REVIEW

Function of gonadotropin-releasing hormone in olfaction

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Abstract. Gonadotropin-releasing hormone (GnRH) is present within neurons of the *nervus terminalis*, the zeroeth cranial nerve. In all vertebrate species, except in sharks where it is a separate nerve, the *nervus terminalis* consists of a chain of neurons embedded within olfactory or vomeronasal nerves in the nasal cavity. The function of the GnRH component of the *nervus terminalis* is thought to be neuromodulatory. Our research on GnRH effects on olfaction confirms this hypothesis. The processes of GnRH neural cell bodies located within chemosensory nerves project centrally into the ventral forebrain and peripherally into the lamina propria of the nasal chemosensory mucosa. GnRH receptors are expressed by chemosensory neurons as shown by RT-PCR/Southern blotting and GnRH agonist binding studies. Patch-clamp studies have shown that GnRH alters the responses of isolated chemosensory neurons to natural or electrophysiological stimulation through the modulation of voltage-gated and receptor-gated channels. Behavioral experiments demonstrate that interfering with the nasal GnRH system leads to deficits in mating behavior. These studies suggest that the function of the intranasal GnRH system is to modify olfactory information, perhaps at reproductively auspicious times. We speculate that the purpose of this altered olfactory sense is to make pheromones more detectable and salient. (Keio J Med 50 (2): 81–85, June 2001)

Key words: *Nervus terminalis*, reproduction, chemosensory systems, neuromodulation

Introduction

GnRH as a coordinating system

The gonadotropin-releasing hormone (GnRH) system is critical for normal reproductive behavior and physiology. In addition to its actions in the brain and pituitary, GnRH influences a number of organ systems such as the breast and ovary. GnRH is also present in the *nervus terminalis*, a neural plexus in the chemosensory mucosa of the nasal cavity. Here it has the potential for influencing the detection of pheromones, those species-specific odors involved with sexual identification and arousal. Thus, the hormone GnRH appears to be in a position to serve as a coordinating system for the multitude of events occurring during reproduction, including changes in olfactory sensitivity to pheromones.

Chemosensory System Involvement in Reproduction

In vertebrates, two nasal chemosensory systems, the olfactory and the vomeronasal systems, play important roles in reproductive physiology and behavior. The peripheral olfactory system is composed of olfactory receptor neurons located in the olfactory epithelium. These neurons send axons to the anterior (main) portion of the olfactory bulb. The vomeronasal system is composed of vomeronasal receptor neurons (housed in a separated portion of the nasal cavity) that send axons to the posterior portion of the olfactory bulb (the accessory olfactory bulb). There has been some controversy as to whether a vomeronasal organ containing a sensory epithelium exists in humans.[1,2]

In mammals, the olfactory system is thought to function in the perception and identification of volatile odors, including volatile pheromones. The vomeronasal system is thought to transmit chemical information, in the form of heavier molecules such as proteins that can also include pheromonal compounds that regulate sex-

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ual development, neuroendocrine responses to pheromones, and sexual attraction and arousal.[3,4] There is evidence that both of these systems may play important roles in reproductive processes in a number of species, including humans.[5–7] The potential role of a human vomeronasal organ (if present) in the detection of and responses to pheromones as well as the utilization of the olfactory system in pheromonal detection has received much attention recently, and is becoming the subject of intense scientific inquiry.

In humans, the olfactory system also appears to be important in detecting pheromones and may be become more sensitive to pheromones at reproductively appropriate times. For example, odors are perceived differently throughout the menstrual cycle in women.[5,6,8] Certain male odors are judged more pleasant by women during ovulation[9] and cause sexual arousal.[10] In addition, male odors have been shown to increase male sociosexual behavior.[11] This interdependence between hormones and olfaction may involve the GnRH system in the nasal cavity.

An understanding of the functional interactions that occur between these nasal chemosensory systems and the GnRH system is central to our basic understanding of the sensory control of reproduction. Neurons containing GnRH are located along the *nervus terminalis* within the brain and in the nasal cavity. Those in the nasal cavity appear to be activated during reproductively appropriate times[12] and could modulate chemosensory reception of pheromones and other related odors. The significance of pheromonal communication in humans has been previously underestimated but is presently considered an important factor in mate selection, maternal behavior, and sexual arousal. Because GnRH levels are altered by pheromones, it is reasonable to suggest that the GnRH system provides feedback information to the chemosensory systems. With knowledge of how this occurs we can develop potentially useful interventions for problems with infertility, maternal bonding, and sexual arousal. With sexual arousal problems being a very common medical complaint among men[13] and woman,[14] further investigation into sensory involvement in sexual arousal is an area that needs exploring.

The Nervus Terminalis – the GnRH System of the Nasal Cavity

Anatomy

The *nervus terminalis* is a diffusely organized GnRH-containing ganglionated nerve associated with the nasal chemosensory systems.[15–17] It extends from structures in the nasal cavity to brain regions that mediate chemosensory processing and reproduction. It is composed of a chain of GnRH-containing neuronal cell bodies comingled with processes that extend peripherally and centrally. These GnRH-containing neurons are generally embedded in other nerve fascicles in the nasal cavity. In rodents, the majority of intranasal GnRH processes are located in the vomeronasal nerves (Fig. 1), whereas in other species such as the salamander the majority of processes extend along olfactory nerve fascicles to the periphery (Fig. 2).

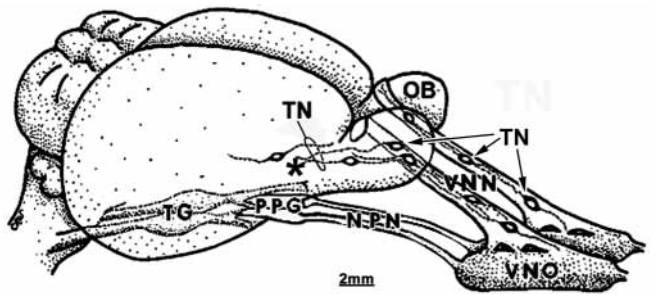

Fig. 1 Diagram of rodent brain and vomeronasal system. The gonadotropin releasing hormone (GnRH)-containing neurons of the *nervus terminalis* (TN) are shown as cell bodies with varicose fibers within vomeronasal nerves (VNN) and brain. These neurons travel along the medial surface of the olfactory bulbs (OB) before entering the brain (brain entry indicated by the asterisk). In the periphery the fibers travel as far as the lamina propria of the mucosa in the vomeronasal organ (VNO). GnRH neurons (not shown in diagram) are also found in the pterygopalatine ganglion (PPG) and nasopalatine nerves (NPN) that innervate the vascular supply and glands of the vomeronasal organ.[31] TG, trigeminal ganglion.

The *nervus terminalis* was discovered more than 100 years ago in sharks in which it is a separate nerve.[18] Interest in the *nervus terminalis* showed a resurgence in the past two decades with the discovery that some of the its neurons contain GnRH.[17] Besides being responsible for gonadotropin release from the pituitary gland when secreted into the portal circulation, GnRH also facilitates sexual behaviors when GnRH receptors are stimulated in specific forebrain and midbrain areas.[19–21] Thus, GnRH acts as a neurohormone or neuromodulator depending on the site of release and/or reception.

Development

Several studies have demonstrated that GnRH-immunoreactive neurons destined for the preoptic area migrate into the brain from the olfactory placode region during development.[22,23] In addition, GnRH-containing *nervus terminalis* neurons migrate along the same path but remain in the nasal cavity. A recent study by Whitlock and her colleagues[24] has shown that GnRH-expressing neurons of the *nervus terminalis* arise from progenitor cells of neural crest origin. Those GnRH neurons that come to reside within the brain

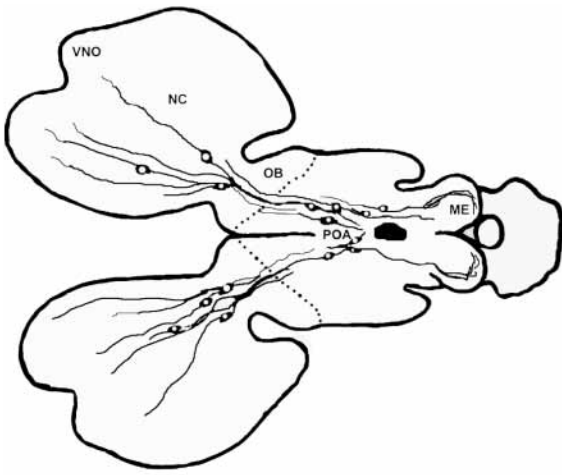

Fig. 2 Diagram of ventral view of tiger salamander brain and nasal cavities (NC). The distribution of GnRH-containing *nervus terminalis* (TN) neurons is represented by cell bodies and fibers extending between the nasal cavity and preoptic area (POA). Within the nasal cavity TN neurons are located within olfactory nerve fascicles and project into the lamina propria of the olfactory mucosa (See Fig. 3). ME, median eminence; OB, olfactory bulb; VNO, vomeronasal organ.

arise from more rostral neural plate regions adjacent to progenitor cells of the anterior pituitary and migrate into the forebrain along the path of the *nervus terminalis*. Some GnRH neurons of the *nervus terminalis* migrate into the ventral forebrain but many remain in the nasal cavity. Except for serving as a migratory path for intracerebral GnRH neurons, the exact functional nature of the *nervus terminalis* neurons in the nasal cavity and ventral forebrain has been difficult to ascertain. However, several investigators[16,25] have hypothesized that the *nervus terminalis* is a functioning neuromodulatory system which can alter sensory processing or behavior. Our patch clamp data on the effects of GnRH on olfactory neurons[26] support this hypothesis and suggest the *nervus terminalis* is a very active and important neuromodulatory component of the nasal chemosensory systems.

Behavioral evidence for nervus terminalis involvement in reproduction

Two studies have demonstrated that lesions of the *nervus terminalis* alter reproductive behavior in mammals and in fish. In hamsters, lesions of the *nervus terminalis* produce deficits in male mating behavior.[27] In fish, lesions of the *nervus terminalis* have been shown to produce deficits in male nest building behavior.[28] While the mechanisms for these behaviors in these two widely separated species are undoubtedly different, the main feature of the effect of the *nervus terminalis* lesions may be to alter pheromone perception or pheromonal influences on the animals' physiology.

Evidence for paracrine secretion of GnRH into the chemosensory mucosa

The *nervus terminalis* is anatomically and developmentally associated with the chemosensory systems.[17,22,23] However, there is no data to indicate a general sensory or chemosensory function. Anatomical[29,30] and neurophysiological[26] studies have suggested a neuromodulatory function of the GnRH component of the *nervus terminalis* in the chemosensory mucosa.

Gonadotropin-releasing hormone-immunoreactive *nervus terminalis* neurons send processes centrally to olfactory and ventral forebrain areas. They also project peripherally to a number of structures related to the nasal cavity including olfactory and vomeronasal mucosa, Bowman's glands, smooth muscle and autonomic ganglia in amphibians[29,30] and mammals.[31] Gonadotropin-releasing hormone-immunoreactive processes project to the region of the vomeronasal organ in humans (unpublished observations). These peripheral GnRH processes do not appear to contact specific cells, rather the processes end within the lamina propria of chemosensory mucosa. Frequently these processes have appeared to contact Bowman's glands in the very rostral regions of the olfactory mucosa in salamanders.[30] Like the GnRH processes in the median eminence, the *nervus terminalis* fibers are frequently varicose, suggesting that GnRH is secreted along the length of the process. However, Oka and Ichikawa[32] (also see Oka and Abe[33] for a review) have provided ultrastructural evidence that GnRH may also be secreted from the membrane of the neural cell body. GnRH may thus be acting as a paracrine hormone/modulator in the nasal cavity.

GnRH Modulation of Chemoreception

GnRH receptors in the nasal cavity

If GnRH modulates chemoreception at the level of the chemoreceptive sensory neurons, it follows that GnRH receptors should be expressed in peripheral nasal tissue. We have previously demonstrated GnRH agonist binding in the olfactory mucosa of tiger salamanders,[34] and have also seen similar binding in the vomeronasal mucosa of prairie voles (unpublished observations). In the salamander, binding was heaviest over the region of olfactory receptor dendrites suggesting that GnRH may act at the level of sensory transduction. In addition to the binding study, we have demonstrated the presence of GnRH receptor mRNA in olfactory and vomeronasal mucosal tissue of the rat using RT-PCR/Southern blotting and DNA sequencing.[35]

Electrophysiological evidence for GnRH modulation of chemosensory systems

The presence of GnRH in *nervus terminalis* neurons and the expression of the GnRH receptor in olfactory mucosa suggest that GnRH modulates the activity of cells related to chemoreception. In support of this hypothesis, we have shown using patch clamp recording techniques that GnRH increases sodium conductances in mudpuppy olfactory neurons.[26] We hypothesized that this may make these neurons more excitable to odor stimulation. We have found in recent patch clamp studies on mouse and rat olfactory or vomeronasal receptor neurons that GnRH can both potentiate or inhibit odor-induced depolarization and hyperpolarization of these neurons.[35]

Human experiments have demonstrated an increased sensitivity to odorants at mid-cycle (i.e. at ovulation[5,6]). This would be the time when GnRH is being released to cause the luteinizing hormone surge. It would be of interest to determine whether GnRH is responsible for these changes in chemoreception in humans.

Conclusion

The GnRH-containing neurons of the *nervus terminalis* have the potential to alter olfaction. The *nervus terminalis* may secrete GnRH into the chemosensory mucosa to control the detection of various odors that are relevant to the species for reproductive purposes. The neurons that release GnRH from the *nervus terminalis* show regular discharges which are produced by endogenous pacemaker potentials in GnRH neurons.[36] The activity of these GnRH neurons can be modified by extrinsic factors such as GnRH from neighboring neurons.[37] It is very likely that other neural inputs from central[38] or peripheral sources (e.g. trigeminal fibers) may also modulate the activity of these neurons (Fig. 3). We hypothesize that the neural activity of and GnRH synthesis by the *nervus terminalis* GnRH neurons is altered by reproductive stimuli and hormones. This would affect the sensitivity of primary chemosensory neurons to pheromones as well as to modulate other areas of the brain involved with sensory detection of reproductively relevant stimuli or behavior. In support of this hypothesis, Propper and Moore[12] have provided evidence for alterations in GnRH activity in the *nervus terminalis* during mating.

There have been several reports of neurons of central origin innervating the ganglion cells of the *nervus terminalis*. In goldfish, neurons in or near the locus coeruleus projecting to *nervus terminalis* have been reported.[39,40] A recent study by Yamamoto and Ito[38] in two teleost species elegantly demonstrated substantial innervation of *nervus terminalis* by multiple central brain areas including mesencephalic tegmentum (nucleus tegmento-olfactorius) and telencephalic areas involved in olfactory processing (e.g. olfactory bulb and area dorsalis telencephali pars posterior).

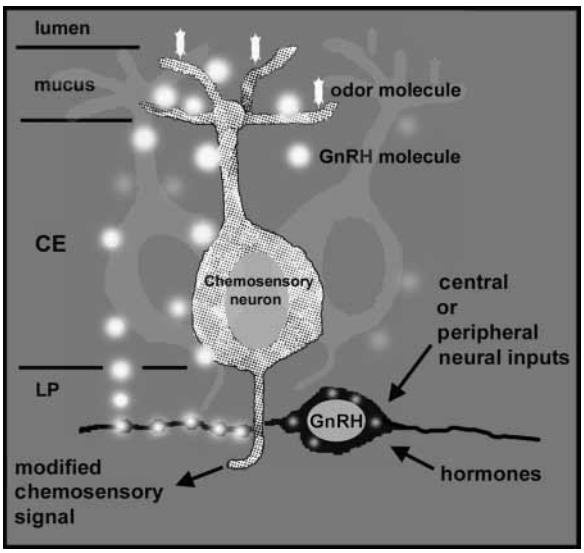

Fig. 3 Hypothetical model of *nervus terminalis* GnRH neuron inputs and outputs. This model illustrates our view of the control of GnRH secretion into the chemosensory mucosa and its modulation of odor responses by chemosensory neurons: Neural and hormonal inputs control the amount of GnRH available in and secreted by GnRH neurons. The level of GnRH secretion influences the response of chemosensory neurons to odor molecules that access the olfactory cilia (the location of olfactory transduction) via the mucous layer covering the chemosensory epithelium. *Nervus terminalis* GnRH fibers are located in the lamina propria (LP) so that GnRH must diffuse into chemosensory epithelium (CE) to act on GnRH receptors located in the membrane of chemosensory neurons. Stimulation of GnRH receptors on chemosensory neurons activates second messenger pathways that in turn alter the odor-induced membrane potential changes, causing modified chemosensory signals to be sent to the brain.

The *nervus terminalis* appears to be a consistent feature of the jawed vertebrate lineage, since it does not seem to be present in lampreys[41] or hagfish.[42] The presence of the *nervus terminalis* in cetaceans[43] that completely lack a nasal chemosensory system may reflect an evolutionary change in *nervus terminalis* function. On the other hand, its presence in cetaceans may indicate that the *nervus terminalis* has more than one function in the nasal cavity or that it may modulate a number of different brain areas. Perhaps, also it can serve as a secondary source of GnRH for endocrine control of pituitary hormone release.

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