 3980, 5770, 7120, 39800 fmol/mg protein), maximal intracellular inositol phosphate production induced by vasopressin and dDAVP was remarkably increased at higher receptor densities. This result is consistent with previous observations in which vasopressin evoked inositol phosphate formation and increased $[Ca^{2+}]_i$ in L cells over-expressing the vasopressin V_2 receptor (Zhu et al., 1994). To determine the pharmacological properties of human vasopressin V_{1B} and V_2 receptors at high receptor densities, inositol phosphate production induced by the vasopressin and oxytocin receptor agonists vasopressin, oxytocin, dDAVP, PO-VT, D3PVP and TG-OT were measured.

In vasopressin V_{1B} receptor-expressing CHO cells, all the tested agonists stimulated inositol phosphate production, although TG-OT was less effective than the other agonists. EC_{50} values were 3.1 (2.3–4.0) nM for vasopressin, 7.8 (6.3–9.4) nM for oxytocin, 5.9 (3.7–4.0) nM for dDAVP, 11 (7.5–16) nM for PO-VT and 3.2 (2.4–4.1) nM for D3PVP. The maximal stimulated signals of oxytocin, dDAVP, PO-VT and D3PVP compared to that of vasopressin were 100%, 101%, 94% and 90%, respectively (Fig. 1a).

In high-density vasopressin V_2 receptor-expressing CHO cells, only vasopressin and dDAVP stimulated inositol phosphate production. Oxytocin had a weak effect on inositol phosphate production while that of PO-VT, D3PVP and TG-OT was insignificant. EC_{50} values were 28 (14–42) nM for vasopressin, 120 (30–220) nM for dDAVP and 1800 (1300–2400) nM for oxytocin. The maximal stimulated signals of dDAVP, oxytocin and D3PVP compared to that of vasopressin were 73%, 34% and 7%, respectively (Fig. 1b). These results confirm that vasopressin V_2 receptors expressed at high-density are capable of evoking inositol phosphate production, and that the agonistic pro-

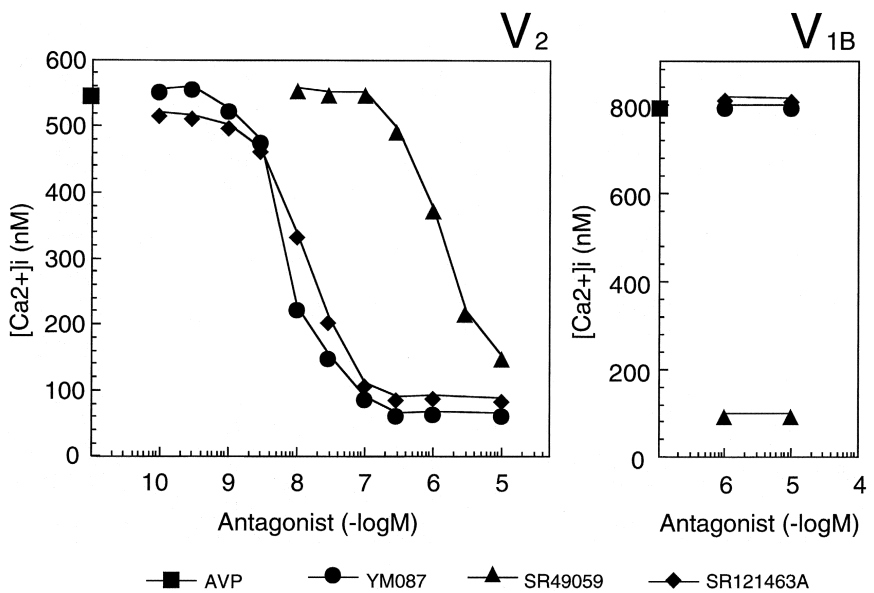

Fig. 3. Inhibitory effects of the vasopressin receptor antagonists SR 49059, SR 121463A, YM087 on 100 nM vasopressin-induced increase in $[Ca^{2+}]_i$ in human V_2 receptor-expressing CHO cells (left) and in human vasopressin V_{1B} receptor-expressing CHO cells (right). Values are means ± S.E.M. from three to five independent experiments.

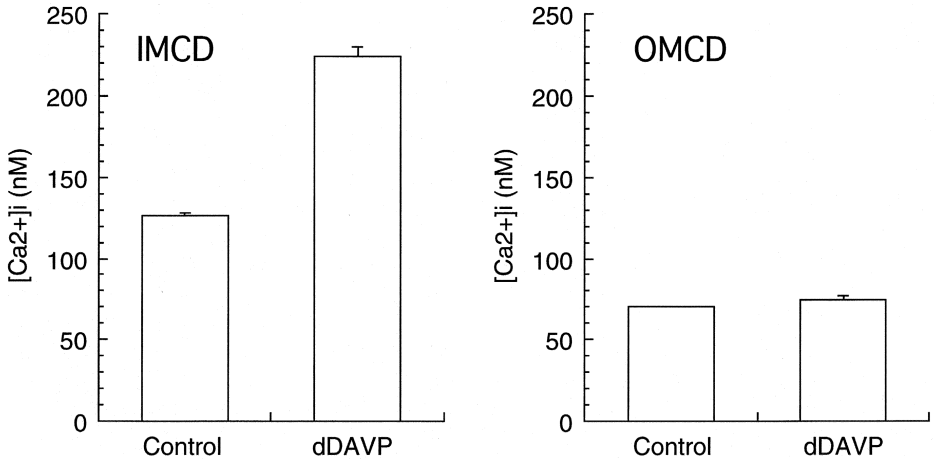

Fig. 4. Effects of dDAVP on $[Ca^{2+}]_i$ in rat inner medullary collecting duct (left) and outer medullary collecting duct cells (right). Values are means ± S.E.M. from four independent experiments.

file of high-density V_2 receptors is different from that of V_{1B} receptors.

3.2. Effect of agonists on intracellular calcium production through human vasopressin V_2 receptor

As shown in Fig. 2, in high-density vasopressin V_2 receptor-expressing CHO cells, vasopressin and dDAVP evoked an increase in $[Ca^{2+}]_i$, whereas D3PVP was less effective. The effective rank order of agonistic activity was vasopressin > dDAVP > oxytocin = PO-VT = D3PVP = TG-OT. EC_{50} values were 14 (8–26) nM for vasopressin, 570 (310–1000) nM for dDAVP and 2300 (220–24 000) nM for oxytocin. The relative maximal stimulated signals of dDAVP, oxytocin and D3PVP compared to that of vasopressin were 102%, 28% and 16%, respectively (Fig. 2).

3.3. Effect of antagonists on intracellular calcium production through human vasopressin V_{1B} and V_2 receptors

In high-density vasopressin V_2 receptor-expressing CHO cells, YM087 and SR121463A potently inhibited the production of intracellular Ca^{2+} on stimulation by 100 nM vasopressin in a concentration-dependent manner. SR49059 had weak antagonistic activity. IC_{50} values were 6.3 (3.1–13) nM for YM087, 9.5 (6.6–13) nM for SR121463A and 1400 (1000–1900) nM for SR49059 (Fig. 3, left). In vasopressin V_{1B} receptor-expressing CHO cells stimulated by 100 nM vasopressin, 1 and 10 μM YM087 and SR121463A had no effect on the production of intracellular Ca^{2+}, whereas 1 μM SR49059 completely blocked it (Fig. 3, right). Furthermore, similarly to vasopressin, upon stimulation by 100 nM dDAVP, 10 μM YM087 completely inhibited the production of intracellular Ca^{2+} in vasopressin V_2 receptor-expressing CHO cells, but had no effect on vasopressin V_{1B} receptor-expressing CHO cells. None of the antagonists (up to 100 μM) changed basal values of Ca^{2+} concentration without stimulation. These results show that YM087 and SR121463A can distinguish between high-density vasopressin V_2 receptors and V_{1B} receptors.

3.4. Effect of dDAVP and d3PVP on intracellular calcium production in isolated inner medullary collecting duct and outer medullary collecting duct cells

Basal $[Ca^{2+}]_i$ values in inner medullary collecting duct and outer medullary collecting duct cells were 120 and 70 nM, respectively. Addition of 10 nM dDAVP evoked an increase in $[Ca^{2+}]_i$ in inner medullary collecting duct cells, but had no effect in outer medullary collecting duct cells (Fig. 4). In inner medullary collecting duct cells, D3PVP also evoked an increase in $[Ca^{2+}]_i$. Further, 1 μM YM087 had no significant antagonistic influence on the $[Ca^{2+}]_i$ increase induced by 10 nM dDAVP and D3PVP (Table 1).

3.5. Distribution of vasopressin V_{1B} receptor mRNA in kidney

By RT-PCR with vasopressin V_{1B} receptor-specific primers and Southern hybridization using PCR products, mRNA of vasopressin V_{1B} receptor was detected in whole

Table 1
Intracellular Ca^{2+} concentration stimulated by dDAVP and D3PVP with and without YM087 in inner medullary collecting duct cells

Substance/treatment (*n*)	$[Ca^{2+}]_i$ (nM)	*P* value
Vehicle (6)	126 ± 1	
1 μM YM087 (6)	127 ± 1	NS
10 nM dDAVP (3)	223 ± 6	< 0.01
1 μM YM087 + 10 nM dDAVP (3)	226 ± 7	< 0.01
10 nM D3PVP (3)	204 ± 5	< 0.01
1 μM YM087 + 10 nM D3PVP (3)	204 ± 3	< 0.01

Cellular Ca^{2+} concentrations represent the means ± S.E.M.. Each group was compared vs. the vehicle group by Dunnett's multiple test.

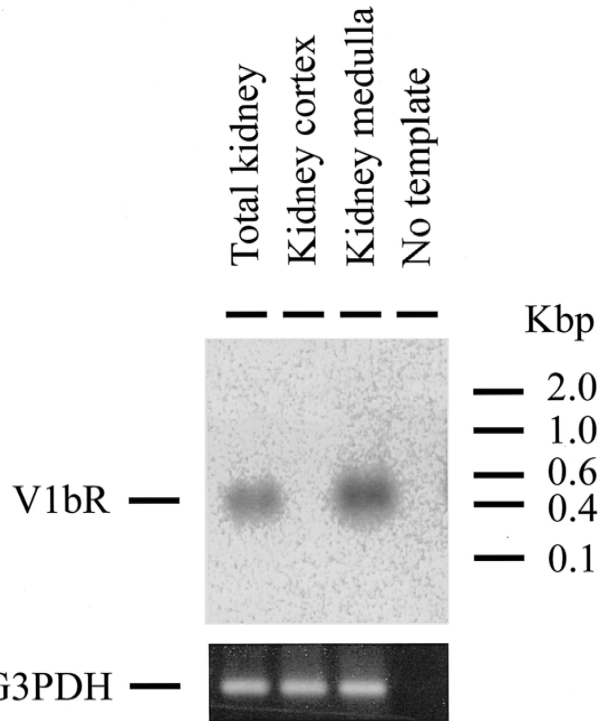

Fig. 5. RT-PCR Southern analysis of whole, medulla and cortex of rat kidney. The same amount of cDNA was applied to each lane after PCR. Rat glyceraldehyde-3-phosphate dehydrogenase as an internal standard.

kidney and medulla, but not in the cortex (Fig. 5). In this RT-PCR analysis, no external band was detected.

4. Discussion

The agonistic and antagonistic properties of vasopressin V_2-like receptors, which are coupled to an increase in $[Ca^{2+}]_i$, are different from those of authentic vasopressin V_2 receptors, which are coupled to an increase in cAMP. If the vasopressin V_2 receptor expressed at high-density does in fact behave as a V_2-like receptor, this would entail it changing its agonistic and antagonistic properties depending on the level of expression or type of secondary messenger signal. In the present study, therefore, we investigated the pharmacological properties of high-density vasopressin V_2 receptors.

Results showed no significant difference in agonistic and antagonistic properties, as measured by inositol phosphate production and the increase in $[Ca^{2+}]_i$, between the high-density vasopressin V_2 receptor and the reported authentic V_2 receptor. Among agonists tested, D3PVP clearly distinguished vasopressin V_2-like receptors in inner medullary collecting duct cells from V_2 receptors in both inner medullary collecting duct cells and CHO cells at high receptor density. D3PVP was less effective at high-density vasopressin V_2 receptor-expressing CHO cells, as measured by inositol phosphate production (Fig. 1b) and the increase in $[Ca^{2+}]_i$ (Fig. 2). In comparison, dDAVP stimulated inositol phosphate production and an increase in $[Ca^{2+}]_i$ via both vasopressin V_{1B} receptors (Fig. 1a, Tahara et al., 1998b) and high-density vasopressin V_2 receptors (Figs. 1b and 2). In inner medullary collecting duct cells, D3PVP and dDAVP stimulated an increase in $[Ca^{2+}]_i$ (Table 1). This is consistent with the previous observations that 10 nM D3PVP and dDAVP induced an increase in $[Ca^{2+}]_i$ (Maeda et al., 1993) and that 0.1 nM D3PVP prevented water permeability induced by a low concentration (0.1 nM) of vasopressin in rat isolated inner medullary collecting duct segments (Han et al., 1993).

D3PVP is known as a vasopressin V_{1B} receptor agonist with weak agonistic activity for vasopressin V_2 receptors and weak antagonistic activity for V_{1A} receptors (Schwartz et al., 1991). In the present study, D3PVP stimulated inositol phosphate production in vasopressin V_{1B} receptor-expressing CHO cells (Fig. 1a). Moreover, we recently reported that dDAVP acted as an agonist at vasopressin V_{1B} as well as V_2 receptors (Saito et al., 1997). Together, the present results suggest that vasopressin V_{1B} receptors may be the candidate vasopressin V_2-like receptors in inner medullary collecting duct.

YM087, a nonpeptide vasopressin V_{1A}/V_2 receptor dual antagonist, had no influence on vasopressin V_{1B} receptors (Tahara et al., 1997, 1998a,b). At 1 μM, YM087 completely inhibited the increase in $[Ca^{2+}]_i$ induced by 100 nM vasopressin in vasopressin V_2 receptor-expressing CHO cells, but had no effect in V_{1B} receptor-expressing cells (Fig. 3). YM087 is therefore the best tool available to distinguish vasopressin V_{1B} receptors from vasopressin V_{1A} and V_2 receptors. In inner medullary collecting duct cells, 1 μM YM087 had no antagonistic influence on the $[Ca^{2+}]_i$ increase induced by 10 nM dDAVP and D3PVP (Table 1). This result clearly indicates that the vasopressin V_2-like receptor in inner medullary collecting duct cells is not the authentic vasopressin V_2 receptor expressed at high receptor density (Ecelbarger et al., 1996), and suggests that the vasopressin V_{1B} receptor is the strongest candidate pharmacologically.

It has been reported that vasopressin V_{1B} receptor mRNA is detected in extra-pituitary tissues, such as pancreas and kidney, in humans (Sugimoto et al., 1995) and rats (Lolait et al., 1995; Saito et al., 1995), though expression levels are lower than in the pituitary. In pancreas, the vasopressin V_{1B} receptor has been pharmacologically identified. It was reported that dDAVP stimulated the secretion of insulin in rat perfused pancreas (Dunning et al., 1984). The secreted insulin comes from β-cells, causing an increase in $[Ca^{2+}]_i$ via vasopressin V_1 receptors (Li et al., 1992) which was not inhibited by a vasopressin V_{1A} receptor antagonist (Richardson et al., 1990). The atypical vasopressin receptor in pancreas, which evokes an increase in $[Ca^{2+}]_i$ after stimulation by dDAVP, termed the V_2-like receptor in inner medullary collecting duct, has been identified as the vasopressin V_{1B} receptor on the basis of the results of agonist studies in cultured hamster beta cell line

(HIT) cells (Richardson et al., 1995) and antagonist studies in RINm5F cells (Lee et al., 1995).

Vasopressin V_{1B} receptor mRNA was detected at the same expression level in kidney as in pancreas. mRNA in kidney is expressed at a level sufficient to be pharmacologically detected to the same degree as in the pancreas. However, the site of vasopressin V_{1B} receptor expression in kidney has yet to be investigated in detail. The present RT-PCR study shows that vasopressin V_{1B} receptor mRNA was located only in the medulla and not in the cortex (Fig. 5). This rough localization to the medulla is consistent with the localization of the vasopressin V_2-like receptor, which is pharmacologically detected solely in the inner medullary collecting duct. However, in previous RT-PCR studies using microdissected nephron, vasopressin V_2 receptor mRNA was detected not only in the inner medullary collecting duct but also in the outer medullary collecting duct and the cortical collecting duct (Terada et al., 1993; Firsov et al., 1994). If the vasopressin V_2-like receptor is the vasopressin V_2 receptor, then this becomes inconsistent with the observation that the vasopressin V_2-like receptor is pharmacologically detected only in the inner medullary collecting duct, and not in the outer medullary collecting duct or cortical collecting duct. It is difficult to explain why the vasopressin V_2 receptor acts as a V_2-like receptor only in the inner medullary collecting duct, even if abundant vasopressin V_2 receptors in the inner medullary collecting duct evoked an increase in $[Ca^{2+}]_i$ after stimulation by dDAVP. The present pharmacological evidence, especially that using D3PVP and YM087, strongly suggests that the vasopressin V_2-like receptor in the inner medullary collecting duct is the vasopressin V_{1B} receptor. The RT-PCR results also support this suggestion. However, the distribution of vasopressin V_{1B} receptors in the nephron has not been determined in detail. Determination of the true nature of the vasopressin V_2-like receptor in the inner medullary collecting duct will require close observation of the localization of vasopressin V_{1B} receptor mRNA or receptor protein in nephron, in situ hybridization, immunohistochemical analysis of renal slices, or RT-PCR analysis with microdissected nephron.

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References

Barberis, C., Tribollet, E., 1996. Vasopressin and oxytocin receptors in the central nervous system. Crit. Rev. Neurobiol. 10, 119–154.

Birnbaumer, M., Seibold, A., Gilbert, S., Ishido, M., Barberis, C., Antaramian, A., Brabet, P., Rosenthal, W., 1992. Molecular cloning of the receptor for human antidiuretic hormone. Nature 357, 333–335.

Champigneulle, A., Siga, E., Vassent, G., Imbert-Teboul, M., 1993. V_2-like vasopressin receptor mobilizes intracellular Ca^{2+} in rat medullary collecting tubules. Am. J. Physiol. 265, F35–F45.

Dunning, B.E., Moltz, J.H., Fawcett, C.P., 1984. Modulation of insulin and glucagon secretion from the perfused rat pancreas by the neurohypophysial hormones and by desamino-D-arginine vasopressin (dDAVP). Peptides 5, 871–875.

Ecelbarger, C.A., Chou, C.L., Lolait, S.J., Knepper, M.A., DiGiovanni, S.R., 1996. Evidence for dual signaling pathways for V_2 vasopressin receptor in rat inner medullary collecting duct. Am. J. Physiol. 270, F623–F633.

Firsov, D., Mandon, B., Morel, A., Merot, J., Le Maout, S., Bellanger, A.C., de Rouffignac, C., Elalouf, J.M., Buhler, J.M., 1994. Molecular analysis of vasopressin receptors in the rat nephron: evidence for alternative splicing of the V_2 receptor. Pfluegers Arch. 429, 79–89.

Grynkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–3450.

Han, J.S., Maeda, Y., Knepper, M.A., 1993. Dual actions of vasopressin and oxytocin in regulation of water permeability in terminal collecting duct. Am. J. Physiol. 265, F26–F34.

Ishikawa, S., Okada, K., Saito, T., 1988. Arginine vasopressin increases cellular free calcium concentration and adenosine 3′,5′-monophosphate production in rat renal papillary collecting tubule cells in culture. Endocrinology 123, 1376–1384.

Jurzak, M., Muller, A.R., Gerstberger, R., 1995. Characterization of vasopressin receptors in cultured cells derived from the region of rat brain circumventricular organs. Neuroscience 65, 1145–1159.

László, F.A., László, F. Jr., De Wied, D., 1991. Pharmacology and clinical perspectives of vasopressin antagonists. Pharmacol. Rev. 43, 73–108.

Lee, B., Yang, C., Chen, T.H., al Azawi, N., Hsu, W.H., 1995. Effect of AVP and oxytocin on insulin release: involvement of V_{1b} receptors. Am. J. Physiol. 269, E1095–E1100.

Li, G., Pralong, W.F., Pittet, D., Mayr, G.W., Schlegel, W., Wollheim, C.B., 1992. Inositol tetrakisphosphate isomers and elevation of cytosolic Ca^{2+} in vasopressin-stimulated insulin-secreting RINm5F cells. J. Biol. Chem. 267, 4349–4356.

Lolait, S.J., O'Carroll, A.M., McBride, O.W., Konig, M., Morel, A., Brownstein, M.J., 1992. Cloning and characterization of a vasopressin V_2 receptor and possible link to nephrogenic diabetes insipidus. Nature 357, 336–339.

Lolait, S.J., O'Carroll, A.M., Mahan, L.C., Felder, C.C., Button, D.C., Young, W.S. 3rd, Mezey, E., Brownstein, M.J., 1995. Extrapituitary expression of the rat V_{1b} vasopressin receptor gene. Proc. Natl. Acad. Sci. U. S. A. 92, 6783–6787.

Maeda, Y., Han, J.S., Gibson, C.C., Knepper, M.A., 1993. Vasopressin and oxytocin receptors coupled to Ca^{2+} mobilization in rat inner medullary collecting duct. Am. J. Physiol. 265, F15–F25.

Michell, R.H., Kirk, C.J., Billah, M.M., 1979. Hormonal stimulation of phosphatidylinositol breakdown with particular reference to the hepatic effects of vasopressin. Biochem. Soc. Trans. 7, 861–865.

Morel, A., O'Carroll, A.M., Brownstein, M.J., Lolait, S.J., 1992. Molecular cloning and expression of a rat V_{1a} arginine vasopressin receptor. Nature 356, 523–526.

Richardson, S.B., Eyler, N., Twente, S., Monaco, M., Altszuler, N., Gibson, M., 1990. Effects of vasopressin on insulin secretion and inositol phosphate production in a hamster beta cell line (HIT). Endocrinology 126, 1047–1052.

Richardson, S.B., Laya, T., Van Ooy, M., 1995. Similarities between hamster pancreatic islet beta (HIT) cell vasopressin receptors and V_{1b} receptors. J. Endocrinol. 147, 59–65.

Saito, M., Sugimoto, T., Tahara, A., Kawashima, H., 1995. Molecular cloning and characterization of rat V_{1b} vasopressin receptor: evidence for its expression in extra-pituitary tissues. Biochem. Biophys. Res. Commun. 212, 751–757.

Saito, M., Tahara, A., Sugimoto, T., 1997. 1-desamino-8-D-arginine

vasopressin (dDAVP) as an agonist on V_{1b} vasopressin receptor. Biochem. Pharmacol. 53, 1711–1717.

Schwartz, J., Derdowska, I., Sobocinska, M., Kupryszewski, G., 1991. A potent new synthetic analog of vasopressin with relative agonist specificity for the pituitary. Endocrinology 129, 1107–1109.

Star, R.A., Nonoguchi, H., Balaban, R., Knepper, M.A., 1988. Calcium and cyclic adenosine monophosphate as second messengers for vasopressin in the rat inner medullary collecting duct. J. Clin. Invest. 81, 1879–1888.

Sugimoto, T., Tsuchimochi, H., McGregor, C.G., Mutoh, H., Shimizu, T., Kurachi, Y., 1992. Molecular cloning and characterization of the platelet-activating factor receptor gene expressed in the human heart. Biochem. Biophys. Res. Commun. 189, 617–624.

Sugimoto, T., Saito, M., Mochizuki, S., Watanabe, Y., Hashimoto, S., Kawashima, H., 1994. Molecular cloning and functional expression of a cDNA encoding the human V_{1b} vasopressin receptor. J. Biol. Chem. 269, 27088–27092.

Sugimoto, T., Saito, M., Mochizuki, S., Tahara, A., Watanabe, Y., Hashimoto, S., Kawashima, H., 1995. Molecular cloning and functional expression of V_{1B} receptor gene. In: Saito, T., Kurokawa, K., Yoshida, S. (Eds.), Neurohypophysis: Recent Progress of Vasopressin and Oxytocin Research: Proceedings of the 1st Joint World Congress of Neurohypophysis and Vasopressin, Nasu, Tochigi, Japan, 16–21 July. Elsevier, Amsterdam, pp. 409–413.

Tahara, A., Tomura, Y., Wada, K.I., Kusayama, T., Tsukada, J., Takanashi, M., Yatsu, T., Uchida, W., Tanaka, A., 1997. Pharmacological profile of YM087, a novel potent nonpeptide vasopressin V_{1A} and V_2 receptor antagonist, in vitro and in vivo. J. Pharmacol. Exp. Ther. 282, 301–308.

Tahara, A., Saito, M., Sugimoto, T., Tomura, Y., Wada, K., Kusayama, T., Tsukada, J., Ishii, N., Yatsu, T., Uchida, W., Tanaka, A., 1998a. Pharmacological characterization of YM087, a potent, nonpeptide human vasopressin V_{1A} and V_2 receptor antagonist. Naunyn-Schmiedeberg's Arch. Pharmacol. 357, 63–69.

Tahara, A., Saito, M., Sugimoto, T., Tomura, Y., Wada, K., Kusayama, T., Tsukada, J., Ishii, N., Yatsu, T., Uchida, W., Tanaka, A., 1998b. Pharmacological characterization of the human vasopressin receptor subtypes stably expressed in Chinese hamster ovary cells. Br. J. Pharmacol. 125, 1463–1470.

Terada, Y., Tomita, K., Nonoguchi, H., Yang, T., Marumo, F., 1993. Different localization and regulation of two types of vasopressin receptor messenger RNA in microdissected rat nephron segments using reverse transcription polymerase chain reaction. J. Clin. Invest. 92, 2339–2345.

Thibonnier, M., Preston, J.A., Dulin, N., Wilkins, P.L., Berti-Mattera, L.N., Mattera, R., 1997. The human V3 pituitary vasopressin receptor: ligand binding profile and density-dependent signaling pathways. Endocrinology 138, 4109–4122.

Zhu, X., Gilbert, S., Birnbaumer, M., Birnbaumer, L., 1994. Dual signaling potential is common among Gs-coupled receptors and dependent on receptor density. Mol. Pharmacol. 46, 460–469.