Chem. Senses 27: 569–572, 2002

Molecular Mechanisms for Migration of Placodally Derived GnRH Neurons

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Abstract

Gonadotropin-releasing hormone (GnRH) neurons, critical for reproduction, are derived from the nasal placode and migrate into the brain along nasal axons. GnRH neurons appear to diverge from olfactory sensory cells during early stages of nasal placode differentiation**.** However, GnRH neurons rely on olfactory/vomeronasal axons as their pathway to the central nervous system (CNS). A novel factor, termed nasal embryonic luteinizing hormone-releasing hormone factor (NELF), was discovered in a differential screen of migrating versus nonmigrating GnRH neurons. NELF is expressed in olfactory sensory cells and GnRH cells in nasal areas. Antisense experiments demonstrated that knock-down of NELF decreased olfactory axon outgrowth and GnRH neuronal migration. These results indicate that NELF plays a role as a guidance molecule for olfactory axon projections and migration of GnRH cells. We hypothesize that NELF acts via a homophilic interaction and that NELF expression is critical for reproduction by insuring that GnRH cells reach the CNS. Furthermore, down-regulation of NELF on GnRH cells as they enter the telencephalon may allow GnRH cells to distinguish a different pathway(s) in the CNS (from those leading to olfactory regions) and thereby facilitate establishment of the appropriate adult-like GnRH distribution.

Introduction

The neuroendocrine gonadotropin-releasing hormone (GnRH) system is essential for vertebrate reproduction [reviewed in (Fink, 1988)]. These cells express the 'mammalian form' of GnRH transcript (Kasten *et al*., 1996). In mammals, these GnRH cells are distributed in a continuum from the olfactory bulbs to the hypothalamus (Figure 1A). Independent of location, the majority of GnRH cells send their axons to the median eminence, where they access the pituitary portal capillary system. Release of GnRH at this site is pulsatile, and affects secretion of gonadotropins from cells in the anterior pituitary and consequently gonadal function [reviewed in (Fink, 1988)].

Although located within the forebrain postnally, GnRH cells originate in the nasal region and thereafter migrate into the forebrain (Figure 1B). Developmental mapping studies led to the hypotheses that (i) GnRH cells originate in the nasal placode and (ii) GnRH cells migrate on olfactory/vomeronasal axons, i.e. are neurophilic in nature [reviewed in (Wray, 2001)]. Olfactory placode ablation and transplantation studies, as well as chick/quail chimeras, dye-labeling studies and *in vitro* models, support the nasal origin for cells expressing the mammalian GnRH transcript [reviewed in (Wray, 2001)]. The relationship between the development of the GnRH system and olfactory systems is illustrated by Kallmann syndrome, a mutation resulting in anosmia and hypogonadism in humans. Examination of tissue from a Kallmann fetus revealed olfactory axons and GnRH cells on the nasal side of the cribriform plate, not within the forebrain (Schwanzel-Fukuda *et al*., 1989). Taken together, these data indicate that the cells that arise in nasal regions and express the mammalian GnRH transcript are, in fact, the major neuroendocrine component of the hypothalamo-pituitary-gonadal axis.

Lineage of neuroendocrine GnRH neurons

The nasal placodes are ectodermal thickenings that give rise to nonsensory respiratory and sensory olfactory epithelia. The olfactory epithelium produces the main olfactory as well as vomeronasal organ epithelia (Halpern, 1987; Farbman, 1992). In many species, GnRH-expressing cells are first detected in the vomeronasal organ epithelia. This observation, together with ablation/transplantation studies, led to the hypothesis that GnRH cells originate in an area of the placode associated with olfactory epithelial-derived structures. However, in chicks, where presumptive olfactory and respiratory areas can be divided prior to placode formation, ablation experiments suggest that GnRH progenitors are more closely associated with respiratory rather than olfactory regions (El Amrauoui and Dubois, 1993), and in fact, GnRH cells have been detected in the respiratory epithelium of normal chicks (Hilal *et al*., 1996).

GnRH cells have also been detected in the respiratory

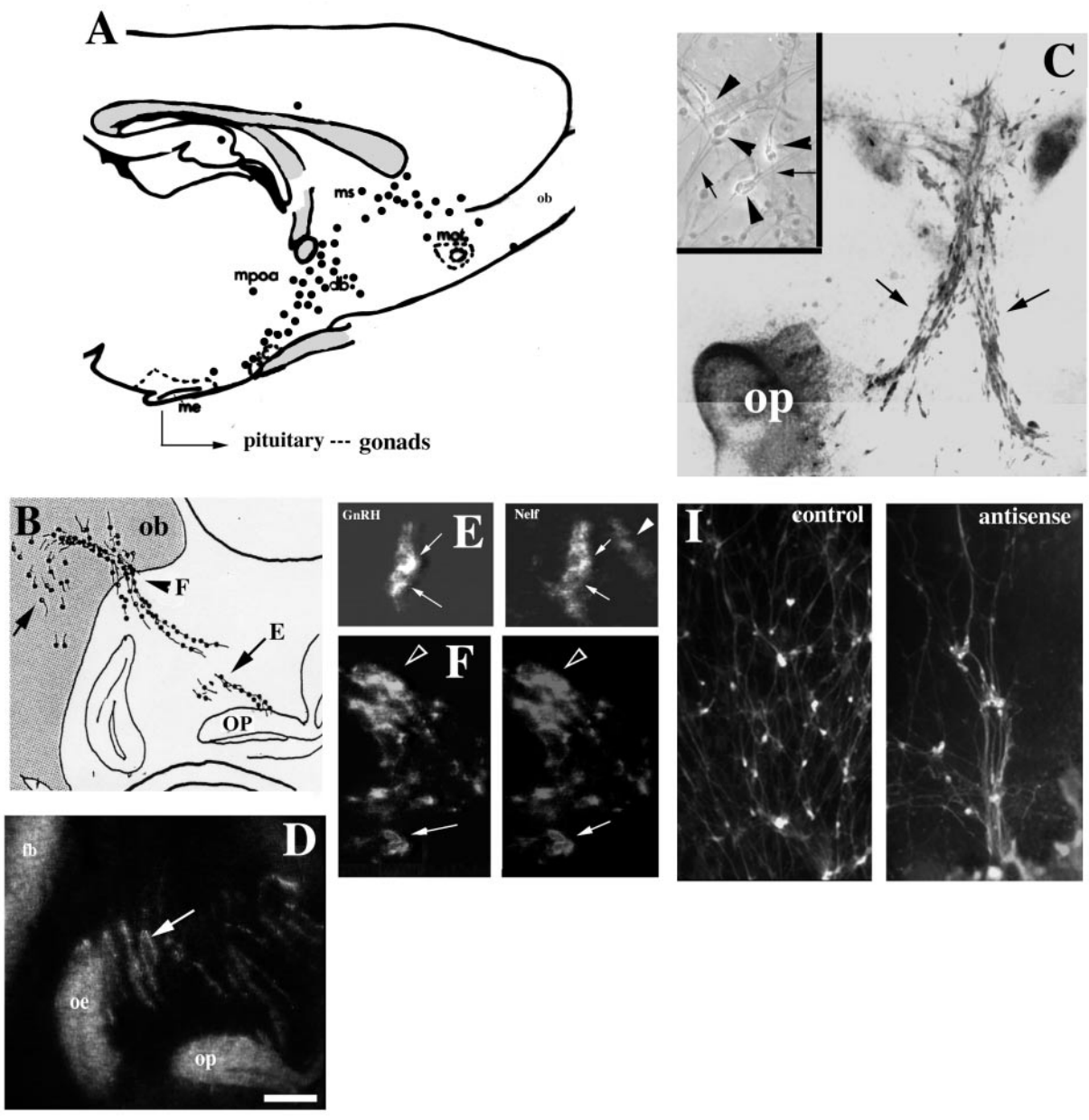

Figure 1. Placodally derived cells of the GnRH neuroendocrine system. **(A)** Parasagittal adult rodent brain section indicating location of GnRH cells (black dots) within the forebrain. The main terminal target of these cells is the median eminence (me), where GnRH via the portal capillary system alters pituitary and subsequently gonadal function. **(B)** Nasal/forebrain region of a mouse embryo depicting migration of GnRH cells (black dots, arrows) from the olfactory pit (op) into the brain. **(C)** Nasal explant maintained for 7 days and immunocytochemically stained for GnRH. On average, 200 GnRH cells (arrows, black cells) are maintained in these explants, ~25% of the GnRH population *in vivo*. Inset: explant observed using phase microscopy. Phase bright GnRH cells (arrowheads) are seen apposed to olfactory axons (arrows). **(D–F)** NELF expression in nasal regions. Areas in these panels are shown in (B). (D) Immunocytochemically stained section indicates NELF expression in olfactory epithelia (oe) and olfactory/vomeronasal axons (arrows). (E and F) Sections immunofluorescently labeled for GnRH (left) and NELF (right). Cells exiting the olfactory pit (E) are positive for both GnRH and NELF (arrows). Olfactory axons in this region were also positive for NELF (arrowhead). (F) GnRH cells co-expressed NELF at the nasal/forebrain junction. **(G)** Knock-down of NELF alters olfactory axon outgrowth and GnRH migration (for location see C). Compare explants with antisense NELF probe to control. ob = olfactory bulb, mot = medial olfactory tract, ms = medial septum, db = diagonal band of Broca, mpoa = medial preoptic area, me = median eminence.

epithelium of mice possessing a mutation in the developmental transcription factor activator protein 2α (Kramer *et al.*, 2000). In this same study, prior to GnRH expression in the nasal placode, an area was identified between respiratory cells and sensory cells. It was speculated that this intermediate region might be the location of GnRH progenitor cells. In addition, GnRH neurons have been screened for olfactory epithelial lineage markers (Kramer and Wray, 2000a). Olfactory epithelial markers were not detected in GnRH cells, while nestin, a marker of central nervous

system (CNS) and neural crest progenitor cells, was. Thus, several studies now argue that olfactory epithelial progenitors are distinct from GnRH progenitors and that GnRH neurons diverge from olfactory sensory cells during early stages of nasal placode differentiation.

Migration of GnRH cells

The exact axonal pathway used by GnRH cells as they migrate across the nasal region is unclear. A variety of molecules expressed on olfactory/vomeronasal axons mark this pathway, but to date none exclusively highlights the route along which GnRH cells move. Nasal explants have been established (Figure 1C) in which GnRH neurons migrate in a manner similar to GnRH cells *in vivo*; for example, they show directed outgrowth of olfactory axons, differentiation of GnRH neurons, migration of GnRH neurons in association with olfactory derived axons, and directed movement of GnRH cells (Fueshko and Wray, 1994). The association of GnRH cells with axons in these explants, and directed movement of GnRH cells, supported cell-adhesion molecules as key molecular mechanisms of GnRH cell migration in nasal regions.

In nasal explants, single GnRH cells can be identified *in situ* (Figure 1C). To determine molecules directly involved in GnRH migration, we used this system to obtained RNA from single GnRH cells (Kramer and Wray, 2000b). A differential screen comparing a migrating GnRH cell and non-migrating GnRH cells was performed. A novel protein, termed nasal embryonic luteinizing hormone-releasing hormone factor (NELF), was identified. NELF is expressed in peripheral nervous system and CNS tissues, including olfactory sensory cells and GnRH cells during embryonic development (Figure 1D–F). As *in vivo*, NELF is expressed on olfactory axons and GnRH cells in nasal explants (Kramer and Wray 2000b). Transfection experiments were performed in nasal explants using NELF antisense probes. Knock-down of NELF decreased olfactory axon outgrowth and GnRH cell migration into the periphery of the explant (Figure 1G). These experiments indicate that NELF plays a role as a common guidance mechanism for olfactory axon projections and subsequently, either directly or indirectly, in the neurophilic migration of GnRH cells across nasal regions.

NELF is not expressed on postnatal GnRH cells (Kramer and Wray, 2000b). Interestingly, NELF is down-regulated on GnRH neurons which enter the forebrain. In particular, NELF is turned off on GnRH cells that migrate toward the hypothalamus, but remains, albeit at low expression levels, on GnRH cells that migrate toward the developing olfactory bulb (Kramer and Wray, 2000b). This raises the possibility that molecules such as NELF, by turning off expression, play an important role in establishing the appropriate GnRH adult-like distribution by insuring that GnRH cells do not end up in forebrain olfactory regions.

NELF is not restricted in its expression pattern to the olfactory and GnRH systems. During development it is robustly expressed in the cortex, hippocampus and thalamus (Kramer and Wray, 2001), and expression is maintained in the brain postnatally in these same regions, as well as in the olfactory bulb. Developmentally, many of the regions that express NELF exhibit active neuronal migration. Thus, although the role of NELF in these areas awaits further investigations, its presence in these structures is consistent with it acting as a migratory signal. The role of NELF postnatally is presently unknown.

Conclusion

It is clear that the development of the olfactory system and GnRH neuroendocrine system are intimately entwined, and probably utilize both cell surface recognition molecules and chemoattractant/repellent molecules. Chemoactive molecules with short diffusion properties are likely involved in directing olfactory axon outgrowth while cell adhesion molecules are more likely responsible for directing GnRH cell movement. NELF is a novel molecule, expressed on both nasal GnRH cells and olfactory axons. Further work is needed to understand the role of NELF in the development of the GnRH neuronal system as well as the olfactory system, but perturbation of this molecule alters olfactory axon outgrowth as well as GnRH cell migration.

Acknowledgements

The author would like to thank Dr P. Kramer for his contributions to the work summarized here.

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Accepted May 1, 2002