Vasopressin Stimulates Insulin Release from Islet Cells through V1b Receptors: a Combined Pharmacological/Knockout Approach

Sayuri Oshikawa, Akito Tanoue, Taka-aki Koshimizu, Yoko Kitagawa, and Gozoh Tsujimoto

Department of Molecular, Cell Pharmacology, National Research Institute for Child Health and Development, Tokyo, Japan (S.O., A.T., T.A., Y.K.); and Department of Genomic Drug Discovery Science, Graduate School of Pharmaceutical Sciences, Kyoto University Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan (G.T.)

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ABSTRACT

Vasopressin receptor subtype(s) responsible for stimulation of insulin release from pancreatic β cells were investigated by using subtype-selective antagonists and mice that were genetically lacking either V1a or V1b receptors. Arginine vasopressin (AVP) increased insulin release from isolated mouse islet cells in a concentration-dependent manner, with a submaximal response at 100 nM. Reverse transcription-polymerase chain reaction (RT-PCR) analysis detected V1b and oxytocin, but not V1a or V2, receptor transcripts in mouse islet cells. We characterized the recently synthesized vasopressin receptor subtype antagonists (2S,4R)-1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl]-3,4-dihydro-2(1H)-quinolinone (OPC-21268), and (2S,4R)-1-[5-chloro-1-[2,4-dimethoxyphenyl]sulfonyl]-3-(2-methoxy-phenyl)-2-oxo-2,3-dihydro-1H-indol-3-yl]-4-hydroxy-N,N-dimethyl-2-pyrrolidine carboxamide (SR149415) using human embryonic kidney 293 cells stably expressing the three cloned mouse vasopressin receptors (V1a, V1b, and V2). A radioligand binding study showed that SR49059 and OPC-21268 potently inhibited [3H]AVP binding to the cloned mouse V1a receptor, with Ki values of 27 and 510 nM, respectively, whereas SSR149415 potently inhibited [3H]AVP binding to the cloned mouse V1b receptor with a Ki value of 110 nM. The inhibitory effects of vasopressin antagonists on AVP-induced insulin release correlate well with the rank order of potency to inhibit [3H]AVP binding to the V1b receptor; pancreatic islet cells were significantly inhibited by SSR149415 but not by SR49059 or OPC-21268. Furthermore, the AVP effect on insulin release was entirely lost in mice lacking the V1b receptor but was preserved in mice lacking the V1a receptor. Our study, which combined pharmacological and knockout approaches, clearly demonstrates that vasopressin-stimulated insulin release from islet cells is mediated via V1b receptors.

The neurohypophysial hormone arginine vasopressin (AVP), and related peptides, including oxytocin (OT), exert their effects through at least four types of G protein-coupled receptors, termed V1a, V1b, V2, and OT receptors (Birnbaumer, 2000; Thibonnier et al., 2001). Among the plethora of physiological processes regulated by AVP (Morel et al., 1992; Thibonnier et al., 2001) is the homeostatic control of blood glucose levels. AVP can decrease the blood glucose level by promoting insulin release, and it can also increase the blood glucose level by promoting the release of glucagon (Dunning et al., 1984b; Chen et al., 1994; Yibchok-Anun and Hsu, 1998) and by enhancing glycogenolysis in the liver (Kirk et al., 1979). The AVP-induced hepatic glycogenolysis is mediated predominantly by the V1a receptor subtype (Morel et al., 1992), whereas the specific receptor responsible for the AVP-promoted insulin release from the pancreas is still unclear. An immunohistological study showed the presence of AVP in the human and rat pancreas, and AVP may work as an important local modulator of pancreatic hormone secre-

ABBREVIATIONS: AVP, [Arg9]vasopressin; OT, oxytocin; OPC-21268, 1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl]-3,4-dihydro-2(1H)-quinolinone; BSA, bovine serum albumin; SR49059, (2S,4R)-1-[2R,3S]-5-chloro-3-[2-chlorophenyl]-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-difydro-1H-indole-2-carboxamide; HEK, human embryonic kidney; EGFP, enhanced green fluorescent protein; OPC-31260, 5-dimethylamino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1H-benzazepine; SSR149415, (2S,4R)-1-[5-chloro-1-[2,4-dimethoxyphenyl]sulfonyl]-3-[2-methoxy-phenyl]-2-oxo-2,3-dihydro-1H-indol-3-yl]-4-hydroxy-N,N-dimethyl-2-pyrrolidine carboxamide; RT-PCR, reverse transcription-polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ELISA, enzyme-linked immunosorbent assay; [Pmp1,Tyr(Me)2]-Arg8-vasopressin, [β-mercaptopo-β-cyclopentamethylene propionic acid, o-methyl-L-tyrosine]-Arg9-vasopressin.
tion (Amico et al., 1988; Folny et al., 2003). In addition, it has been shown that stimulation of vasopressin receptors in the pancreatic islet cells leads to stimulation of phospholipase C, resulting in the formation of inositol 1,4,5-trisphosphate and diacylglycerol (Gao et al., 1990).

Pharmacological studies have been performed to determine the vasopressin receptor subtype that is responsible for insulin secretion (Dunning et al., 1984a; Monaco et al., 1988). The application of V1b receptor agonist desamino-o-(3'-

-pyriddy1)-Ala²,Arg⁸)-VP was reported to increase insulin release from hamster β cells (Richardson et al., 1995). In addition, it was reported that receptor blockade by a mixed V1a/b antagonist, but not by a V1a antagonist, reduced AVP-stimulated insulin release (Lee et al., 1995; Richardson et al., 1995). Because of the lack of a sufficiently specific V1b-blocker, however, definitive conclusions based on these studies have remained elusive (Gao and Henquin, 1993). Recently, highly selective peptide and nonpeptide vasopressin receptor antagonists have been developed (Hirasawa et al., 1994; Thibonnier et al., 2001). Nonpeptide V1a receptor-selective antagonists such as OPC21268 and SR49059 have been developed for potential therapeutic use in treating hypertension and congestive heart failure (Yamamura et al., 1991; Serradeil-Le Gal et al., 1993). More recently, a V1b receptor-selective antagonist, SSR149415, has been developed. Pharmacological studies showed that it inhibits the exogenous AVP-induced increase in circulating corticotropin and produces anxiolytic- and antidepressant-like effects (Griebel et al., 2002; Serradeil-Le Gal et al., 2002a,b).

In addition to these pharmacological characterizations, V1a and V1b receptor knockout (KO) mice have been generated successfully by our group as well as by another group (Wersinger et al., 2002; Tanoue et al., 2004; A. Tanoue, Y. Nasa, R. Oikawa, Y. Kawahara, S. Oshikawa, Y. Kitagawa, T. Koshimizu, N. Hatae, T. Kuwaki, S. Takeo, and G. Tsujimoto, submitted for publication). V1a- and V1b-KO mice are not lethal and the mice have no apparent anatomical anomaly. The V1a and V1b receptor-mediated AVP responses were completely lost in V1a-KO and V1b-KO, respectively; thus, AVP-stimulated pressor response and corticotropin release were completely lost in V1a-KO and V1b-KO, respectively. These results indicated that both the V1a and V1b receptors were indispensable for these effects, and deleting these receptor genes apparently did not trigger any compensatory mechanism (Wersinger et al., 2002; Tanoue et al., 2004; A. Tanoue, Y. Nasa, R. Oikawa, Y. Kawahara, S. Oshikawa, Y. Kitagawa, T. Koshimizu, N. Hatae, T. Kuwaki, S. Takeo, and G. Tsujimoto, submitted for publication). Hence, these KO mice are good animal models to study the physiology of each receptor subtype.

In the present study, we investigated vasopressin receptor subtype(s) responsible for AVP-induced insulin release from pancreatic cells. This was done pharmacologically, using subtype-selective antagonists, and by using mice that genetically lacked either the V1a or the V1b receptor.

**Materials and Methods**

**Materials.** HEK293 cells were obtained from the American Type Culture Collection (Manassas, VA). Cell culture media and geneticin were from Invitrogen (Carlsbad, CA). Fetal bovine serum was from HyClone (Logan, UT). Restriction and modification enzymes were from Promega (Madison, WI). [³H]AVP (68.5 Ci/mmol) were from PerkinElmer Life and Analytical Sciences (Boston, MA). The pEGFP-N₃ expression vector was from BD Biosciences Clontech (Palo Alto, CA). The pCDNA3.1 His B expression vector was from In VitroGen. AVP and [Pmp¹,Tyr(Me)²]-Arg⁸-vasopressin were from Peptide Institute Inc. (Osaka, Japan). OPC-21268 and OPC-31260 were gifts from Sanofi-Synthelabo (Montpelier, France). Standard reagents, unless otherwise stated, were purchased from Sigma (Sigma-Aldrich, Tokyo, Japan).

**Animals.** Mice deficient in V1a or V1b receptors were generated by gene targeting as described elsewhere (Tanoue et al., 2004; A. Tanoue, Y. Nasa, R. Oikawa, Y. Kawahara, S. Oshikawa, Y. Kitagawa, T. Koshimizu, N. Hatae, T. Kuwaki, S. Takeo, and G. Tsujimoto, submitted for publication). Corresponding wild-type mice (12 weeks of age) were used as control mice. Mice were maintained under controlled condition at 25°C, with food and water available ad libitum. All animal experiments described were approved and conducted in accord with our institutional standards of animal care.

**Isolation of cDNA for Mouse Vasopressin Receptors.** cDNAs of the mouse V1a, V1b, and V2 receptors were obtained by the RT-PCR method. Total RNAs from the mouse liver, pituitary, and kidney were prepared using isoegen reagent (Nippon Gene, Tokyo, Japan). Total RNA (5 µg) was treated with RNase-free DNase (Takara, Tokyo, Japan) and reverse-transcribed using random hexamers, as described previously (Tanoue et al., 1990). One tenth of each cDNA sample was amplified by PCR with receptor-specific primers (10 pmol of each) in a buffer containing 0.25 mM of each dNTP, 50 mM KCl, 10 mM Tris-HCl, pH 8.6, 1.5 mM MgCl₂, and 2 units of Taq DNA polymerase (Takara, Tokyo, Japan). Thermal cycling was performed for 1 min at 94°C, 1 min at 58°C, and 2 min at 72°C for 30 cycles. The upstream and downstream primers (5′→3′) were AATGAAATGATGAGATTTCCCGGAGGC and TCCGATCCACTGGAGAGACCGTAAA for V1a (1672 bp), CAA

-GAAGCATGATTCTGAGCCCTTCT and AAGGGATCCAGAGATGCCTTCCAT for V1b (1574 bp), and ATGACCTGTGAGTCTACCC and TAGAAAGAGCCCCAGTAGCTAC for V2 (1137 bp). The primers were derived from the mouse V1a and V1b genomic sequences (Kikuchi et al., 1999) (GenBank accession numbers are AB030013 and SEG AB034488S for V1a and V1b, respectively) and primers for V2 were from a previous report (Oksche et al., 2002) (GenBank accession number is NM 019404). The amplified cDNAs were then subcloned into pGEM-vectors and sequenced.

**Analysis.** Our preliminary Northern blot analysis revealed that the expression level of the V1b receptor transcript was very restricted and that this mRNA was scarce. Therefore, studies on its tissue distribution were performed by RT-PCR. cDNA samples from different mouse tissues were amplified by PCR with primer sets specific to the receptors or to glyceralde- hyde-3-phosphate dehydrogenase (GAPDH). The upstream and downstream primer sequences, located within the first exon of the mouse V1a, V1b, and V2X genes, were ATTGCTGAGGCTACTCTCATCATTCC upstream and CTTTGGCAATTTTGCGCTGCTG downstream (for V1a (532 bp), TCACTTGGCACCCATGCGCCAT upstream and AAGAGCTGTGAGGTGACCC downstream for V1b (335 bp), GACCCCTCTTTGTGTCTCCTA upstream and TCAGGAGGTTGTACCCCTCAT downstream for V2 (206 bp), and TCAGGTCAAGTCGCGCAG upstream and TCTACCCACTGC- CCAGAAC downstream for OXT (771 bp). The GAPDH sequences (5′→3′) were GGTACATCATCTGCCGCCCCCTTC for sense and ACCACCCCTTTGTGGCTT for antisense primers (662 bp), respectively. Control PCR reactions were also performed on non-reverse-transcribed RNA to exclude any contamination with genomic DNA.

**HEK293 Cells Stably Expressing Mouse V1a, V1b, and V2 Receptors.** The mouse V1a, V1b, and V2 cDNAs were subcloned into the mammalian expression vectors pcDNA3.1 and pEGFP-N₃. HEK293 cells were stably transfected with each of the pEGFP-N₃


constructs using the LipofectAMINE reagent (Invitrogen). Individual colonies of transfected HEK293 cells resistant to G418 were picked up and selected according to the intensity of their fluorescent signal using an EPICS XL-MCL flow cytometer (Beckman Coulter, Fullerton, CA). As a negative control, HEK293 cells were also stably transfected with the pcDNA3.1. Nucleotide sequences of all these clones were verified using the dye terminator technique, with a model 377 DNA sequencer from Applied Biosystems (Foster City, CA).

Ligand Binding Assay. Cellular membrane fractions were prepared from HEK293 cells that were stably expressing mouse V1a, V1b, or V2, as described previously (Shibata et al., 1995). Briefly, the collected cells were placed in 2 ml of ice-cold buffer A (250 mM sucrose, 5 mM Tris-HCl, and 1 mM MgCl₂, pH 7.4), and disrupted in a Branson sonicator (SONIFIER 250; Branson, Danbury, CT) at setting 5 for 8 s. The mixture was then centrifuged at 100,000 g for 10 min to remove nuclei. The supernatant was centrifuged at 35,000 g for 20 min at 4°C. The resulting pellet was suspended in binding buffer B (100 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 1 mM EDTA, and 1 mg/ml BSA, pH 7.4) and was frozen at −80°C until use. Protein concentration was measured using the biocinchoninic acid protein assay kit (Pierce, Rockford, IL).

Radioligand binding studies using [³H]AVP were performed as described previously (Hirasawa et al., 1994). The reaction was initiated by adding the membranes in 250 μl of a buffer containing 100 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 1 mM EDTA, 1 mg/ml BSA, and [³H]AVP (0.1–10.0 nM), incubated for 30 min at 30°C. The reaction was terminated by addition of 4 ml of ice-cold washing solution (100 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 1 mM EDTA, and 0.1 mg/ml BSA), immediately filtered onto Whatman glass fiber (0.8 μm), and binding was measured by liquid scintillation spectrometry. To determine nonspecific binding, 1 μM concentrations of unlabeled AVP was used. The specific binding was calculated as the difference between the total and nonspecific counts. For competition binding, each assay contained about 1 nM of [³H]AVP. HEK293 cells transfected with pcDNA3.1 vector did not have any detectable binding sites for [³H]AVP (data not shown).

Insulin Secretion from Isolated Islets. Pancreatic islets were isolated from 12-weeks-old male mice by collagenase digestion and centrifugation in a Ficoll gradient (Shibata et al., 1976). Briefly, after clamping the common bile duct at its entrance to the duodenum, 3 ml of Hanks’ medium containing 2 mg/ml of collagenase S-1 (Nitta, Japan) was injected into the duct. The swollen pancreas was surgically removed, incubated at 37°C for 20 min, and digestion was terminated by adding 40 ml of ice-cold RPMI medium containing 10% FBS. Digested pancreata were then dispersed by pipetting and rinsed twice with 30 ml of the same medium. After filtering through a Spectra-mesh (40 μm; Spectrum Laboratories, Inc., Ft. Lauderdale, FL), the digested tissue was resuspended in a 4-ml layer of Ficoll (specific gravity, 1.22) and overlaid with a 2-ml layer of Ficoll, pH 7.4 until assay. Protein concentration was measured using the bicinchoninic acid protein assay kit (Pierce, Rockford, IL). The mixture was then centrifuged at 1000 g at 4°C. The resulting pellet was suspended in binding buffer B (100 mM Tris-HCl, 5 mM MgCl₂, 1 mM EDTA, and 1 mg/ml BSA, pH 7.4) and was frozen at −80°C until use. Protein concentration was measured using the biocinchoninic acid protein assay kit (Pierce, Rockford, IL).

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Data Analysis. The radioligand binding data were analyzed by the iterative nonlinear regression program, LIGAND (Munson and Rodbard, 1980). The dissociation constant (Kᵢ) and the maximal binding capacity (Bₘₐₓ) were deduced from Scatchard plotting. The inhibitory dissociation constants (Kᵢ) for unlabeled AVP analogs were calculated from competition binding experiments according to Cheng and Prusoff, using the following equation (Cheng and Prusoff, 1973): Kᵢ = IC₅₀/[L]/Kᵢ, where IC₅₀ is the concentration of unlabeled antagonist leading to half-maximal inhibition of specific binding and [L] is the concentration of the radioligand present in the assay. Experiments were repeated at least three times and data were expressed as mean ± S.E. Statistical analysis was performed with the Statview software package (Abacus Concepts Inc., Berkeley, CA). Significant differences with P < 0.05 were determined by the one-way analysis of variance with Newman-Keuls multiple comparison test.

Results and Discussion

mRNA Expression of AVP Receptors in Mouse Islet Cells. Expression of the AVP receptor mRNA in various mouse tissues was first examined by RT-PCR. As shown in Fig. 1A, both V1a and V2 receptor mRNA were widely detected in brain and other peripheral tissues, including mesenteric artery, heart, lung, liver, pancreas, spleen, kidney, and adrenal gland. In contrast, the V1b receptor had a limited tissue distribution. It was initially defined as a pituitary-specific subtype in rat and human (Jard et al., 1986; Lolait et al., 1995; Grazzini et al., 1996; Hurbin et al., 1998; Vaccari et al., 1998); indeed, its mRNA was markedly abundant in pituitary glands. A faint but positive signal for the V1b receptor subtype was consistently detected in brain and adrenal gland.

In cDNA samples prepared from whole pancreas tissue, the mRNA of AVP receptor subtype was consistently detected in brain and adrenal gland.

Fig. 1. Tissue distributions of the vasopressin receptor transcripts. A, RT-PCR analysis of RNA from mouse tissues. The V1a, V1b, V2, and GAPDH mRNA transcripts were amplified and detected by staining with ethidium bromide. The amplified PCR products for V1a, V1b, V2, and GAPDH were 532-, 333-, 206-, and 662-bp, respectively, as indicated by the arrows. M, marker; Br, brain; Hi, hippocampus; Pi, pituitary; Ma, mesenteric artery; He, heart; Lu, lung; Li, liver; Pa, pancreas; Sp, spleen; Ki, kidney; Ad, adrenal. B, RT-PCR analysis of RNA from mouse islets. Total RNA was isolated from cultured islet as described under Materials and Methods and subjected to RT-PCR analysis. The V1a, V1b, V2, and OT receptor transcripts were detected as 532-, 333-, 206-, and 711-bp fragments, respectively. Is, islets; Ki, kidney; Pt, pituitary; Ut, uterus.
transcripts for the V1a and V2 receptors were barely detectable, whereas the V1b receptor was not detected (Fig. 1A). However, when total RNA was extracted from isolated islet cells, the transcripts for V1b and OT receptors were clearly detected (Fig. 1B). This may have been a result of minor populations of islet cells, because pancreatic exocrine cells comprise more than 90% of the whole tissue.

**Binding Properties of Vasopressin Receptor Subtype-Selective Antagonists in the Cloned Mouse Vasopressin Receptors.** Using cells that stably expressing the mouse AVP receptor subtypes, we first examined pharmacological properties of recently developed antagonists by carrying out radioligand binding studies with $[^3H]$AVP. The maximum binding capacity ($B_{\text{max}}$) was 8.9 ± 0.1 (n = 6), 0.3 ± 0.01 (n = 3), and 1.0 ± 0.01 (n = 3) pmol/mg protein in cell lines expressing V1a, V1b, and V2 receptor, respectively.

![Fig. 2. Inhibition of specific $[^3H]$AVP binding by AVP and vasopressin antagonists in membrane preparations from stable cell lines. Specific $[^3H]$AVP binding to membrane preparations from HEK293 cells expressing V1a, V1b, and V2 receptors was determined in the presence of AVP, SSR149415, OPC-31260, OPC-21268, and [Pmp$^1$,Tyr(Me)$^2$]-Arg$^8$-vasopressin (○). Non-specific binding was defined as binding in the presence of 1 μM AVP. Data are plotted as the percentage of specific binding remaining in the presence of the indicated concentration of antagonists. Each point reported is the average of a single experiment performed in triplicate. Experiments were repeated three to five times and pK$_i$ values were calculated.

Scatchard plot analysis showed a single class of high-affinity binding site in each stable cell line, with K$_D$ values of 1.3 ± 0.1 (n = 6), 0.5 ± 0.1 (n = 5), and 0.3 ± 0.1 (n = 3) nM for the V1a, V1b, and V2 receptors, respectively. As shown in Fig. 2 and Table 1, competition binding experiments with AVP and five antagonists demonstrated that [Pmp$^1$,Tyr(Me)$^2$]-Arg$^8$-vasopressin was a nonselective vasopressin receptor antagonist in mice and that SR49059 was a V1a antagonist with ~20 times more selective for the V1a receptor over V1b receptor. SSR149415 exhibited a higher affinity for the mouse V1b receptor (pK$_i$ = 7.0 ± 0.2, n = 4) than the V1a (pK$_i$ = 5.2 ± 0.6, n = 4) and V2 (pK$_i$ = 5.2 ± 0.5, n = 4) receptors, indicating that SSR149415 is useful for identifying the V1b mediated effects in mice. Affinity of OPC-21268 for the mouse V1a receptor was 100 times higher than its affinity for V1b and V2 receptors, whereas OPC-31260 was selective for the V2 subtype.

The new V1b receptor antagonist, SSR149415, has been reported to show high affinity for both human and rat V1b receptors; K$_i$ values are 1.5 and 1.3 nM for human and rat V1b receptors, respectively (Serradeil-Le Gal et al., 2002a). However, we found that K$_i$ value of SSR149415 for mouse V1b receptor was approximately 80-fold larger compared with those observed for human and rat V1b receptors, although SSR149415 still displayed more than 50-fold higher affinity for the V1b receptor than for other vasopressin and oxytocin receptors in mice. In addition, we found that [Pmp$^1$,Tyr(Me)$^2$]-Arg$^8$-vasopressin was nonselective for three vasopressin receptor subtypes in mice, although it had been reported to be selective for V1a receptor in rat (Kruszynski et al., 1980; Morel et al., 1992; Lolait et al., 1995). A similar species-dependent difference in the binding properties at vasopressin receptor has been noted for synthetic vasopressin receptor ligand OPC-21268; thus, the rat V1a receptor exhibited more than 1000 times higher affinity than the human homolog (Hirasawa et al., 1994).

**Effects of Receptor Antagonists on AVP-Induced Insulin Release from Mouse Islet Cells.** AVP caused a significant, concentration-dependent increase in insulin release from mouse islets in the agonist dose range from 1 to 100 nM, and the dose of 100 nM AVP was used for a further series of experiments to examine the effects of antagonists. As shown in Fig. 3, a nonselective vasopressin receptor antagonist, [Pmp$^1$,Tyr(Me)$^2$]-Arg$^8$-vasopressin, inhibited AVP-induced insulin release in a concentration-dependent manner in a range from 10 to 1000 nM (Fig. 3). However, SR49059 weakly antagonized insulin release at high concentrations (over 1 μM), and the V1a receptor antagonist, OPC-21268, was inef-

**TABLE 1** The pK$_i$ values of AVP and reference compounds for the mouse AVP receptors expressed in HEK293 cells

<table>
<thead>
<tr>
<th>Ligands</th>
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<th>V1b</th>
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<tr>
<td>[Pmp$^1$,Tyr(Me)$^2$]-Arg$^8$-</td>
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<tr>
<td>OPC-31260</td>
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effective even at 1 μM. In contrast, V1b-selective SSR149415 potently inhibited the AVP action even at 1 nM (n = 6), and this inhibitory effect was concentration-dependent. At all concentrations tested, application of the antagonists without AVP did not change insulin release (data not shown). Hence, the V1b receptor affinity for the antagonist correlates well with its efficiency in inhibiting AVP-induced insulin release from islet cells.

**AVP-Induced Insulin Release from Islet Cells in V1a- and V1b-KO Mice.** We further examined the consequence of deleting individual V1 receptor genes in AVP-induced insulin release. V1a and V1b receptor transcripts were absent in the islets from V1a-KO and V1b-KO, respectively (Fig. 4A). AVP-induced accumulation of insulin release from cultured islet cells was similar for the V1a-KO and wild-type control mouse. However, AVP-stimulated insulin release from the islet cells was completely lost in V1b-KO mice (Fig. 4B). This is in good agreement with the results obtained from pharmacological characterization. Both studies show that the V1b receptor subtype is fully operative in mouse pancreatic islet cells and that AVP-mediated insulin release seems to occur solely via the V1b receptor. When a fasting blood glucose level was obtained from control and V1b-KO mice, no significant difference was observed between two groups: 57 ± 2 mg/dL for control mice (n = 12) and 52 ± 3 mg/dL for V1b-KO mice (n = 10), respectively.

Glucose stimulates an increase in the intracellular Ca²⁺ concentration in pancreatic β cells and causes the insulin secretion. This process can be mainly regulated by plasma membrane depolarization as well as by the CD38-cyclic ADP ribose signal system (Okamoto and Takasawa, 2002). Vasopressin can work as a positive modulator for the glucose-stimulated insulin release by acting on both signal cascades. Upon the stimulation of the β cells by AVP, intervals between

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**Fig. 3.** Effects of AVP receptor antagonists on AVP-induced insulin release in mouse islet cells. Cells were stimulated with AVP for 20 min in the presence or absence of the antagonists. The antagonists were pre-treated for 5 min before the stimulation. The level of secreted insulin was determined with the ELISA method. SSR, SSR149415; OPC, OPC-21268; SR, SR49059; PTM, [Pmp¹,Tyr (Me)²]-Arg⁸-AVP. Values are mean ± S.E. for five to six experiments. *, p < 0.05, significant difference from islets treated with vehicle.

**Fig. 4.** AVP-induced insulin release in V1a and V1b-KO mice. A, RT-PCR analysis of the vasopressin receptor transcripts in the islets from wildtype, V1a-KO, and V1b-KO mice. The sizes of detected DNA fragments are indicated in base pairs (bp). B, the AVP-induced increase in insulin release was not observed in the islets obtained from V1b-KO mice. The level of secreted insulin was determined with the ELISA method. Values are mean ± S.E. for six experiments. *, p < 0.05, indicates significant difference from wild-type mouse islets.


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**Address correspondence to:** Dr. Gozoh Tsujimoto, Department of Genomic Drug Discovery Science, Graduate School of Pharmaceutical Sciences, Kyoto University Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan. E-mail: gozoh.tsujimoto@nch.go.jp