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Research Report

Differential co-localization with choline acetyltransferase in nervus terminalis suggests functional differences for GnRH isoforms in bonnethead sharks (*Sphyrna tiburo*)

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ABSTRACT

The nervus terminalis (NT) is a vertebrate cranial nerve whose function in adults is unknown. In bonnethead sharks, the nerve is anatomically independent of the olfactory system, with two major cell populations within one or more ganglia along its exposed length. Most cells are immunoreactive for either gonadotropin-releasing hormone (GnRH) or RF-amide-like peptides. To define further the cell populations and connectivity, we used double-label immunocytochemistry with antisera to different isoforms of GnRH and to choline acetyltransferase (ChAT). The labeling patterns of two GnRH antisera revealed different populations of GnRH-immunoreactive (ir) cell profiles in the NT ganglion. One antiserum labeled a large group of cells and fibers, which likely contain mammalian GnRH (GnRH-I) as described in previous studies and which were ChAT immunoreactive. The other antiserum labeled large club-like structures, which were anuclear, and a sparse number of fibers, but with no clear labeling of cell bodies in the ganglion. These club structures were choline acetyltrasferase (ChAT)-negative, and preabsorption control tests suggest they may contain chicken-GnRH-II (GnRH-II) or dogfish GnRH. The second major NT ganglion cell-type was immunoreactive for RF-amides, which regulate GnRH release in other vertebrates, and may provide an intraganglionic influence on GnRH release. The immunocytochemical and anatomical differences between the two GnRH-immunoreactive profile types indicate possible functional differences for these isoforms in the NT. The club-like structures may be sites of GnRH release into the general circulation since these structures were observed near blood vessels and resembled structures seen in the median eminence of rats.

1. Introduction

The nervus terminalis (NT), which is found in all vertebrate groups, is the most rostral cranial nerve. Although its function has yet to be determined, cells and fibers within the NT contain the hormone gonadotropin-releasing hormone (GnRH; Phillips et al., 1980; Schwanzel-Fukuda and Silverman, 1980; Munz et al., 1982; Wirsig and Getchell, 1986; Wirsig and Leonard, 1986b; White and Meredith, 1995; Kim et al., 1999). In elasmobranchs, the NT contains a higher concentration of GnRH than any other part of the forebrain (Nozaki et al., 1984; Demski et al., 1987),

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suggesting that NT may be important for the reproductive function of GnRH.

The central trunk of the NT extends as a bundle of GnRH-positive and -negative fibers from the peripheral NT ganglion to ventral–caudal regions of the telencephalon, towards the hypothalamus (Stell, 1984; Wright and Demski, 1991; Lovejoy et al., 1992a; Oka and Matsushima, 1993; Forlano et al., 2000). There is no pituitary portal system in elasmobranchs (Dodd, 1983), and it is unknown where GnRH is released or how it reaches gonadotropes in the ventral lobe of the pituitary. It has been proposed that some of the NT fibers lead to the ventral telencephalon or septal area where they might release GnRH (Nozaki, 1985; Lovejoy et al., 1992a). Numerous GnRH-immunoreactive (ir) fibers also extend throughout the forebrain, suggesting a neuromodulatory action on other neural systems (Pfaff et al., 1987; Oka and Matsushima, 1993; Millar, 2003). In Atlantic stingrays, stimulation of the peripheral trunk of NT led to an increase in measurable levels of GnRH in the cerebrospinal fluid, presumably by synaptic release from NT fibers in the brain (Moeller and Meredith, 1998).

The NT has also been suspected of having a chemosensory component since it connects the nose and the brain, and fibers from the peripheral trunks extend into the nasal epithelium (Demski and Northcutt, 1983; Wirsig and Leonard, 1986a; Demski et al., 1987; Koza and Wirsig-Wiechmann, 2001). However, an intrinsic chemosensory function has not been demonstrated and NT fibers appear not to influence olfactory sensory signals directly, at least in elasmobranchs (Meredith and White, 1987). GnRH itself does modulate some olfactory responses in axolotls (Park and Eisthen, 2003) and mudpuppies (Zhang and Delay, 2007). NT is present in mammals, and in male golden hamsters, NT damage caudal to the olfactory bulb leads to some deficits in mating behavior (Wirsig and Leonard, 1987; Wirsig-Wiechmann, 1997). A response generally considered to be GnRH-dependent, the testosterone secretion induced by odors from female hamsters in estrus was not decreased by NT lesions (Wirsig-Wiechmann, 1993), although a NT-dependent pathway might have survived, via NT connections to the accessory olfactory bulb (Wirsig and Leonard, 1986a) and on to medial amygdala.

It is now understood that GnRH decapeptides are produced from three genes (in fish) and can be classified into three corresponding families (GnRH-I, -II, and -III) (Fernald and White, 1999; Millar et al., 2004). Differences in anatomical location suggest functional differences in the release and action of different GnRH isoforms (Phillips et al., 1987; Muske, 1993; Sherwood et al., 1993; Muske et al., 1994; Rissman et al., 1995; King and Miller, 1995; Latimer et al., 2000; Millar, 2003; Millar et al., 2004; Temple et al., 2003). The putative ancestral form, GnRH-II (His^{5}Trp^{7}Tyr^8-GnRH; formerly chicken-GnRH-II; Millar, 2003), generally expressed in a cluster of cells in the midbrain, is completely conserved across species. It can act as a neuromodulator (Troskie et al., 1998) and can facilitate reproductive behavior in birds (Maney et al., 1997) and food-restricted musk shrews (Temple et al., 2003). At least one additional isoform is expressed elsewhere in the brain (Millar et al., 2004), generally a GnRH-I or GnRH-III-family peptide responsible for LH release from the pituitary. GnRH-I peptides are quite variable in structure and are most visible in a separate population of cells along the NT–septum–preoptic axis (Jennes and Stumpf, 1986; Peter et al., 1987; Lehman et al., 1987; Silverman et al., 1987; Forlano et al., 2000; Dubois et al., 2002). The originally identified peptide, mammalian GnRH-I (mGnRH), is the releasing peptide in most mammals, amphibia, and probably in some teleost fish (Dubois et al., 2002). A variant GnRH-I (Gln^8-GnRH; formerly chicken-I GnRH) is the releasing peptide in birds and reptiles, and other variants have been identified in the preoptic area of several teleosts and one mammal (Grove-Strawser et al., 2002). Most teleosts have the (well-conserved) GnRH-III peptide (Trp^7-Leu^8-GnRH, originally named salmon GnRH) replacing GnRH-I as the LH-releasing peptide (Palevitch et al., 2009), but some also express additional isoforms (Pandolfi et al., 2005). Mammalian GnRH-I (mGnRH) can also act as a neuromodulator if applied exogenously (Pan et al., 1988) and has been suggested to mediate a neural (intracerebral) facilitation of reproductive behavior in male and female mammals (Dorsa and Smith, 1980; Moss and Dudley, 1989; Fernandez-Fewell and Meredith, 1995; Blake and Meredith, 2010) independently of its LH-releasing function.

Elasmobranchs, which lack a developed pituitary portal system, express GnRH-II in the midbrain, and often two or more other isoforms, concentrated more in neurons of the NT nerve and ganglia than in the preoptic area, and generally considered to be involved in gonadotropin release.

Within the NT, a close relationship has been shown in elasmobranchs and some other species, between cells expressing GnRH and fibers immunoreactive to antisera raised against RF-amide (RFa) peptides, e.g., FMRF-amide (Phe-Met-Arg-Phe-NH_2; Stell, 1984; Muske and Moore, 1988; Wirsig-Wiechmann and Basinger, 1988; Chiba, 2000) or LPLRF-amide (Leu-Pro-Leu-Arg-Phe-NH_2; White and Meredith, 1995).

A similar close relationship has recently been demonstrated in the ventral forebrain of birds and mammals (see Tsutsui et al., 2010; Johnson et al., 2007; Simonneaux et al., 2009). The RF-amide family peptides, including kisspeptin, gonadotropin inhibitory peptide (GnIH) and RFRP1/3, are critically involved in regulating release of GnRH acutely and according to season in birds, mammals, and reptiles and probably in teleosts (Johnson et al., 2007; Kriegsfeld et al., 2006; Simonneaux et al., 2009; Clarke et al., 2009; Oka, 2009). In elasmobranchs, the function of the RFa-ir cell groups in NT is unknown, but they are placed in an ideal location to influence GnRH release from the GnRH system(s) in NT.

In bonnethead sharks (*Sphyrna tiburo*), one or more ganglia are situated along the exposed length of the NT nerve between the olfactory bulbs and the nerve's entry into the forebrain. Cells within the large main ganglion can be classified into two types. One cell class is GnRH-ir as described above and is co-localized with a plexus of catecholamine-ir fibers. A second, distinct class is immunoreactive to antibodies raised against RFa peptides (including LPRFa). Both were acetylcholinesterase-positive (White and Meredith, 1995).

Here we report on two classes of GnRH-ir cell profiles in the NT ganglion in addition to the RF-amide-ir cells. They express different isoforms of GnRH, and one (only) shows co-localization of choline acetyltransferase (ChAT) immunoreactivity. Their anatomical and cytochemical differences suggest potential differences in function. Early parts of this study were published in abstract form (Moeller et al., 1997).