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AMPHIBIAN NEUROHYPOPHYSIAL PEPTIDES

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ABSTRACT

The amphibian neurohypophysis releases primarily two peptides – arginine vasotocin (AVT) and mesotocin (MT). Both are nonapeptides synthesized in multiple brain areas. Four specific receptor subtypes have been identified. The VT1aR is specific for AVT, found in brain and kidney, and is coupled to a protein kinase C-mediated pathway. The VT1bR differs only in restricted expression exclusively in brain and pituitary. The VT2R has a high affinity for AVT and hydrins and is found primarily in the skin and bladder, where it utilizes the cAMP signaling pathway. The MTR is specific for MT, couples to a protein kinase C-linked pathway, and is found in brain and peripheral tissues. Biological actions of AVT include control of osmoregulation, behavior, reproductive tract contractions, and the cardiovascular system. Actions of MT are poorly understood. Finally, the amphibian neurohypophysis produces hydrins which are extended forms of AVT with specific osmoregulatory effects on skin and urinary bladder.

KEYWORDS

vasotocin; mesotocin, hydrin; osmoregulation; sexual behavior

1. DISCOVERY

The class Amphibia contains three orders: the Anurans (frogs and toads), the Urodeles (salamanders and newts), and the Gymnophionans (caecilians). In the early 1900's, it was already recognized that the neurohypophysis (posterior pituitary) of frogs contained "active principles" that altered blood pressure. The structure of these principles was first elucidated in an anuran amphibian for arginine vasotocin (AVT) and mesotocin (MT) in the 1960's [2]. Characterization of AVT and MT structure in caecilians and urodeles, however, has only occurred within the last 15 years [16, 27]. In all amphibians investigated, AVT and MT have been found, with the exception of *Bufo regularis* and *Plethodon shermani* in which mesotocin is replaced by [Ser5,Ile8]-oxytocin (seritocin) or [Val4]-mesotocin, respectively (Table 1; [2, 27]). Finally, anuran amphibians also possess unique closely-related neurohypophysial peptides called hydrins (section 6).

2. PEPTIDE STRUCTURE AND PROCESSING OF PRECURSOR mRNAs

The nonapeptides AVT and MT are part of a conserved family of neurohypophysial hormones. Representative species from all vertebrate classes examined to date possess AVT and it is thus considered the evolutionary precursor peptide in this family [2]. AVT is replaced by arginine vasopressin (AVP) in most mammals. Birds and reptiles also possess MT, in common with amphibians. Isotocin replaces MT in most fish and oxytocin (OT) is the mammalian homolog. All have a nine amino acid peptide backbone with a disulfide bridge between conserved cysteine residues at positions 1 and 6. All possess an NH2-blocked C-terminal glycine.

The nucleotide sequences coding for AVT and MT precursors have been reported for only four species of amphibians: the toad *Bufo japonicus*, two urodeles (*Taricha granulosa* and *Plethodon shermani*) and the caecilian, *Typhlonectes natans* (reviewed in [27]). Following a hydrophobic signal sequence, the amino acids of AVT or MT are represented. Next, a tripeptide is found which may facilitate processing or become incorporated into hydrin. This tripeptide is followed by a 93 amino acid neurophysin domain and a 36 or 37 amino acid glycoprotein domain. Although these domains result in the production of two peptides in mammals, this is not the case in amphibians where a single large neurophysin is produced [2]. In the MT precursor, a 93 or 94 amino acid peptide neurophysin also follows but a glycoprotein is not present.

3. DISTRIBUTION OF AVT AND MT

Amphibian neurohypophysial peptides are synthesized in a variety of brain nuclei [17, 28]. In all three amphibian orders, the most prominent cell group is in the magnocellular preoptic area. It is likely that these cells are the primary source of AVT and MT released from the neurohypophysis. AVT cell bodies can also be found in the pallium, bed nucleus of the stria terminalis, ventral thalamus, suprachiasmatic nucleus, hypothalamic nucleus and midbrain tegmentum. On the other hand, MT cells are restricted primarily to diencephalic regions. Fibers are widespread and these peptides likely function as neuromodulators. There can be significant variation in the location of AVT cell populations across species, especially outside the diencephalic regions. AVT concentrations in some brain areas are sexually dimorphic and steroid-sensitive [6, 7, 28]. Current evidence indicates this is primarily due to the effects of androgens.

4. RECEPTOR STRUCTURE AND DISTRIBUTION

The MT receptor (MTR) and three subtypes of AVT receptor have been cloned in representative species of anuran and urodele amphibians [1, 4, 15, 18, 26]. All four belong to the G-protein coupled receptor family, with typical seven transmembrane domains. One AVT receptor subtype closely resembles the mammalian AVP receptor subtype V1a. The cDNA for this receptor (VT1aR) encodes a protein of 418 or 419 amino acids. The potency of ligands for this receptor is AVT > OT >AVP ≈ MT in an anuran and AVT > AVP > MT ≈ OT in a urodele amphibian. The VT1aR preferentially couples to the protein kinase C-mediated signaling pathway. The second AVT receptor subtype (VT2R) is more similar to the mammalian V2R. The receptor protein is 363 or 367 amino acids. The potency of ligands for this receptor is AVT = hydrin 1 > hydrin 2 = AVP > MT = OT. The VT2R likely utilizes the adenylate cyclase-cAMP signaling pathway. The third subtype of AVT receptor has only been recently characterized in a newt [15]. This subtype VT1bR shares high sequence identity with the mammalian V1b receptor. Finally, the cDNA for the MTR encodes a receptor of 384 to 393 amino acids that shares significant homology with the OT receptor. The sensitivity of the MTR for ligands in this peptide family is MT > OT > AVT ≥ AVP or MT > AVT = OT > AVP > hydrin 1= isotocin = hydrin 2, depending on species. The frog MTR likely couples exclusively to the protein kinase C-linked signaling pathway.

The VT1aR mRNA is abundant in brain, anterior pituitary, heart, adrenal gland, kidney and oviduct [1]. In the brain, the distribution of binding sites detected with 3H-AVP autoradiography is very similar to the distribution of the receptor mRNA [1, 6, 15]. Putative receptors are abundant in accumbens, pallium, striatum, lateral amygdala, preoptic area, magnocellular nucleus, hypothalamus,

thalamus, and optic tectum. In the pituitary, VT1aR mRNA is found in the anterior pituitary and the intermediate lobe but not in the neurohypophysis [1]. Binding studies with 3H-AVP show receptors in the anterior pituitary but, in contrast, also show receptors in the neurohypophysis. This may reflect the presence of another subtype of AVT receptor in amphibians, not detected by the VT1aR probe. The presence of a VT1R subtype (either 1a or 1b) in amphibian kidney is also supported by autoradiographic and physiological evidence [5, 8, 24]. Binding sites for 3H-AVP or 125I-OVTA are found specifically located over the glomeruli and not renal tubules. These sites have ligand specificity more similar to mammalian V1 receptors than V2 receptors. The mRNA for the VT2R is expressed in the brain, heart, kidney, urinary bladder, and pelvic skin patch [14, 15, 18]. Lastly, the VT1b receptor is expressed almost exclusively in the brain and anterior pituitary in a newt [15].

The MTR mRNA is abundant in the brain of anurans [1, 4, 18]. *In situ* hybridization shows highest levels in pallium, amygdala, ventral striatum, anterior preoptic area, nucleus of the periventricular organ, posterior tuberculum, ventral hypothalamic nucleus, thalamic nuclei, and optic tectum [1]. The MTR mRNA, like that for VT1aR, is found in the anterior pituitary and intermediate lobe but not in the neurohypophysis. The neural function of MT is unknown. The MTR mRNA is also found in several peripheral tissues, including heart, adrenal gland, kidney, urinary bladder, skeletal muscle, fat body, and testis [1, 4, 18]. Importantly, the receptor was not found in the oviduct [1]. This suggests that, despite the structural similarities with the OT receptor, the MTR does not play a homologous role in reproductive tract smooth muscle contraction (section 5.3).

5. MAJOR BIOLOGICAL ACTIONS OF AVT AND MT

5.1 Osmoregulatory Effects:

AVT control of osmoregulation in amphibians is complex and involves several different target organs – the kidneys, the urinary bladder, and the skin. At the renal level, AVT causes antidiuresis first by constricting preglomerular arteries and causing a reduction in glomerular filtration rate by as much as 85% [33]. The glomerular action is supported by the location of receptors over the glomeruli and presence of VT1aR mRNA (section 4). Whether AVT alters tubule reabsoption in amphibians is more controversial. AVT can cause antidiuresis by acting directly on tubules in a few species [33]. In addition, anuran amphibian kidney possesses an AVT-dependent aquaporin water channel protein [30, 32]. Binding sites for radiolabeled AVT analogs have not been located over tubules, however, and it is clear in some species that AVT has no tubular effects [5, 8]. Mesotocin increases glomerular filtration rate and is diuretic in amphibians [13, 32, 33].

The anuran urinary bladder served as the archetypal tissue for research on the water permeability of membranes for decades. Anurans can store fluid equivalent to 50% of body weight in the bladder. AVT stimulates the movement of water from the bladder back into the vasculature and this likely occurs via the VT2R [33]. AVT increases cAMP levels and the expression and translocation of aquaporin proteins [30, 32]. Interestingly, while mammals posses a single AVP-dependent aquaporin in the kidney, anuran amphibians have three AVT-dependent proteins -- aquaporin forms unique for the kidney, urinary bladder and ventral pelvic skin. The anuran bladder also shows AVT-modulated sodium and urea reabsorption [19, 32]. Mesotocin has a limited ability to stimulate water and sodium uptake across the anuran bladder and the MTR has been located in this tissue [32].

The permeable skin of amphibians allows the unique movement of water across that surface. AVT increases water permeability of ventral pelvic skin and facilitates rehydration of anurans and urodeles [33]. The VT2 receptor mRNA and protein is expressed in the pelvic skin and AVT regulates expression of water channel aquaporins in this tissue [23, 30]. Mesotocin has been reported to increase cutaneous water permeability, however MTR mRNA has not been found here [1, 4, 18]. Neither AVT nor MT alter water permeability of the skin of a caecilian amphibian [29]. Finally, active sodium transport across skin is also facilitated by AVT and MT in some, but not all, species [31, 33].

5.2 Behavioral Effects:

In recent decades, the behavioral effects of neurohypophysial peptides in amphibians have received the most attention [7]. In many anurans, AVT modulates the display of vocal behavior [21, 34]. The peptide facilitates the advertisement call used to attract mates, while it inhibits the release call given by unreceptive females. In addition, AVT stimulates the attraction of female anurans to the calls of males and alters locomotor behaviors. AVT also alters reproductive behaviors of urodeles. AVT stimulates amplectic clasping, pheromone release, and egg-laying behavior [21, 34]. Effects of AVT are likely occurring via action in the central nervous system and there is extensive interaction between AVT, androgens and corticosterone in modulation of amphibian reproductive behaviors [7].

5.3 Reproductive Tract Effects:

As in other vertebrate classes, the amphibian oviduct shows robust smooth muscle contractions following exposure to neurohypophysial peptides [11]. Unexpectedly, however, the oxytocin

homolog, MT, is not the most potent peptide. Instead, AVT causes oviduct contractions. Sex steroids increase the responsiveness of the oviduct to AVT. In an analogous fashion, AVT stimulates contractions of the male urodele Wolffian duct [35].

5.4 Cardiovascular Effects:

It has been known since 1904 that pituitary extracts alter the circulatory system of the frog however this has received little attention. AVT is vasopressor and causes constriction of vessels *in vitro* [9]. Further, although AVT causes bradycardia *in vivo*, when autonomic receptors are blocked, AVT increases heart rate and contractile force. On the other hand, MT acts as a vasodepressor in amphibians [9]. Three receptor subtypes (VT1R, VT2R, and MTR) are found in the heart but it is unknown which receptors are present in the vasculature [1, 4, 18]. It has been proposed that the cardiovascular effects of AVT are the most primitive and that these gave rise to the osmotic effects of the peptide in amphibians and other tetrapods.

5.5 Other Effects:

In amphibians, the adrenal gland homolog is the interrenal gland, where chromaffin cells are intermingled with steroidogenic cells. AVT-immunoreactivity is found in virtually all chromaffin granules and may serve in a paracrine role to stimulate secretion of steroids [20]. AVT causes a rapid increase in corticosterone and aldosterone output from the interrenal gland [12]. Rank order potency of ligands suggests a VT2R is responsible [20]. However, the effect is via phosphoinositide-specific phospholipase C and VT2R mRNA has not been found in the interregnal gland [1, 4, 18, 20].

AVT stimulates hepatic glycogenolysis and increases glycogen phosphorylase *a* activity in representative anuran and urodele amphibians [3]. This effect differs significantly from the stimulation of glycogenolysis in mammalian liver by AVP, however, because it appears to work via a VT2 subtype receptor and coupling to adenylate cyclase. VT2R mRNA has not been reported in the liver but the liver receptor subtype may differ from the bladder VT2R [14, 18].

6. STRUCTURE AND FUNCTION OF HYDRINS

Neurohypophysial extracts from anuran amphibians were first reported in 1961 to contain a third class of peptides. These peptides were sequenced and named "hydrins" in 1989 (Table 1: [25]). Hydrin 2 is broadly distributed across anuran families. In *Xenopus laevis*, two variants of hydrin (termed hydrin 1 and 1') are found instead. Hydrins have not been detected in any urodele or in any other vertebrate. Hydrins likely result from differential processing of the AVT preprohomone. The result is an extended peptide that is not amidated. Hydrins are as active as vasotocin in the anuran bladder, more active on anuran skin, but inactive in the kidney [2, 25]. Given the high affinity of the VT2R for hydrins (section 4), it is likely that a receptor of this subtype is responsible for the biological actions of hydrins. Indeed, AVT, hydrin 1 and hydrin 2 have very similar effects on the translocation of aquaporin proteins in anuran ventral pelvic skin [22]. In addition to effects on water reabsorption, hydrin 2 has been shown to influence cutaneous ion transport [10]. Differences in physiological effects of AVT and hydrins on target tissues, however, suggest that two subtypes of VT2R may exist.

Table 1

Amphibian Neurohypophysial Peptides.

	1	2	3	4	5	6	7	8	9	
Vasotocin	Cys-	Tyr-	Ile-	Gln-	Asn-	Cys-	Pro-	Arg-	Gly-	(NH2)
Mesotocin	—	—	—	—	—	—	—	Ile-	—	(NH2)
[Val4]-Mesotocin	—	—	—	Val-	—	—	—	Ile-	—	(NH2)
Seritocin	—	—	—	—	Ser-	—	—	Ile-	—	(NH2)
Hydrin 1	—	—	—	—	—	—	—	—	—	Gly-Lys-Arg(OH)
Hydrin 1'	—	—	—	—	—	—	—	—	—	Gly-Lys(OH)
Hydrin 2	—	—	—	—	—	—	—	—	—	Gly(OH)

Dashes indicate residues identical with those of vasotocin.

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