

Characterization and Distribution of an Arginine Vasotocin Receptor in Mouse

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ABSTRACT. A cDNA, which has a high homology with teleost *Platichthys flesus* [Arg⁸] vasotocin (AVT) receptor (GenBank: AK033957), was found in mouse genome database. Analyses of the deduced amino acid sequence revealed that a cDNA has several features of AVT receptor. We tentatively named it as a mouse vasotocin receptor (MVTR). A two-electrodes voltage clamp technique was applied to characterize the MVTR expressed in *Xenopus laevis* oocytes. AVT induced Ca²⁺-dependent Cl⁻ currents in *Xenopus* oocytes injected with MVTR cRNA. On the other hand, [Arg⁸] vasopressin, oxytocin and isotocin did not induce such currents. RT-PCR showed that MVTR mRNA was specifically expressed in the brain. *In situ* hybridization analysis demonstrated significant expression of MVTR mRNA in suprachiasmatic nucleus, arcuate nucleus and medial habenular nucleus of mouse brain. These results suggest that MVTR may mediate a variety of physiological functions in mouse.

KEY WORDS: [Arg⁸] vasotocin, G protein-coupled receptor, *in situ* hybridization, voltage clamp.

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[Arg⁸] vasotocin (AVT) is one of neurohypophyseal hormones [40]. Neurohypophyseal hormones are composed of nine amino acid structures with a disulphide bridge linking two cysteines at position 1 and 6 to give a ring structure. AVT possess a hybrid structure, having the C-terminal sequence of [Arg⁸] vasopressin (AVP) and the N-terminal ring of oxytocin (OT).

The role of AVT and its receptor have been well characterized in lower vertebrates including lungfish, amphibians, reptiles and birds [12]. AVT is synthesized in the cell bodies of magnocellular neurons of the hypothalamic pre-optic nucleus as a larger precursor molecule, called pro-vasotocin, and is stored in and released from the pituitary [28]. In the central nervous system, AVT works as a neuromodulator to control reproductive behavior [34, 38]. In the periphery, AVT plays a role in regulating osmotic and electrolyte balance and blood pressure [13].

A few studies about AVT and its receptor have been reported in mammals. Some reports showed the presence of AVT in the pineal gland of sheep [24], bovine [2] and rat [31, 33]. In addition, *in vitro* experiments also showed that rat pineal releases AVT when it is stimulated by acetylcholine [37]. It has been observed that AVT has several functions in mammals [15]. Most effects of AVT on mammalian brain have been explained by cross-reaction with AVP or OT receptors. However, the effects on sleep-wake cycles such as the cat REM sleep and LH releasing are selective to AVT. In mature cats, injection of AVT into third ventricle decreases the amount of REM sleep, whereas injection of AVT antiserum increases REM sleep. Peripheral administration of AVT in newborn rats increases the quiet state sleep and decreases the active state sleep [17]. Electrophysiological studies on neurons in the rat brain have also shown

that the effects of AVT are more potent than either those of AVP and OT [18, 26]. From all these data, it has been speculated that there exist AVT-specific receptor in mammals.

Recent reports took advantage of the FANTOM2 (Functional Annotation Meeting of Mouse cDNA 2) project, which aimed to collect full-length cDNAs inclusively from mouse tissues, and found 410 candidates for G protein-coupled receptor (GPCR) cDNAs [20]. Forty-eight genes of them were new in mouse and their characters are still not known. In this experiment, we searched for cDNAs encoding AVT receptor in mouse genome database using several sequence analyses. One of the cDNAs was predicted to be a cDNA encoding AVT receptor in mouse. To investigate whether it is a functional AVT receptor, we expressed it in *Xenopus* oocytes and measured the response against AVT. Distribution of its mRNA was examined by RT-PCR and *in situ* hybridization.

MATERIALS AND METHODS

Preparation of cDNA: cDNAs having high sequence homology with *Platichthys flesus* AVT receptor (GenBank: AF184966) [41] were searched in mouse genome database using FASTA program (<http://fasta.genome.jp/>). Several cDNAs were selected as candidates for mouse AVT receptor cDNA. For such cDNAs, hydrophobicity analysis of the deduced amino acid sequences was done using TMHMM program (<http://www.cbs.dtu.dk/service/TMHMM/>). To search for sequence motifs, the amino acid sequences were scanned using PROSITE program (<http://kr.expasy.org/prosite>). Homology analysis was carried out using Clustal W program (<http://align.genome.jp>). From these analyses, one of the cDNA (GenBank: AK033957) was predicted to be a cDNA encoding mouse AVT receptor. It was tentatively named as a mouse vasotocin receptor (MVTR). Plasmids pFLC1 containing the MVTR cDNA was purchased

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from Dnaform (Tokyo, Japan).

Preparation of cRNA: Routine molecular cloning techniques were used as described briefly [35].

DNA fragment containing MVTR cDNA was isolated by cleavage of plasmid pFLC1 with *Sfi*I (TaKaRa, Otsu, Japan). After blunting with T4DNA polymerase (TaKaRa), the DNA fragment was ligated into pBluescript SK (+) (Stratagene, La Jolla, CA) which was cleaved with *EcoRV* (TaKaRa). After cleaving the plasmid with *Bam*HI, complementary RNA of the MVTR was synthesized *in vitro* using RiboMAX Large Scale RNA Production Systems (Promega, Madison, WI). For a 5' prime capping of the cRNA, m⁷G5' ppp5'G Cap Analog (Promega) was added to the reaction mixture. All reactions were carried out according to the manufacture's instructions. After reaction cRNA was purified by phenol-chloroform treatment and free nucleotides were removed using a Micro Bio-Spin Columns P-30 (Bio-Rad, Marnes-la-coquette, France). Then, the cRNA was recovered by ethanol precipitation, and it was dissolved in RNase-free water.

Oocyte expression: Oocytes were obtained from *Xenopus laevis* left in ice for 1 hr and incubated for 1 hr at room temperature with 1 mg/ml collagenase in Barth's medium (5 mM Tris-HCl, pH 7.6, containing 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 0.33 mM Ca(NO₃)₂, 0.41 mM CaCl₂, 0.82 mM MgSO₄) containing 18 units/ml penicillin and 18 µg/ml streptomycin. After follicular cell layers were removed with forceps, MVTR cRNA was injected into the oocyte at stage V or VI. Before recording, oocytes were incubated at 19°C for 2–5 days in Barth's medium.

Currents were recorded with two-electrodes voltage clamp technique using an amplifier (TEV-200A., Dagan Co., Minneapolis, MN). Electrodes were filled with 3 M KCl. During experiments, oocytes were perfused with a constant stream of frog Ringer's solution (Tris-HCl, pH 7.2, containing 115 mM NaCl, 1 mM KCl, 1.8 mM CaCl₂, 5 mM) at 21°C. The oocyte membrane was voltage-clamped at -40 mV. To detect physiological responses with peptide ligands, peptides diluted with frog Ringer's solution were applied to the perfusion chamber every 15 min to prevent desensitization.

AVP was purchased from Calbiochem-Novabiochem AG (Lauferfingen, Switzerland). AVT, OT and isotocin (IT) were purchased from Peptide Institute (Osaka, Japan).

As a positive control, plasmids containing cDNA encoding the AVP receptor (V1aR) was gifts from Dr. Tanoue (National Research Institute for Child Health and Development). Control experiments were carried out by the procedures described above.

RT-PCR: Total RNAs were extracted from mouse (C57BL/6 adult male) tissues using Rneasy Kit (QIAGEN, Santa Clarita, CA). RT-PCR was performed with the gene specific primers, forward: 5'-CCG GAT GAC TCC TAC TGG ACC-3' and reverse: 5'-CGG CTT GGA GAG AAT CTG CAT-3', which corresponds to position 604–624 and 1084–1104 of MVTR sequence, using RT-PCR high -plus- (TOYOBO, Osaka, Japan). RT-PCR reactions were per-

formed with one cycle of 60°C for 30 min, 94°C for 2 min, then 40 cycles of 94°C for 1 min, 49°C for 1.5 min, followed by one cycle of 49°C for 7 min. Control RT-PCR was performed with primers specific for G3PDH, forward: 5'-TCC ACC ACC CTG TTG CTG TA-3' and reverse: 5'-ACC ACA GTC CAT GCC ATC AC-3'. RT-PCR reactions were performed with one cycle of 60°C for 30 min, 94°C for 2 min, then 40 cycles of 94°C for 1 min, 60°C for 1.5 min, followed by one cycle of 60°C for 7 min.

To confirm the specificity of the RT-PCR reaction, nested PCR was performed with the primers, forward: 5'-CCG GAT GAC TCC TAC TGG ACC-3' and reverse: 5'-GGC ATA GAA ACG CTC CTT GGT-3', which corresponds to position 604–624 and 913–933 of MVTR sequence, using Taq DNA polymerase (TaKaRa). Nested PCR reactions were performed with 30 cycles of 95°C for 30 sec, 45°C for 30 sec, 72°C for 1 min.

In situ hybridization: DNA fragments containing nested PCR product of 330 bp was cloned into a pGEM T easy vector (Promega), and the sequence and insert direction were confirmed by sequence analysis. Digoxigenin (DIG)-labeled antisense and sense cRNA probes were synthesized with above clones using a DIG RNA labeling kit (Roche Diagnostics, Basel, Switzerland). To prepare mouse tissue sections, brains were dissected from adult male (C57BL/6), fixed in 4% paraformaldehyde and embedded in paraffin. All steps prior to and during hybridization were conducted under RNase-free conditions. Sections (6 µm) were deparaffinized with xylene and rehydrated through descending ethanol concentrations (3 min each) and PBS. The sections were treated with proteinase K, 20 µg/ml in PBS, pH 7.4 at room temperature for 5 min. Then, they were immersed in 0.2% (w/v) glycine in PBS (5 min), followed by hybridization in a humidified chamber overnight at 50°C with prehybridization solution containing 10% dextran sulfate and 300 pg/µl of the DIG-UTP labeled antisense or sense RNA probes. The sections were then washed three times for 10 min each time with 2 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M Na citrate, pH 7.4) and 0.5 × SSC at 50°C. After washing, sections were prepared for immunodetection by incubating them in Buffer A (100 mM Tris-HCl, pH 7.5, containing 150 mM NaCl) containing 3% normal goats serum and 1% bovine serum albumin for 30 min at room temperature. The sections were then exposed to anti-DIG-Alkaline phosphatase conjugate (1:500 dilution, Roche Diagnostics) in the same buffer for 1.5 hr at room temperature followed by extensive washing with Buffer A and then with Buffer B (100 mM Tris-HCl, pH 9.5, containing 100 mM NaCl and 50 mM MgCl₂). The bound antibody was detected by incubating the sections with 5-Bromo-4-Chloro-3-Indolyl Phosphate/Nitroblue Tetrazolium (BCIP/NBT) substrate (Roche Diagnostics), producing precipitate reaction product. The reaction was stopped by washing sections with distilled water followed by mounting in entellan (MERCK, Darmstadt, Germany). Sections were examined with a Zeiss microscope (Axiophot 2).

Similar experiments were carried out using different sets

of probes. PCR was performed with the gene specific primers, forward: 5'-CAG TAG CTT GCA CTG AAA TTG-3' and reverse: 5'-GGC CAT GAA GTC TCC TGT GAA-3', which corresponds to position 68–88 and 347–367 of MVTR sequence.

RESULTS

Sequences analysis of MVTR: Nucleotide sequence and deduced amino acid sequence of MVTR is shown in Fig. 1. Hydrophobicity analysis of the deduced amino acid sequence of MVTR revealed seven hydrophobic regions characteristic for the seven transmembrane domains of GPCR. Scanning of the PROSITE database revealed a number of motifs characteristic for GPCR in the MVTR sequence, a GPCR signature at position 134–150, N-glycosylation sites at position 4–7, 13–16 and 250–253, phosphorylation sites for protein kinase C at positions 47–49, 74–76, 352–354 and 357–359. The N-glycosylation sites may have a function in targeting the receptor protein to the plasma membrane. The sites of phosphorylation have been mapped mainly to the C-terminal tail and have been linked regulatory processes, such as desensitization and internalization [11]. The D-R-Y sequence, positions at 145–147, is highly conserved (Fig. 2). This amino acid triplet is present in the cytoplasmic end of transmembrane III and is thought to be important in G-protein coupling [10]. Two cysteine residues composing a disulphide bridge, which are important for ligand binding, are conserved at position in the extracellular II and III (positions at 121 and 197). Other important site for ligand binding analyzed of the white sucker AVT receptor by mutational analyses also conserved at Q in the transmembrane III and F in the transmembrane IV [16]. MVTR also have these residues (positions at Q128 and F185). The sequence comparison of MVTR with other members of AVT receptor was carried out using their deduced

amino acid sequences. MVTR has 68.3% sequence similarity (34.3% identity) with *P. fesus* AVT receptor, 65.2% similarity (33.3% identity) with *R. catesbeiana* AVT receptor (GenBank: AY277924), and 67.8% similarity (32.8% identity) with *G. gallus* AVT receptor (GenBank: AF147743). These data show that MVTR has significant sequence relationship with AVT receptors and retains common conserved sequence elements.

Expressions in the oocyte: In *Xenopus* oocytes, the interaction of an agonist with its GPCR induces an increase in the intracellular Ca²⁺ via phospholipase C (PLC) and leads to the activation of a Ca²⁺-dependent Cl⁻ channel, which can be evaluated by direct observation of the resultant inward Cl⁻ current. This system has been employed for functional analyses of AVP/OT receptors [21, 30]. To define which ligands activate the MVTR, we expressed its cRNA in *Xenopus* oocytes. The physiological response of the receptor was tested by application of various neurohypophysal hormones, which are structurally similar to AVT. A significant inward current was observed when injected oocytes were exposed to 10 nM AVT. This physiological response was dose-dependent over the range of 50 nM to 10 nM of AVT (Fig. 3), retaining the same response until 200 nM. In order to ascertain whether this experiments was carried out under optimal condition, AVP were administered to oocytes injected with V1aR that is well known AVP receptor. In these oocytes, high responses were elicited with AVP (Data not shown).

RT-PCR analysis: To know tissue distribution of MVTR mRNA, RT-PCR was carried out in several tissues of mouse. It revealed that MVTR mRNA was expressed in the brain (Fig. 4). Brain was divided into three regions: anterior, middle and posterior, followed by RT-PCR. There was no significant difference in intensity of transcript signals. In order to confirm specificity of RT-PCR reaction, nested RT-PCR was performed, and similar results were obtained

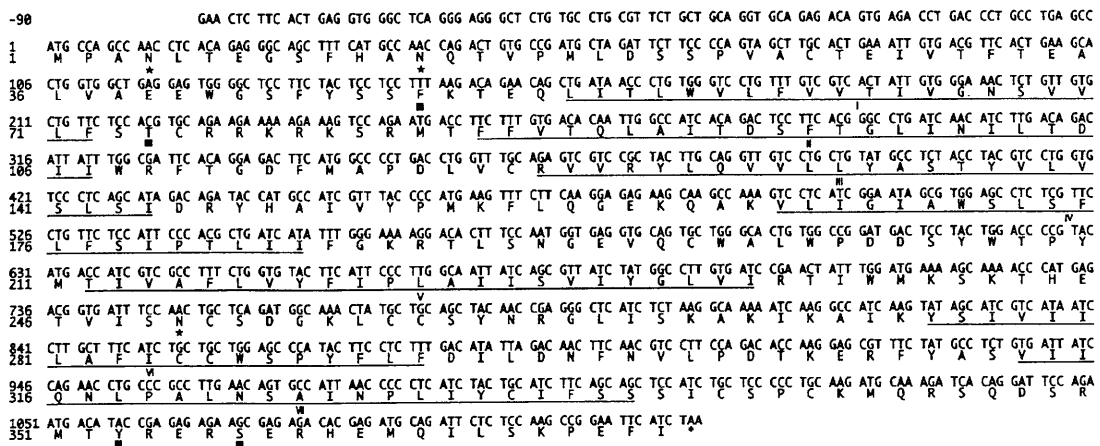


Fig. 1. Nucleotide sequence and deduced amino acid sequence of the MVTR (AK033957). Only the open reading frame and a part of the 5' untranslated leader sequence are shown. In-frame stop codon is indicated by asterisk. The seven transmembrane domains are underlined and numbered. The potential phosphorylation sites for protein kinase C are indicated by ■. Potential N-glycosylation sites are marked by ★.

VTR-Pf	-----MEKPG NTLHPNGSD PFCRNEEVAQ IEDMVLSTF YWAVIGAVSV LEAMYNKKK MSRMHLFIKH	65
VTR-Rc	MGFSKLGSSG QELSEGNQST LDNAESETPF LFLSASPNEIS STVKSMSGSD LLNRDEEELAK IETAVLAVIE VAAVLGNCSV LLEGYKSKKK MSRMHLFIKH	100
VTR-Gg	-----ARKN FSPFMQSDTH QTESPPHRLI SLTNKSDPYG RPERDEQLAQ VEIAYLVVIE LTASVGNFIL ILEVWRRRKK LSRMYVEHLH	83
MVTR	MPANLETEGSF HANQEVPMLD SSPVACTEIV TFEALVAEE WGSFYSSFKT EQLITLWLF VVTIVGNSVW LFSTCR-RKR KSRMTEFVTV	83
VTR-Pf	LSLADLVVAF FQVLPOLONE ITYRFFGPDE LCRIVKHLQV TOMFASTYIM VMTLDRYIA ICHPLKTLQO PTORSYIMEV STNMCSLVFS TPQYEFPSLS	165
VTR-Rc	LSLADLVVAF FQVLPOLONE ITYRFFGPDE LCRIZIRHLQV FOMFASTYIM VMTADRYIA ICHPLKTLHQ PTKRSYLHEG SARIISFILS TPQYGFYLYK	200
VTR-Gg	LSLADLVVAF FQVLPOLONE ITDYVEIGPDE LCRIEIKYLOL LDMFASTYIM VMTVDRYQA VCYPMVTFQK KRALWNIPEK TSNISLILS LPQVETFSKI	183
MVTR	LAETDSFTGL INIETDIHR FTGDPMARDL VCRVRYLQV VLLYASTYVL YSLSIDRYHA IVYPMKFLQG E-KQAKVLEG IANSLSELS IPTLITFGKR	188
VTR-Pf	EVKNGSTYKD CNAHFIEPNG ARAYITWITG GIEFLVPVIL VMCYGFICHT IWNKIKYKRR KTIPTGAASKN GLIGKNSYSS VITTSRAKLR TVKMTFVIVL	265
VTR-Rc	DLGDG--YYD CNADEFSPKG LKAYITWITL SIFVVPYIIL LTCYGEICYN IWRNIZKCKTK RGETDRKRSN GLLS-TSVSS VRTTSRAKLR TVKMTFVIVT	297
VTR-Gg	EISPG--JFE CNAEFIQPNG PRAYVTWILV VIFFEIPSTIL ITICQVKECKT IKRNIVYKRR NEYQVTNQQK VLPSS--RASS VNCISKAMIK TVKMTIVTVV	279
MVTR	TLSNG--EVQ CNAALWDDSY WTPYMTLVAF LVYEIPLAIT SVINGLVIRT IMMSKTHET VISNCSGDK -----LCCSYN RGLISKAKIK AIKYSIVIEL	281
VTR-Pf	AYIICNAPFE TVQMWVWDE HFQYADSENT AVTISALLAS LNSCCNPWEY NFFSGHLLQD FMNCFAWCR ANADFKKEDS DSSIRRTILE TKMTNRSPTG	365
VTR-Rc	AYIICNTPYE TIQMWVYAD NTNKIENENT VYTVSALLAS LNSCCNPWEY NFFSGHLLQD FILSVLCCSR FKHNLSKEDS DSSIRRTISF TRIQTRSPTH	397
VTR-Gg	AYVLENSPFE IAQLNSVWFP SG---ITEGS AFTIIMLLGN LNSCCNPWEY NYFCGHEP-- -----YETH KQLENTSAGE DSVVTSIHEL VDRDPEENST	368
MVTR	AFIICNSPYE LFDIIONNFP LP-DTKERFY ASVTIONLPA LNSAIHPLIY CIFSSSICSP CKMQRSDSR MTYRERSERH EMQILSKPEF I.....	371
VTR-Pf	STGNRDLDN SPKTSIQME.....	384
VTR-Rc	STDTNKDSPK SSRSLKFLPL QI.....	419
VTR-Gg	CA.....	370
MVTR	

Fig. 2. Comparison of deduced amino acid sequence for the MVTR with sequences of AVT receptor of *P. flesus* (VTR-Pf; AF184966), the AVT receptor of *Rana catesbeiana* (VTR-Rc; AY277924) and the *Gallus gallus* AVT receptor (VTR-Gg; AF147743). Lines under the MVTR sequence indicate transmembrane regions. The important sequence in G-protein coupling site is boxed. Two cystine residues in extracellular II and III, which composed a disulfide bridge, are marked by ▲. The important sequence in ligand binding sites are marked by ◆.

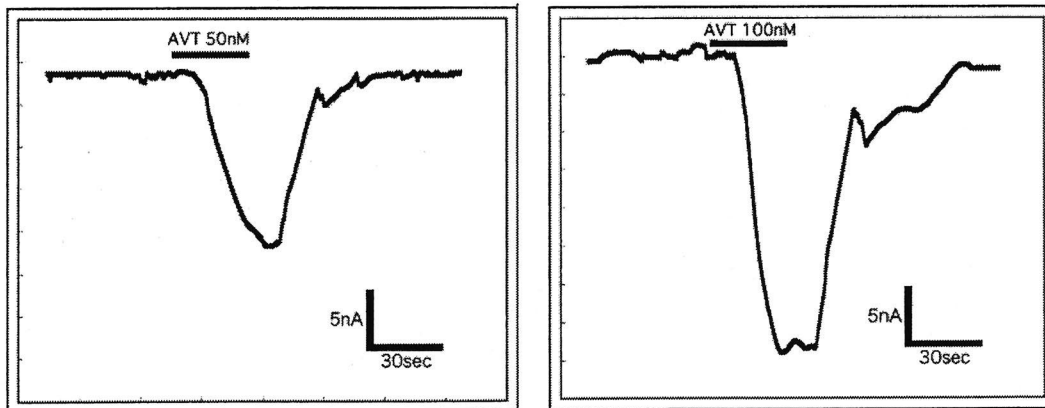


Fig. 3. Functional expression of the MVTR in *Xenopus* oocytes as recorded using voltage clamp techniques. Recorded membrane current trace of an oocyte expressing the MVTR protein was shown when 50 nM AVT was applied (A). After a washout, 100 nM AVT was applied (B). An inward current is shown as a downward curve and the upper bars show the application of AVT at the described concentration. AVP, OT and IT induced no response, whose concentrations were equivalent to that of AVT. Application of AVT to noninjected oocytes failed to elicit the current.

(Data not shown).

In situ hybridization: *In situ* hybridization using a cRNA probe complementary to MVTR mRNA (antisense probe) was performed on coronal sections through various regions of mouse brain. MVTR mRNAs were expressed highly in the neurons of suprachiasmatic nucleus (SCN)(Fig. 5A), arcuate nucleus (Arc)(Fig. 5C) and medial habenular nucleus (MHb)(Fig. 5D). High magnification micrograph showed that cytoplasm were uniquely stained in all these regions. Another locus did not show a significant signal. Sense probe did not stain in these regions (Fig. 5B). *In situ* hybridization performed using a cRNA probe made from different region of the cDNA produced similar results (Data

not shown).

DISCUSSION

From data accumulated in the GenBank database, we attempted to find a DNA encoding MVTR. *P. flesus* AVT receptor had already been well characterized using receptor expression experiments in *Xenopus* oocytes. Therefore, we searched for candidates for cDNA encoding MVTR by comparing with the cDNA of *P. flesus* AVT receptor. We found that deduced amino acid sequence of MVTR, one of the candidates, has significant homology with that of *P. flesus* AVT receptor and other AVT receptors. Moreover amino acid

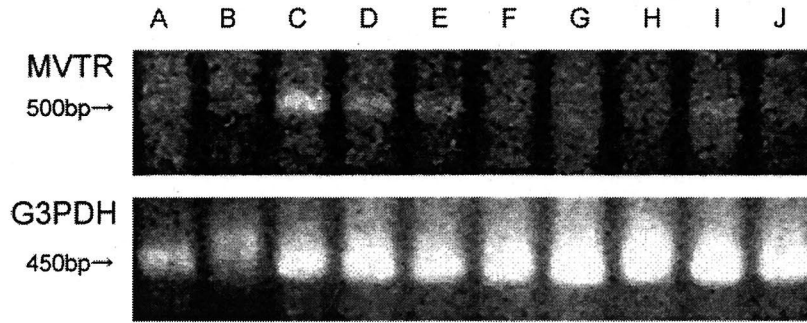


Fig. 4. Tissue distribution of MVTR mRNA as determined by RT-PCR. Respective sizes of the amplified fragment are indicated by bar. Only in brain the MVTR mRNA could be detected. In all cases the integrity of the RNA preparation was ascertained by a control PCR with primers specific for G3PDH (about 450 bp). A, liver; B, spleen; C, anterior brain; D, middle brain; E, posterior brain; F, cerebellum; G, kidneys; H, testes; I, eyes; J, intestine.

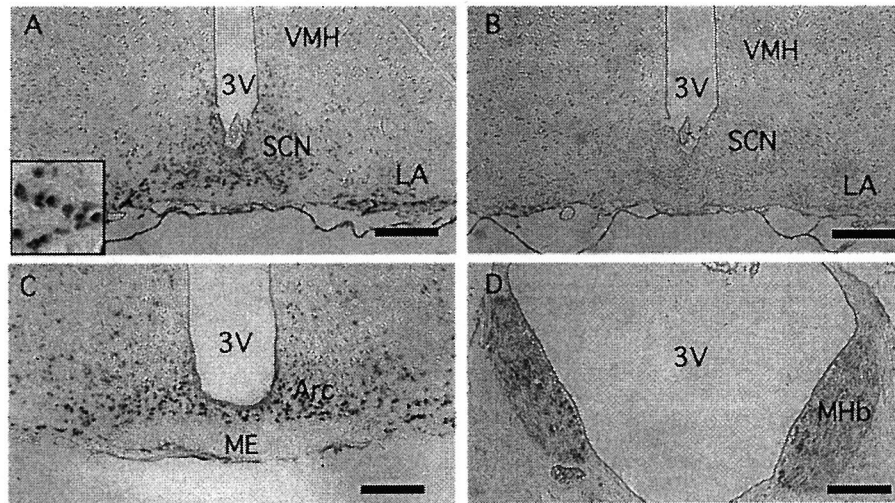


Fig. 5. Distribution of MVTR mRNA expression in mouse brain. Coronal sections containing SCN (A, B), Arc (C) and MHb (D) which were hybridized with antisense cRNA probe (A, C, D) or sense cRNA probe (B). Cells are shown in more detail, at a higher magnification, in the insert. Bars=10 μ m. Abbreviations: Arc, arcuate nucleus; LA, lateroanterior hypothalamic nucleus; ME, medial eminence; MHb, medial habenular nucleus; SCN, suprachiasmatic nucleus; VMH, ventromedial hypothalamic nucleus; 3V, 3rd ventricle.

sequence of MVTR shows many standard characteristic of the AVP/OT receptor family.

A dose-dependent increase of current in response to AVT was observed under voltage-clamp when the MVTR was expressed in *Xenopus* oocytes (Fig. 3). This demonstrates that MVTR is working functionally in cells. AVP, OT and IT are structurally similar to AVT, did not elicit significant current in injected oocytes, indicating that MVTR is specific for AVT.

In general, G protein coupled receptors are linked to several kinds of G protein. And G proteins transduce some signaling pathways. AVP and OT receptors have been well characterized in mammals [6, 43]. The actions of AVP are mediated through three different AVP receptors: V1aR, V1b

receptor (V1bR), and V2 receptor (V2R). Upon ligand stimulation, V1aR and V1bR activate the PLC/Ca²⁺ signaling pathway mediating the Gq proteins, while V2R activates the adenylate cyclase/protein kinase A pathway mediating the Gs proteins. OT receptor also activates PLC/Ca²⁺ signaling pathway mediating the Gq proteins in response to ligand. In *Xenopus* oocytes, Go and Gq proteins activate the PLC/Ca²⁺ signaling pathway [5, 19, 29]. However, in few cases, Gi and Gs proteins also activate this signaling pathway [9]. At present, it is not clear which G protein intermediates the MVTR in *Xenopus* oocytes.

In the present study, the current detected was weaker, compared to the current observed in other responses involving Gq-PLC activation, such as V1aR responses, mGluR1

metabotropic glutamate receptor responses [25] and M1-type muscarinic acetylcholine receptor responses [22]. Similar weak responses were observed in receptor responses mediating other PLC/Ca²⁺ signaling pathway. For example, δ opioid receptor activates PLC activation via Gi proteins in *Xenopus* oocytes [27]. But the intrinsic activity to activate PLC is less potent, compared to Gq proteins. In addition, Gi proteins stimulate the PLC/Ca²⁺ signaling pathway less effective than Go proteins [7].

The distribution of the MVTR mRNA in mouse brain indicates new and as yet undetermined roles for AVT in brain. *In situ* hybridization analyses demonstrated the significant expression of MVTR mRNA in SCN, Arc and MHb.

SCN is well known to regulate circadian rhythm and reproduction in mammals [1, 3]. The excitatory effect of AVP and its potential contribution to the circadian cycle of electrical activity in the SCN of the rat was investigated using extracellular recording from hypothalamic slices of rats [26]. The majority of neurons tested for their responses to AVP and AVT displayed coincident, dose-dependent excitation by both peptides, although the relative efficacy varied between neurons, with some showing a highly preferential excitation by AVT. These results show that AVT works specifically in SCN and does not conflict with our results that show the expression of MVTR mRNA in this region.

Arc, known to play a role in energy homeostasis and reproduction [8, 23], is labeled by a [³H]d(CH₂)₅[Tyr(Me)]VP, AVP antagonist, in rat brain [39], but it is not labeled by either [³H]AVP or [³H]OT. These results suggest that AVT specific receptor or novel AVP receptor subtype would be present in this region.

Habenular complex (Hb) is well known to take part in a variety of biological functions such as, pain processing, reproductive behavior, reward, food and water intake, stress response, sleep wake cycles and learning [36]. AVT produces its specific effects when injected into the third ventricle of the brain. The electrolytic destruction of the Hb completely suppressed the AVT effects and neither AVP nor OT was able to mimic these effects [32]. In addition, Hb specifically bound synthetic AVT [14]. These results predict the action of AVT on the Hb, which in turn consist with our results of MVTR mRNA expression in this region.

MVTR distribution is partially similar to V1aR [4]. But in this study, MVTR did not respond to AVP, indicating that MVTR is different from V1aR.

Recently a neuropeptide S receptor (NPSR) has been reported [42]. Its gene sequence is consistent with that of MVTR. NPSR mRNA was widely distributed in rat brain, the strongest expression signals were found in several discrete nuclei or regions, such as the anterior olfactory nucleus, endopiriform nucleus, amygdala, precommissural nucleus, paraventricular thalamic nucleus, and subiculum. High levels of expression were found in multiple nuclei of hypothalamus. Their results using rat coincided with our results that were obtained using mouse. However, there

were some differences between them. In mouse, MVTR mRNA was distributed especially in SCN, Arc and MHb.

In conclusion, mouse has an AVT reactive receptor. It is responsive to AVT but also neuropeptide S and it may be playing roles related to sleep, reproduction and a variety of physiological functions.

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