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Expression of arginine vasotocin receptors in the developing zebrafish CNS

Kenichi Iwasaki [a], Meari Taguchi [b], Joshua L. Bonkowsky [c,d], John Y. Kuwada [a,b,*]

[a] Neuroscience Graduate Program, University of Michigan, United States
[b] Department of Molecular, Cellular and Developmental Biology, University of Michigan, United States
[c] Department of Pediatrics, University of Utah School of Medicine, United States
[d] Department of Neurobiology and Anatomy, University of Utah School of Medicine, United States

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ABSTRACT

Vasotocin/vasopressin is a neuropeptide that regulates social and reproductive behaviors in a variety of animals including fish. Arginine vasotocin (AVT) is expressed by cells in the ventral hypothalamic and preoptic areas in the diencephalon during embryogenesis in zebrafish suggesting that vasotocin might mediate other functions within the CNS prior to the development of social and reproductive behaviors. In order to examine potential early roles for vasotocin we cloned two zebrafish vasotocin receptors homologous to $AVPR_{1a}$. The receptors are expressed primarily in the CNS in similar but generally non-overlapping patterns. Both receptors are expressed in the forebrain, midbrain and hindbrain by larval stage. Of note, $AVTR_{1a}$-expressing neurons in the hindbrain appear to be contacted by the axons of preoptic neurons in the forebrain that include *avt+* neurons and sensory axons in the lateral longitudinal fasciculus (LLF). Furthermore, $AVTR_{1a}$-expressing hindbrain neurons extend axons into the medial longitudinal fasciculus (MLF) that contains axons of many neurons thought to be involved in locomotor responses to sensory stimulation. One hypothesis consistent with this anatomy is that AVT signaling mediates or gates sensory input to motor circuits in the hindbrain and spinal cord.

Signaling within the CNS via the nonapeptide, arginine vasopressin (AVP) in mammals and its homolog arginine vasotocin (AVT) in nonmammalian vertebrates, regulates social and reproductive behaviors in a wide variety of species (reviewed in Donaldson and Young, 2008). Dysfunction of signaling by AVP and oxytocin, another nonapeptide implicated in social behaviors, is thought to contribute to psychiatric disorders such as autism, affective disorders, obsessive–compulsive disorder, posttraumatic stress disorder and schizophrenia (reviewed in Heinrichs et al., 2009). Furthermore, AVP/AVT released into the circulation by the posterior pituitary in response to sexual stimulation, stress and dehydration mediates a variety of peripheral effects including antidiuretic activity by the kidney (Leng and Bicknell, 1986; Nishimura and Fan, 2003).

AVP is expressed by neurons of the supraoptic, paraventricular and suprachiasmatic nuclei of the hypothalamus in mammals (Brownstein et al., 1980; Young and Gainer, 2003) and AVT primarily by neurons of the preoptic area in fish (Venkatesh and Brenner, 1995; Acher et al., 1997). Additionally AVP is expressed by the bed nucleus of the stria terminalis and amygdala in the mammalian brain (DeVries and Buijs, 1983; DeVries et al., 1985). There are 3 AVP receptors in mammals with the $AVPR_{1a}$ (V1a) and $AVPR_{1b}$ (V1b) receptors expressed primarily in the CNS and the $AVPR_2$ (V2) receptor in the periphery (Caldwell et al., 2008). AVT receptors have been identified in a number of teleosts as well (Mahlmann et al., 1994; Conklin et al., 1999; Warne, 2001; An et al., 2008). In the pupfish and perhaps other teleosts there appear to be two V1a receptors and a V2 receptor for AVT (Lema, 2010). Like the mammalian V1a receptor, RT-PCR found that the pupfish V1a receptors are widely expressed throughout the CNS.

AVT is expressed by cells in the ventral hypothalamus and the preoptic area of the diencephalon, and isotocin in the preoptic area (Tessmar-Raible et al., 2007; Eaton et al., 2008; Blechman et al., 2011) during embryogenesis in zebrafish prior to the development of social or reproductive behaviors. During embryogenesis behaviors exhibited by zebrafish embryos are restricted to simple motor responses such as escape swimming evoked by sensory stimulation (Saint-Amant and Drapeau, 1998). The embryonic expression of AVT suggests that it may participate in the development of sensory and/or motor circuits early in development. As a first step in examining the function of AVT signaling in the embryonic CNS, we cloned two V1a type receptors in zebrafish and determined their expression patterns during early stages of development. The

* Corresponding author. Address: Department of Molecular, Cellular and Developmental Biology, University of Michigan, 830 North University Ave., Ann Arbor, MI 48109-1048, United States. Tel.: +1 734 936 2842; fax: +1 734 647 0884.
E-mail address: kuwada@umich.edu (J.Y. Kuwada).

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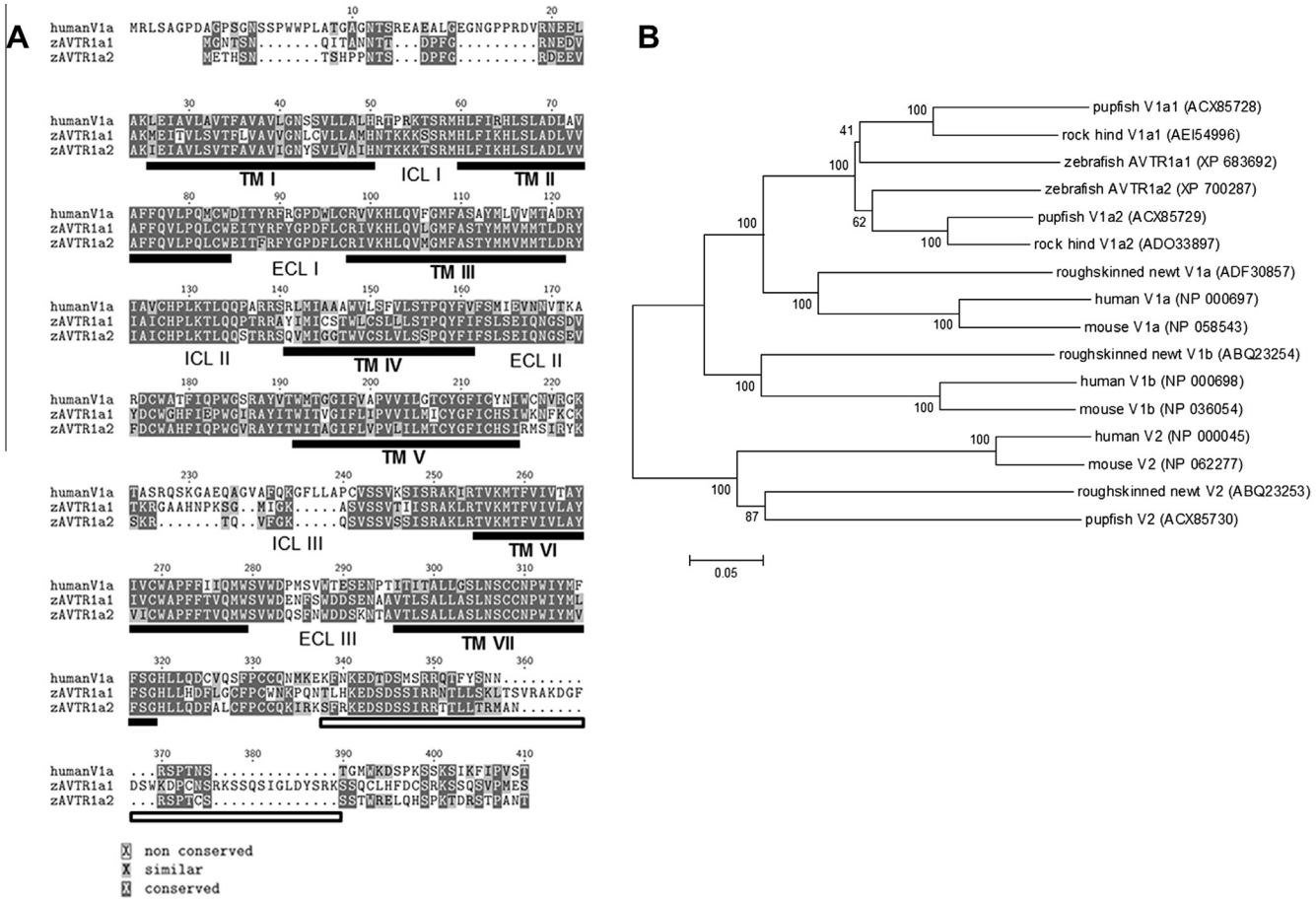

Fig. 1. Zebrafish $AVTR_{1a1}$ and $AVTR_{1a2}$. (A) Amino acid alignment for human V1a (NP_000697), zebrafish $AVTR_{1a1}$, and zebrafish $AVTR_{1a2}$. There is a high degree of amino acid conservation between human V1a and zebrafish AVTRs. The dark gray boxes indicate amino acid identity and light gray boxes similarity. The amino acid number corresponds to that for zebrafish $AVTR_{1a1}$. The black bars mark the transmembrane domains (TMs) for zebrafish $AVTR_{1a1}$. The white box indicates the DUF1856 domains conserved in the C-termini of various AVP receptors. The locations of the intracellular loops (ICL) and extracellular loops (ECL) are also indicated. (B) The phylogeny of AVP/AVT receptors.

expression pattern of the V1a receptors is concordant with the hypothesis that AVT signaling may play a role in early sensory/motor function.

1. Results

1.1. Zebrafish contain two AVT receptors homologous to $AVPR_{1a}$

We cloned two AVT receptors by RT-PCR from adult zebrafish brain tissue, $AVTR_{1a1}$ and $AVTR_{1a2}$, that were 60% and 62% identical at the amino acid level with human V1a receptor and 54% and 52% identical with human V1b receptor, respectively (blastp, www.ncbi.nlm.nih.gov) (Fig. 1A). A phylogenetic analysis of zebrafish $AVTR_{1a1}$ and $AVTR_{1a2}$ was consistent with the assignment of the zebrafish receptors as V1a type (Fig. 1B). These findings are consistent with previous findings of a duplicated V1a receptor in teleosts (Lema, 2010).

1.2. avtr1a1 and avtr1a2 are expressed primarily in the CNS in early stage zebrafish

By 25 h postfertilization (hpf) *avtr1a1* and *avtr1a2* are expressed by a cluster of cells in the forebrain and by a small number of discrete cells in the hindbrain (Fig. 2A). The earliest expression seen via *in situ* hybridization was *avtr1a1* in the forebrain and hindbrain at 22 hpf (not shown). Examination of *avtr1a1* expressing cells in embryos in which all axons are labeled with anti-acetylated α-tubulin found that the forebrain cells are in apparent contact with the postoptic commissure (POC) and/or tract of the postoptic commissure (TPOC) (Fig. 2B; Chitnis and Kuwada, 1990), which is consistent with these cells projecting axons into the POC/TPOC. These *avtr1a1+* neurons as the putative *avtr1a1+* epiphyseal and nucleus of the posterior commissure neurons (see below) may also express HNK-1 since the pattern of early neurons/axons labeled with anti-HNK-1 and anti-acetylated α-tubulin are similar (Wilson et al., 1990). Additionally there are irregularly spaced, occasional dorsal cells in the spinal cord of unknown identity that express *avtr1a1* (Fig. 2C).

By 48 hpf *avtr1a1* is expressed by a cluster of ventral forebrain cells (I), forebrain cells near the dorsal midline (II), dorsal cells located at the forebrain/tectum boundary (III), ventral cells near the forebrain/tegmentum border (IV), and two longitudinal stripes of cells in the midbrain/anterior hindbrain region (V and VI) when viewed from a dorsal perspective as well as the posterior hindbrain cells (Fig. 3A–C). Lateral views show that in the ventral forebrain cluster I is located anterior and ventral to cluster IV (Fig. 3D). Examination of cluster I *avtr1a1+* cells in 48 hpf embryos with all axons labeled with anti-acetylated α tubulin showed that these neurons appear to project axons into the TPOC (Fig. 4A) suggesting that these are the POC/TPOC forebrain neurons observed earlier (Fig. 2B). Examination of forebrain *avtr1a1+* cells in *otpb.A:GAL4; UAS:gfp* embryos that express GFP in preoptic neurons (Fujimoto et al., 2011) showed that cluster I *avtr1a1+* cells are adjacent to