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Short Communication

Molecular cloning of three types of arginine vasotocin receptor in the newt, *Cynops pyrrhogaster*

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Abstract

Three types of cDNA encoding the arginine vasotocin (AVT) receptors from the newt, *Cynops pyrrhogaster* were cloned and the gene expression of each receptor analyzed in the organs and tissues of the newt. The deduced amino acid sequence of one type of AVT receptor, consisting of 418 amino acid residues, showed a high degree of sequence identity with the mammalian arginine vasopressin (AVP) V1a receptors (61–68%). The second type of cDNA, encoding an amino acid sequence consisting of 367 amino acid residues, exhibited a relatively high sequence identity with mammalian AVP V2 receptors (50–51%). The third cDNA, encoding a sequence of 415 amino acid residues, possessed high sequence identity with mammalian AVP V3/V1b receptors (59–63%). Phylogenetic analysis revealed that the first, second and third types of receptor were close to mammalian AVP V1a, V2 and V3/V1b receptors, respectively, and RT-PCR using gene specific primers for each type of receptor indicated that the first and second types of receptor mRNA were expressed in various organs and tissues, including the circulatory, osmoregulatory, and reproductive organs of both male and female newts. In contrast, mRNA expression of the third cDNA was mainly detected in the brain and pituitary, and its expression pattern was distinctly different from that of the other two. We suggest that the first, second and third types of newt AVT receptor obtained in the present study are counterparts of mammalian AVP V1a, V2 and V3/V1b receptors, respectively.

Keywords: Arginine vasotocin; Arginine vasotocin receptor; Newt

1. Introduction

Arginine vasotocin (AVT), a neurohypophyseal hormone found in non-mammalian vertebrates is the ortholog of arginine vasopressin (AVP) in mammals. In amphibians, besides its well-known antidiuretic function, AVT exhibits various functions, particularly in reproduction. It elicits calling behavior in male frogs (Boyd, 1994), enhances sexual receptivity in female frogs (Boyd, 1992), and causes egg-laying behavior (Moore et al., 1992), and amplectic clasping behavior in the newt, *Taricha granulosa* (Moore and Zoellar, 1979; Moore and Miller, 1983). We have shown that AVT is also involved in various aspects of reproductive events in the Japanese red-bellied newt, *Cynops pyrrhogaster*. It induces the release of female-attracting pheromone sodefrin (Kikuyama et al., 1995) from the abdominal gland, spermatophore deposition from the cloaca, and acts centrally to enhance courtship behavior. Studies using antagonists of mammalian AVP V1a and V2 receptors showed that AVT acts via V1a-like receptor to induce these phenomena (Toyoda et al., 2003).

The actions of neurohypophyseal hormones are known to be mediated through specific G protein-coupled receptors. In mammalian species, at least three subtypes of receptor for AVP (V1a, V2, V3/V1b) have been reported and characterized (Lolait et al., 1992; Morel et al., 1992; Sugimoto et al., 1994). These three types of receptor have also

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been found to be present differentially in various tissues and organs. The AVP V1a receptor mainly expressed in vascular smooth muscle cells and hepatocytes (Morel et al., 1992), the V2 receptor expressed in the kidney, where it regulates water reabsorption (Lolait et al., 1992), and the V3/V1b receptor exclusively located in the pituitary gland (Sugimoto et al., 1994; de Keyzer et al., 1996). Moreover, AVP actions through the V1a and V3/V1b receptors are mediated by the phospholipase C (PLC)/protein kinase C (PKC) signaling pathway, and AVP acting through the V2 receptor is mediated through the adenylyl cyclase (AC)/protein kinase A (PKA) pathway (Liu and Wess, 1996; Gimpl and Fahrenholz, 2001).

The AVT receptor cDNA was first isolated in white sucker by Mahlmann et al. (1994) who, based on the deduced amino acid sequences of the AVT receptors and pharmacological characteristics, concluded that this receptor has a functional relationship with mammalian AVP V1a receptor. Two types of frog AVT receptor cDNAs have subsequently been cloned. The sequence identity and pharmacological properties of one type of AVT receptor, which was isolated from *Hyla japonica*, were found to be similar to those of the mammalian AVP V2 receptor (Kohno et al., 2003). The other AVT receptor, isolated from *Rana catesbeiana* and *R. esculenta*, was found to have characteristics similar to those of mammalian AVP V1a receptor (Acharjee et al., 2004). Further, a counterpart of the mammalian AVP V3/V1b receptor, named the pituitary vasotocin receptor, has been characterized in chicken (Cornett et al., 2003).

As a step to elucidate the actions of AVT in the newt *Cynops* at the receptor level, an attempt was made to obtain AVT receptor cDNAs. Here we report the sequences of three types of newt AVT receptor and the distribution of these receptor mRNAs in several organs and tissues of male and female newts.

2. Materials and methods

2.1. Animals

Male and female newts *Cynops pyrrhogaster* were purchased from Ohuchi Aquatic Animal Supply (Misato, Japan). They were captured in March. We selected only sexually developed male and female newts for use. They were kept in a tank at 22 °C under a 12/12-h (light/dark) photoregimen and fed daily with *Tubifex* worms until use. All experimental procedures were conducted in accordance with Waseda University's guidelines for the care and use of laboratory animals.

2.2. Cloning of V1-type AVT receptor cDNA

A cDNA fragment of newt AVT V1-type receptor was obtained by reverse-transcriptase (RT)-PCR using primers (V1S, V1A; see Table 1) designed on the basis of alignment with mammalian AVP receptors (Morel et al., 1992; Thibonnier et al., 1994) and bullfrog AVT V1 receptor mRNA sequences (Acharjee et al., 2004). Newt brain total RNA was extracted using ISOGEN (Nippon Gene, Japan) according to the manufacturer's instructions. Genomic DNA contaminating the total RNA sample was digested with DNase I (Takara, Japan). One microgram of brain total RNA was reverse transcribed using Superscript II RNase H-reverse transcriptase (Invitrogen, Carlsbad, Calif.) and dT $_{(12–18)}$ primers. PCR amplification was performed with Ex *Taq* DNA polymerase (Takara) under the following cycling conditions: denaturation at 94 °C for 5 min, followed by 40 cycles at 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s. PCR products of the expected size were excised, purified, and subcloned into pT7 blue T-vector (Novagen, Madison, Wis.). Poly(A)+ RNA was purified from the brain total RNA using an Oligotex-(dT)30 Super RNA purification kit (Takara). Double-stranded cDNA was then synthesized from Poly(A)+ RNA using a Timesaver cDNA synthesis kit (GE Healthcare, UK). *Eco*RI-*Not*I adaptor-added cDNA was ligated into the lambda ZAP II (Stratagene, La Jolla, Calif.) and packaged into Packagene (Promega, Madison, Wis.). About 3×10^5 recombinant plaques were screened with the partial AVT V1-type receptor cDNA as a probe. This probe was ^{32}P-labeled by the random priming method using the BcaBEST labeling kit (Takara). The nylon membranes (Hybond N+, GE Healthcare) were first prehybridized in hybridization buffer (50% formamide, 5 × SSPE, 0.5% SDS, 5 × Denhardt's, 20 μg/ml denatured salmon sperm DNA) at 42 °C for 2 h, then hybridized for 16 h under the same conditions in the presence of the radiolabeled probe. The membranes were washed successively in 2 × SSPE-0.1% sodium dodecyl

Table 1
Oligonucleotide sequence of primers used fo RACE and PCR

Application	Name	Oligonucleotide sequence (5′–3′)
RT-PCR	V1S	TGCTGGGAGATCACTTACCGCTTC
RT-PCR	V1A	TGCTGGGAGATCACTTACCGCTTC
RT-PCR	nV1S	CAGGTCTTCGGCATGTTCGCT
RT-PCR	nV1A	GACGTAGGCGGACACAATGAC
RT-PCR	V2S	GCMCRWTTTGYRRARCCATGGGG
RT-PCR	V2A	GATCCAAGGGTTLGTACAGCTG
RT-PCR/3′RACE	nV2S	ATTTGTCCTGCCTACCCTAGTG
RT-PCR/5′RACE	nV2A	TGGACGTTCCGTTTCTGCCTT
5′RACE	nV2A2	CAAGCCACGAAAACCGGAACA
RT-PCR/3′RACE	V3S	ACYGTGAAGATGACCTTTTGT
RT-PCR/5′RACE	V3A	AGRAGCATGGWGATGGTGAA
RT-PCR/3′RACE	nV3S	TTTGCTGGACGCCATTCTTCAG
RT-PCR/5′RACE	nV3A	GCGTTCGCATCCCAGACAGA
RT-PCR	nV3A2	TCTGCATGGGTCTGACATTGCTAC
RT-PCR	nACTS	CCTTCCTTGGTATGGAATCG
RT-PCR	nACTA	CATCATACTCCTGCTTGCTG
3′RACE	3′RACE adaptor primer	GGCCACGCGTCGACTAGTAC
3′RACE	Adaptor-dT primer	GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTT
5′RACE	5′RACE abridged anchor primer	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG
5′RACE	Abridged universal amplification primer	GGCCACGCGTCGACTAGTAC