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1-Desamino-8-D-Arginine Vasopressin (DDAVP) as an Agonist on V_{1b} Vasopressin Receptor

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ABSTRACT. 1-desamino-8-D-arginine vasopressin (DDAVP) is considered a standard vasopressin V_2 receptor-selective agonist with a potent antidiuretic effect through V_2 receptor without the induction of vasoconstriction through V_{1a} receptor. Furthermore, DDAVP was reported to act as an agonist on non-V_{1a}, non-V_2 receptor to cause the accumulation of intracellular Ca^{2+} in several tissues. However, the agonistic activity of DDAVP against the other vasopressin receptor, V_{1b} (or V_3), which can accumulate intracellular Ca^{2+} and which we recently cloned, has not been clarified. Hence, we compared the characteristics of DDAVP on V_{1b} receptor with those on the other vasopressin receptors. In binding experiments, DDAVP more strongly inhibited [^{3}H]arginine vasopressin binding to V_{1b} than to V_2 receptor (K_i: 5.84 nM vs 65.9 nM). In addition, DDAVP dose-dependently stimulated inositol turnover in human V_{1b} receptor-expressing COS-1 cells. DDAVP acted as a full agonist on human V_{1b} receptor (EC_{50}: 11.4 nM) as well as on human V_2 receptor (EC_{50}: 23.9 nM). However, DDAVP behaved as a partial agonist toward rat V_{1b} receptor (intrinsic activity: 0.7, EC_{50}: 43.5 nM), while there was no significant difference in the agonistic properties of arginine vasopressin on human and rat V_{1b} receptor. In conclusion, DDAVP acts as an agonist on V_{1b} receptor, as it does on V_2 receptor. These findings will allow us to better understand the physiological role of V_{1b} receptor in pancreatic β cells and in the renal inner medullary collecting duct, and help us to identify as yet unknown vasopressin receptors through which DDAVP cause the accumulation of intracellular Ca^{2+} in other tissues. BIOCHEM PHARMACOL 53;11:1711–1717, 1997.

KEY WORDS. vasopressin; receptor; binding; agonist; desmopressin; second messengers

1-desamino-8-D-arginine vasopressin (DDAVP), desmopressin, is well-known as a synthetic analog of 8-arginine vasopressin (AVP) with a selective agonistic effect on antidiuretic type (V_2) receptor [1]. Although the affinities of DDAVP for human V_2 and human vascular type (V_{1a}) receptors are similar in competitive binding [2, 3], DDAVP has strong antidiuretic activity but less potent vasoconstrictor activity [4] because of its agonistic effect on V_2 receptor and antagonistic activity to V_{1a} receptor. In addition, its resistance to degradation results in 20-fold greater antidiuretic activity than AVP *in vivo* [5]. Thus, DDAVP is considered to be a 'strong' and 'selective' V_2 receptor agonist, and is sometimes utilized as a standard reagent of V_2-selective agonists in investigation of the vasopressin receptor signal pathway. In addition to its effect on V_2 receptor in kidney, DDAVP has also been shown to stimulate another type of vasopressin receptor, which was neither of the V_{1a} nor V_2 type, in several tissues such as inner medulla collecting duct (IMCD) of the kidney [6, 7, 8], pancreas [9], hypothalamus [10] and endothelium [11]. In these tissues, atypical receptors mediated intracellular Ca^{2+} accumulation without cyclic adenosine 3′,5′-monophosphate (cAMP) accumulation [7, 9, 12]. In addition, agonistic effects through these receptors were antagonized by several V_1 antagonists but not by several V_2 antagonists [9, 11, 12]. However, based on the idea that DDAVP is a specific agonist for V_2 receptor, it has been concluded that these receptors were probably V_2 or V_2-like receptors.

V_{1b} receptor, which is known as a vasopressin receptor subtype, has been pharmacologically identified in rat pituitary [13, 14], as have V_{1a} and V_2 receptors. Although this receptor is classified as a V_1 type because it evokes Ca^{2+} accumulation as a second messenger, it is clearly differentiated from V_{1a} receptor by binding characteristics for agonists and antagonists. Receptor binding studies and *in vitro* physiological studies have revealed that this receptor is localized mainly in the anterior pituitary gland [14], where it modulates the release of adrenocorticotropic hormone (ACTH) [15, 16, 17]. Recently, we were the first to succeed in the molecular cloning of V_{1b} receptor cDNA from

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Abbreviations: DDAVP, 1-desamino-8-D-arginine vasopressin; AVP, 8-arginine vasopressin; IMCD, inner medulla collecting duct; cAMP, cyclic adenosine 3′,5′-monophosphate; ACTH, adrenocorticotropic hormone; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; IPs, inositol-monophosphate, inositol-disphosphate and inositol-trisphosphate; RT-PCR, reverse transcription-polymerase chain reaction.

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human pituitary gland [18]. cDNA sequences of this receptor have since been reported in humans [19] and rats [20, 21]. As a consequence of the cloning of this receptor, reverse transcription-polymerase chain reaction (RT-PCR) analysis revealed that its messenger RNA is expressed not only in anterior pituitary gland but also in many extra-pituitary tissues such as brain, pancreas and kidney [20, 21, 22]. Although the physiological functions of V_{1b} receptor in extra-pituitary tissues remain unknown, several tissues expressing V_{1b} receptor were also reported to express the atypical receptor through which DDAVP evoked Ca^{2+} accumulation. Thus, a finding that DDAVP acts as an agonist of V_{1b} receptor would strongly suggest that the atypical receptors are V_{1b} receptors. However, it has also been reported that DDAVP induced only a small release of ACTH from rat anterior pituitary *in vitro* [16] and *in vivo* [23], and only then at high concentrations. These findings indicate that DDAVP has little effect on V_{1b} receptor, and its effects on this receptor have been concluded to be pharmacologically insignificant. To better understand these issues, we investigated the characteristics of DDAVP on cloned human and rat V_{1b} receptors transiently expressed in COS-1 cells.

MATERIALS AND METHODS

Materials

Materials were obtained from the following sources: AVP, DDAVP and oxytocin from Peptide Research Inc. (Osaka, Japan); [d$(CH_2)_5^1$,Tyr(Me)^2]AVP from Sigma (St. Louis, MO, U.S.A.); [^{3}H]AVP and cAMP measurement assay kit from Amersham (Chicago, IL, U.S.A.); myo-[^{3}H]inositol from DuPont-NEN (Boston, MA, U.S.A.); Dulbecco's modified Eagle's medium (DMEM), inositol-free DMEM, penicillin, streptomycin and fetal calf serum (FCS) from GIBCO-BRL (Grand Island, NY, U.S.A.); and AG1-X8 resin formate form from Bio-Rad (Hercules, CA, U.S.A.). pEF-BOS was provided by Dr. S. Nagata (Osaka Bioscience Institute, Osaka, Japan) and pME-18S by Dr. T. Saito (Chiba University, School of Medicine, Chiba, Japan). COS-1 cells were provided by Dr. H. Handa (Tokyo Institute of Technology, Yokohama, Japan).

Transfections in COS-1 Cells

cDNA fragments containing the entire coding region of vasopressin receptors human V_{1b}, human V_2, and rat V_{1b} were subcloned into the mammalian expression vector pEF-BOS [24], and human V_{1a} was subcloned into the mammalian expression vector pME-18S [25]. COS-1 cells were grown in monolayer culture at 37° in 5% CO_2 and supplied with DMEM supplemented with 10% FCS, penicillin (100 units/mL) and streptomycin (100 μg/mL). COS-1 cells (2×10^6 cells) were incubated overnight, exposed to the plasmid DNA (40 μg) with DEAE-dextran (0.25 mg/mL) for 14 hr, and then exposed to 0.1 mM chloroquine for 2.5 hr. After 48 hr culture, the transfected cells were used for binding experiments and second messenger formation assays.

Crude Membrane Preparations and Radioligand Binding Studies

COS-1 cells were homogenized at 72 hr after transfection in ice-cold 10 mM Tris-HCl, pH 7.4, containing 5 mM EDTA, and centrifuged at 35,000 × *g* for 20 min at 4°. Membranes (30 μg protein) were incubated with 0.1–3 nM concentrations of [^{3}H]AVP and agonists for 1 hr at 22°. The reactions were terminated by rapid filtration and washing with ice-cold Tris-HCl buffer through 96-well UniFilter Plates using a MicroMate 196 Cell Harvester (Packard, Meriden, CT, USA). Radioactivity was counted with a TopCount Microplate Scintillation Counter (Packard). Non-specific binding was determined in the presence of 1 μM AVP. Data were analyzed with a non-linear curve fitted to the Hill equation. K_i values were calculated according to the Cheng and Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_d)$.

cAMP Measurements

COS-1 cells at 24 hr after transfection were plated into 12-well plates (1×10^5 cells/well), cultured to 90–95% confluence and incubated in DMEM with 0.5 mM 3-isobutyl-1-methylxanthine and 0.1% BSA containing vehicle or various concentrations of agonists for 10 min at 37°. At the end of incubation, the cell monolayers were washed three times with PBS followed by lysis with boiling 0.5 mM sodium acetate, pH 6.2, containing 0.2 mM 3-isobutyl-1-methylxanthine. Extracts were then boiled for 3 min and kept at −20° before determination of cAMP using the competitive protein assay kit [26].

Inositol-monophosphate, Inositol-disphosphate and Inositol-trisphosphate (IPs) Measurements

COS-1 cells were plated into 12-well plates (1×10^5 cells/well) at 24 hr after transfection, and cultured at 37° for 24 hr. At 24 hr after plating, the culture medium was replaced with inositol-free DMEM containing 2 μCi/mL myo-[^{3}H]inositol, 2% FCS, penicillin (100 units/mL) and streptomycin (100 μg/mL). After a 16–22 hr labeling period, cells were washed twice with DMEM and then incubated in DMEM containing 20 mM LiCl at 37° for 15 min with various concentrations of vasopressin receptor agonists. Incubation was stopped by the addition of perchloric acid (5% v/v). Extracts were neutralized with 4 M $KHCO_3$ and then centrifuged at 4,000 × *g* for 15 min. The supernatant was applied to an AG1-X8 resin column. Tritium-labeled IPs were separated by the addition of an ammonium formate/formic acid mixture of increasing ionic strength, as described by Berridge *et al.* [27].

TABLE 1. Vasopressin receptor affinity (K_d) and density (B_{max}) of [^{3}H]AVP in transiently transfected COS-1 cells expressing human V_{1a}, V_{1b}, and V_2 vasopressin receptors

	V_{1a}	V_{1b}	V_2
K_d (nM)	0.665 ± 0.069 (6)	0.275 ± 0.081 (4)	2.14 ± 0.66 (5)
B_{max} (fmol/mg protein)	2180 ± 170 (6)	368 ± 26 (4)	2660 ± 760 (5)

K_d and B_{max} for AVP were determined by Scatchard analysis of saturation isotherms of [^{3}H]AVP with plasma membranes of COS-1 cells transfected with human V_{1a}, V_{1b}, and V_2 receptor. Each value represents the mean ± SEM of at least four independent duplicate experiments. The number of experiments is given in parentheses.

RESULTS

Effect of DDAVP on Binding of [^{3}H]AVP to Human Vasopressin Receptors

Mammalian expression plasmid vectors containing the cDNAs encoding human vasopressin receptors V_{1a}, V_{1b}, and V_2 were transfected into COS-1 cells. Binding assay was performed with [^{3}H]AVP and the respective membranes of each receptor-transfected cell. The K_d and B_{max} values for each vasopressin receptor are shown in Table 1. K_d values for human V_{1a}, V_{1b}, and V_2 receptors in the present study were 0.665, 0.275, and 2.14 nM, respectively, these values being similar to those observed in previous studies with cloned vasopressin receptors expressed in mammalian cells [18, 28, 29]. B_{max} values for these receptors were 2180, 368, and 2660 fmol/mg protein, respectively.

To confirm the binding properties of the vasopressin receptors transfected in COS-1 cells, binding inhibition experiments with unlabeled vasopressin analogs AVP, oxytocin, DDAVP, and V_{1a} antagonist [d(CH$_2$)$_5$^{1},Tyr(Me)^2]AVP were performed. Table 2 shows the K_i values for these vasopressin analogs. Among these analogs, AVP was the strongest inhibitor of [^{3}H]AVP binding. The V_{1a} antagonist [d(CH$_2$)$_5$^{1},Tyr(Me)^2]AVP strongly inhibited human V_{1a} receptor (0.658 nM), but had less effect on human V_2 receptor (207 nM), while the inhibitory effect of [d(CH$_2$)$_5$^{1},Tyr(Me)^2]AVP on human V_{1b} receptor (94.2 nM) was intermediate between those for human V_{1a} and human V_2 receptors. The rank order of K_i values for oxytocin was the same as that for [d(CH$_2$)$_5$^{1},Tyr(Me)^2]AVP. These results were consistent with previous observations [2, 3, 18] and clearly showed that the receptors were authentically expressed in these COS-1 cells. In the present study, the K_i value for DDAVP on human V_{1b} receptor (5.84 nM) was approximately 10-fold less than K_i values on V_{1a} receptor (58.0 nM) and V_2 receptor (65.9 nM) (Table 2). These K_i values for DDAVP on human V_{1a} and V_2 receptors were similar to those observed in previous reports [2, 3]. These results show that DDAVP had the most potent affinity for V_{1b} receptor among the three human vasopressin receptors tested.

Effect of DDAVP on Intracellular Levels of cAMP and IPs through Each Human Vasopressin Receptor

As shown in Fig. 1, 1×10^{-7} M AVP evoked intracellular cAMP accumulation in human V_2 receptor-expressing COS-1 cells, and IPs accumulation in human V_1 (V_{1a} or V_{1b}) receptor-expressing COS-1 cells. Moreover, 1×10^{-7} M DDAVP evoked cAMP accumulation in V_2 receptor-expressing cells, but little IPs accumulation was observed in V_{1a} receptor-expressing cells even at 1×10^{-5} M DDAVP (data not shown). In the V_2 receptor-expressing cells, DDAVP and AVP evoked appreciable but insignificant IPs production, as was observed by previous investigators [30, 31]. AVP and DDAVP did not affect cAMP or IPs accumulation in mock transfected cells, and no change occurred in any receptor-expressing cells in the absence of agonist (data not shown). These results showed that the vasopressin receptors expressed in COS-1 cells intactly converted an agonistic stimulus into an intracellular second messenger. In the V_{1b} receptor-expressing cells, 1×10^{-7} M DDAVP evoked IPs accumulation. Moreover, the amount of IPs accumulated by DDAVP was the same as that accumulated by AVP in these cells (Fig. 1). In conclusion, these results show that DDAVP caused human V_{1b} receptor to evoke IPs accumulation in these COS-1 cells.

TABLE 2. K_i values for different neurohypophysial agonists and analogs on human V_{1a}, V_{1b}, and V_2 vasopressin receptors

	K_i (nM)		
Agonist	V_{1a}	V_{1b}	V_2
AVP	0.660 ± 0.250 (5)	0.340 ± 0.079 (5)	2.70 ± 1.04 (7)
Oxytocin	18.8 ± 5.1 (5)	319 ± 116 (5)	3550 ± 1080 (7)
DDAVP	58.0 ± 25.7 (5)	5.84 ± 2.44 (5)	65.9 ± 28.4 (7)
[d(CH$_2$)$_5$^{1},Tyr(Me)^2]AVP	0.658 ± 0.198 (5)	94.2 ± 22.4 (5)	207 ± 51 (7)

K_i values for AVP, oxytocin, and DDAVP were determined from IC_{50} values based on competition studies using [^{3}H]AVP and membranes of COS-1 cells transfected with human V_{1a}, V_{1b}, V_2 vasopressin receptors, respectively. Each value represents the mean ± SEM of at least five independent duplicate experiments. The number of experiments is given in parentheses.

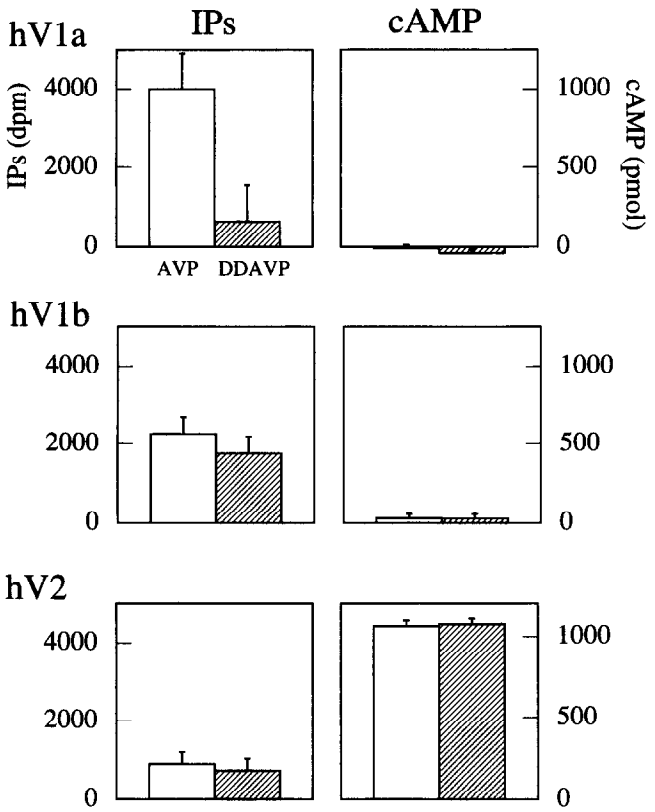

FIG. 1. Agonistic effects of AVP and DDAVP in COS-1 cells transfected with human vasopressin receptors. The left panel shows IPs production induced by 100 nM AVP (open bars) and DDAVP (hatched bars). The right panel shows cAMP production induced by each agonist. Neither agonist affected respective cAMP or IPs accumulation in mock transfected cells, and no change occurred in any receptor-expressing cells in the absence of agonist. Data represent the mean ± SEM of at least three independent duplicate experiments.

Agonistic Effect of DDAVP on IPs or cAMP Production through Human V_{1b} and V_2 Receptors

To compare the agonistic potency of DDAVP on IPs production through human V_{1b} receptor with that on cAMP production through human V_2 receptor, we examined EC_{50} values for increasing concentrations of AVP and DDAVP on these two receptors, as measured by IPs accumulation in human V_{1b} receptor-expressing COS-1 cells and by cAMP accumulation in human V_2 receptor-expressing COS-1 cells. Each EC_{50} value was obtained between the basal level with no stimulation and the level obtained at each maximal stimulation. The respective EC_{50} values showed that the agonistic activity of DDAVP on human V_{1b} receptor in evoking IPs accumulation (11.4 nM) was slightly stronger than its activity on human V_2 receptor in evoking intracellular cAMP accumulation (23.9 nM) (Table 3).

TABLE 3. Agonistic potencies of AVP and DDAVP on human V_{1b} and V_2 receptors

Agonist	EC_{50} (nM)	
	V_{1b} (IPs production)	V_2 (cAMP production)
AVP	1.51 ± 0.23 (3)	2.87 ± 0.81 (3)
DDAVP	11.4 ± 1.8 (4)	23.9 ± 5.8 (3)

EC_{50} values for AVP and DDAVP were determined from IPs production studies with COS-1 cells expressing human V_{1b} receptor and from cAMP production studies with COS-1 cells expressing human V_2 receptor. Data for human V_{1b} receptor were derived from data in Fig. 2. Each value represents the mean ± SEM of at least three independent duplicate experiments, and the number of experiments is given in parentheses.

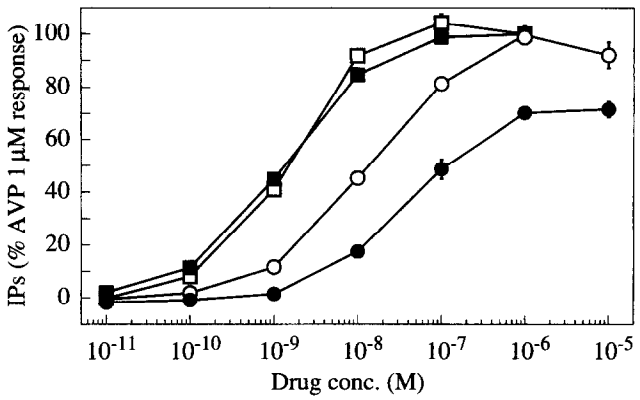

FIG. 2. Agonistic effects of AVP and DDAVP in COS-1 cells expressing human and rat vasopressin V_{1b} receptors. IPs production was induced by 0.01–1000 nM AVP (square) and DDAVP (circle) through human (open) and rat (closed) V_{1b} receptor expressed in COS-1 cells. Each point represents the mean ± SEM of at least three independent duplicate experiments. Values are plotted as % responses of 1 μM AVP.

Effect of DDAVP on Intracellular IPs through Human and Rat V_{1b} Receptors

In previous observations using rat pituitary, DDAVP was reported to have only weak agonistic activity on V_{1b} receptor [16, 23]. In the present study, in contrast, DDAVP acted as a strong agonist on human V_{1b} receptor-expressing cells (Table 3). We therefore compared the agonistic activity of DDAVP on rat V_{1b} receptor with that on human V_{1b} receptor. As shown in Fig. 2, DDAVP showed a partial agonistic activity (intrinsic activity: 0.7) on IPs production in rat V_{1b} receptor-expressing cells, in contrast to a full agonistic activity in human V_{1b} receptor-expressing cells. Moreover, the agonistic potency of DDAVP on rat V_{1b} receptor was approximately 4 times weaker than that on human V_{1b} receptor (EC_{50}: 43.5 nM vs 11.4 nM). In contrast, AVP equally stimulated IPs production in rat and human V_{1b} receptor-expressing COS-1 cells (EC_{50}: 1.31 nM vs 1.51 nM) (Table 3, Table 4). On the other hand, binding inhibition analysis showed that the K_i value for DDAVP on rat V_{1b} receptor was equivalent to that on human V_{1b} receptor (K_i: 9.29 nM vs 5.84 nM), and that the K_i value for AVP on rat V_{1b} receptor was equivalent to that on human V_{1b} receptors (K_i: 0.206 nM vs 0.340 nM) (Table 2, Table 4). These results show that there is a species difference between human and rat V_{1b} receptors in agonistic efficacies and potencies of DDAVP.

TABLE 4. K_i values and EC_{50} values for AVP and DDAVP on rat V_{1b} receptor

Agonist	K_i (nM)	EC_{50} (nM)
AVP	0.206 ± 0.049 (3)	1.31 ± 0.13 (3)
DDAVP	9.29 ± 0.62 (3)	43.5 ± 11.1 (4)

K_i values for AVP and DDAVP were determined from competition studies using [^{3}H]AVP and membranes of COS-1 cells transfected with rat V_{1b} vasopressin receptors. The [^{3}H]AVP specifically bound to these membranes with a K_d value of 0.211 ± 0.008 nM and a B_{max} value of 390 ± 100 fmol/mg protein (N = 3). EC_{50} values for AVP and DDAVP were determined from IPs production studies with COS-1 cells expressing rat V_{1b} receptor, and were derived from data in Fig. 2. Each value represents the mean ± SEM of at least three independent duplicate experiments, and the number of experiments is given in parentheses.

DISCUSSION

Selectivity of DDAVP as a Vasopressin Receptor Agonist

In the present study using various receptor-expressing COS-1 cells, we showed that DDAVP not only induced an agonistic response in human V_2 receptor, but also induced an equally potent response in human V_{1b} receptor. This finding clearly suggests that DDAVP pharmacologically acts on V_{1b} receptor in the same manner as on V_2 receptor. It is also demonstrated that DDAVP stimulated IPs accumulation only through human V_{1b} receptor, whereas it stimulated cAMP accumulation only through human V_2 receptor (Fig. 1). In the binding studies, DDAVP was 10 times more potent on human V_{1b} than human V_2 receptors (Table 2). However in the studies of agonistic potencies, DDAVP was only twice as potent (Table 3). V_2 receptor activates adenylate cyclases through $G\alpha_s$, whereas V_{1b} receptor activates phospholipase C through $G\alpha_q$. Although the exact mechanism is unclear, apparent differences in the ratio of the activities of DDAVP between binding affinity and agonistic potency may be due to dissimilarities in the interaction of each vasopressin receptor with its respective G-protein and effector. As regards IPs accumulation through V_2 receptor, this receptor had not been thought to evoke PI turnover. It was recently reported that AVP stimulated IPs accumulation through V_2 receptor in the presence of murine G-protein α-subunit $G\alpha_{15}$ or its human counterpart $G\alpha_{16}$ [30]. However, because $G\alpha_{15}$ and $G\alpha_{16}$ are expressed only in a subset of hematopoietic cells [32, 33], V_2 receptor could mediate IPs accumulation only when this receptor was expressed in hematopoietic cells. In the other tissues lacking $G\alpha_{15}$ or $G\alpha_{16}$, therefore, DDAVP could evoke IPs accumulation only through V_{1b} and not through V_2 receptor.

Species Differences in Affinity of DDAVP for V_{1b} Receptors

In previous pharmacological studies, DDAVP weakly inhibited the binding of [^{3}H]AVP to V_{1b} receptor in rat pituitary membrane [13] and induced the minor release of ACTH from rat anterior pituitary tissue [15, 16]. In addition, in binding inhibition experiments using cloned rat V_{1b} receptor, DDAVP only moderately inhibited the binding of [^{3}H]AVP (K_i: 51.3 nM) [20]. These less evident pharmacological effects with rat V_{1b} receptor led us to regard the effects of DDAVP on V_{1b} receptor as pharmacologically insignificant.

However, some differences in DDAVP activity do exist between our present results with the human V_{1b} receptor and these previous pharmacological observations using rats. The present study showed that DDAVP acted as a partial agonist on rat V_{1b} receptor, but as a full agonist on human V_{1b} receptor. In addition, the agonistic potency of DDAVP on rat V_{1b} receptor was less effective than that on human V_{1b} receptor (EC_{50}: 43.5 nM vs 11.4 nM), though the affinity of DDAVP for rat V_{1b} receptor seemed equivalent to that for human V_{1b} receptor in the binding experiments (K_i: 9.29 nM vs 5.84 nM) (Table 3, Table 4). It was previously reported that intravenous infusion of DDAVP in humans resulted in the stimulation of plasma cortisol secretion as a secondary effect of ACTH release (DDAVP: 0.4 μg/kg) [34], whereas its ACTH-releasing activity in rats was weak (DDAVP: 3.3–100 μg/100 g) [23] or absent (DDAVP: 0.05–0.8 μg/100 g) [16]. This species difference between rats and humans in terms of the agonistic effects of DDAVP in our present study is consistent with those in these previous *in vivo* studies, and clearly explains why DDAVP showed weak effects on rat pituitary tissue in previous *in vitro* studies [13, 15, 16].

Possible V_{1b} Receptor in Extra-pituitary Tissues, Considered an Atypical Vasopressin Receptor Subtype

The existence in various tissues of DDAVP-sensitive atypical vasopressin receptor subtypes distinguishable from V_{1a} and V_2 receptors has been suggested. DDAVP stimulated the secretion of insulin in a rat perfused islet [35]. In a hamster insulinoma cell line (HIT), AVP caused the dose-dependent stimulation of insulin secretion through the induction of IPs production. However, AVP did not induce the accumulation of intracellular cAMP, nor did forskolin have any effect on this insulin secretion. Furthermore, neither V_{1a}- nor V_2-selective antagonists had any effect on AVP-stimulated insulin secretion in HIT cells. Thus, these finding suggested that the insulin secretion was induced through a V_1-like and not a V_{1a} receptor [12]. Recently, Richardson *et al.* reported that a functional vasopressin receptor which stimulated insulin secretion through the activation of phospholipase C in HIT cells belonged to a V_{1b} rather than a V_{1a} receptor subclass [9]. In their study, DDAVP stimulated insulin secretion (EC_{50}: 73 nmol/L) and inositol-monophosphate production (EC_{50}: 44.3 nmol/L) in HIT cells. These EC_{50} values appear comparable to those in our present study of IPs production on cloned human and rat V_{1b} receptors (11.4 nM and 43.5 nM, respectively) (Table 3, Table 4). Furthermore, we and others have detected the expression of V_{1b} receptor mRNA on pancreas in humans [22] and rats [20, 21] using a RT-PCR technique. Lee *et al.* recently used a variety of

vasopressin antagonists on rat islet and RINm5F cells to suggest that the vasopressin receptor in rat β cell might be V_{1b} receptor [36]. These and the present findings strongly suggest that the DDAVP-sensitive receptor in islet β cells is a V_{1b} receptor, and that it is through this receptor that AVP induces pancreatic β cells to secrete insulin.

Furthermore, the existence of a DDAVP-sensitive vasopressin receptor which evoked intracellular calcium has also been reported in IMCD [6, 7, 37] and in neurons of the organum vasoculosum laminae terminalis, a part of the hypothalamus, in rat brain [10]. RT-PCR Southern blot analysis revealed the expression of V_{1b} receptor mRNA in human hypothalamus [22] and rat inner medulla.* These findings indicate that V_{1b} receptor is expressed and seemingly plays a physiological role in these tissues. In IMCD of kidney, in particular, it was reported that, as with DDAVP, the V_{1b} agonist [deamino[1],D-3-(pyridyl)Ala[2]]AVP significantly increased intracellular calcium, but that V_{1a} agonist did not [8]. In this tissue, AVP showed both stimulatory action (at 0.1 nM) through cAMP production and inhibitory action (at 10 nM) through the effect of intracellular calcium accumulation on osmotic water permeability, and [deamino[1],D-3-(pyridyl)Ala[2]]AVP inhibitory action on vasopressin-stimulated osmotic water permeability [38]. These and the present findings therefore suggest that the DDAVP-sensitive atypical vasopressin receptor in IMCD of the kidney may be identical to V_{1b} receptor and play a role in controlling osmotic water permeability in this tissue.

The present study clearly suggests that the pharmacological activity of DDAVP is through V_{1b} receptor, at least in pancreas and IMCD of kidney. Until now, few V_{1b}-specific agonists and antagonists have been available for investigation of the physiological role of V_{1b} receptor in extrapituitary tissues. Our data also reveal that the effects of DDAVP through V_{1b} receptor are greater in humans than in rats. These newly elucidated properties of DDAVP may help us better understand the physiological role of V_{1b} receptor, especially in humans.

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