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New insights into the neuroanatomical distribution and phylogeny of opioids and POMC-derived peptides in fish

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ABSTRACT

This review re-evaluates the use of immunological probes to map enkephalinergic, dynorphinergic, and endorphinergic circuits in the CNS of lobe-finned fishes, ray-finned fishes, and cartilaginous fishes in light of the characterization of proenkephalin, prodynorphin, and POMC sequences from representatives of these groups of fish over the past 20 years. The use of α-MSH specific antisera is a reliable method for detecting POMC immunopositive cell bodies and fibers. Since α-MSH and β-endorphin are co-localized in the same neurons, these studies also reveal the distribution of endorphinergic networks. Met-enkephalin specific antisera can be used to detect enkephalinergic circuits in the CNS of gnathostomes because of the ubiquitous presence of this pentapeptide in the proenkephalin sequences of gnathostomes. However, the use of leu-enkephalin specific antisera to detect enkephalinergic networks is more problematic. While this immunological probe is appropriate for analyzing enkephalinergic networks in mammals and perhaps teleosts, for the lungfishes and cartilaginous fishes this probe is more likely able to detect dynorphinergic circuits. In this regard, there is a need to re-examine dynorphinergic networks in non-mammalian gnathostomes by using species specific antisera directed against dynorphin end-products.

1. Introduction

The seminal discovery of the opiate-like peptides (opioids), met-enkephalin and leu-enkephalin, in 1975 [20] quickly led to the realization that neurons in the central nervous system of mammals are capable of synthesizing several types of opioids which are collectively referred to as the enkephalins, the dynorphins, and the endorphins [14]. Not long after the discovery of these various opioids it became clear that in the case of mammals enkephalins, dynorphins, and endorphins are encoded on distinct genes which constitute the opioid precursor-coding gene family [14]. For mammals, proenkephalin is the common precursor for met-enkephalin, leu-enkephalin, and two C-terminally extended forms of met-enkephalin [39]. Prodynorphin is the common precursor for three C-terminally extended forms of leu-enkephalin: dynorphin A, dynorphin B, and α-neo-endorphin [21]. Finally, proopiomelanocortin (POMC) is the common precursor for the opioid, β-endorphin, and the melanocortin-related peptides, ACTH, α-MSH, β-MSH, and γ-MSH [35]. About a decade later the discovery of the peptide nociceptin (orphanin) and the characterization of the precursor pronociceptin (proorphanin) [33,34,49] revealed that these four neuropeptide-coding precursors were members of the opioid/orphanin gene family (for review see: [10]). This review will focus on the anatomical distribution of enkephalin, β-endorphin, and melanocortin immunoreactivity in the CNS of representatives from the major classes of fishes. In some case inferences will be made on the presence of dynorphinergic circuits in these organisms.

The discovery of these various opioid-related peptides quickly led to the development of immunological probes to map the distribution of enkephalinergic, dynorphinergic, and endorphinergic circuits in the CNS of mammals. These studies were reviewed by Khachaturian et al. [22] and the following generalizations emerged for mammalian opioid networks: (a) a given neuron in the CNS of mammals will only express a single opioid precursor-coding gene; (b) proenkephalin cell bodies are the most numerous in the CNS of mammals and are found in several nuclei in the telencephalon, diencephalon, midbrain and brain stem; (c) the distribution of prodynorphin and proenkephalin circuits may overlap, although for mammals the enkephalinergic circuits appear more numerous than the dynorphinergic circuits; and (d) the POMC network has the most restricted distribution and is limited to two cell body groups, one in the hypothalamus and another in the brain stem. Since an individual mammalian neuron will only express one opioid gene, met-enkephalin and leu-enkephalin will be present in the same secretory vesicles. Similarly, prodynorphin cell bodies will package dynorphin A, dynorphin B, and α-neo-endorphin (collectively referred to as the dynorphins) in the same secretory vesicles,

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and POMC neurons package β-endorphin and the melanocortins in the same secretory vesicles. This segregation of the three types of opioids in the mammalian CNS is consistent with the ligand selectivity of the opioid receptor gene family [25]. Initially it was assumed that the opioid receptor gene family had three members, the mu receptor gene, the delta receptor gene, and the kappa receptor gene [25]. However, with the discovery of nociceptin/orphanin and its receptor, the ORL receptor [49] it is now clear that the opioid/ORL receptor-coding gene family also has four members [10,56].

With respect to the opioid receptors (i.e., mu, delta, and kappa), met-enkephalin and leu-enkephalin are the preferred ligands for the delta opioid receptor, whereas, the prodynorphin opioids are the preferred ligand for the kappa opioid receptor, and β-endorphin is the preferred ligand for the mu opioid receptor [25]. However, it is appreciated that all of the mammalian opioid ligands can in fact bind to all of the mammalian opioid receptors with different affinity [25]. Furthermore, Mansour et al. [30] observed that there was not always a best fit match between a type of opioid ligand and the corresponding opioid receptor at some opioid terminal fields in the CNS of mammals. However, co-localization of met-enkephalin and leu-enkephalin in the same neurons, coupled with the localization of the dynorphins and β-endorphin in distinct neurons are common features of opioid networks in the CNS of mammals.

While the co-evolution of opioid peptide-coding genes and opioid receptor-coding genes has been explored in other reviews [31,56,57], this review addresses the question of whether the anatomical distribution of opioid peptide-secreting neurons in the mammalian CNS [22] represents the ancestral condition for the evolution of opioid networks. Alternatively, the organization of the mammalian opioid networks may be a more derived condition unique to mammals, or perhaps to the amniotes, or even to all of the sarcopterygian vertebrates. One approach to address this question would be to look at the anatomical distribution of met-enkephalin, leu-enkephalin, and β-endorphin in representative cartilaginous fishes, ray-finned fishes, and lobe-finned fishes. Based on the mammalian opioid networks, β-endorphin would be present in POMC neurons, and met-enkephalin and leu-enkephalin should be co-localized in the same neurons. However, as subsequent immunocytochemical analyses over the past thirteen years have revealed, the actual distribution of these opioids is more complex than originally anticipated.

Based on studies on mammals, opioid peptides, functioning as inhibitory neurotransmitters, modulate a number of circuits within the central nervous that are involved in pain regulation, fine motor control, reproduction, and cardiovascular functions [25]. It is beyond the scope of this review to include an overview of these physiological actions in this phylogenetic study. However, once the neuroanatomical circuits have been clearly delineated in these various groups of fishes, it should be possible to use a phylogenetic approach to understand the functional evolution of opioid and melanocortin circuits.

2. Neuroanatomical distribution of met-enkephalin and leu-enkephalin

2.1. Cartilaginous fishes

In the cartilaginous fishes (Fig 1), the neuroanatomical distribution of enkephalins has been studied in several species including the following elasmobranches: the horn shark *Heterodontus francisci* [60,62,63], the spiny dogfish *Squalus acanthias* [40,41,59,62,63], the small spotted dogfish *Scyliorhinus canicula* [70], the skates *Raja binoculata* [61,62] *Raja rhina*, the bat ray *Myliobatis californica* [61],

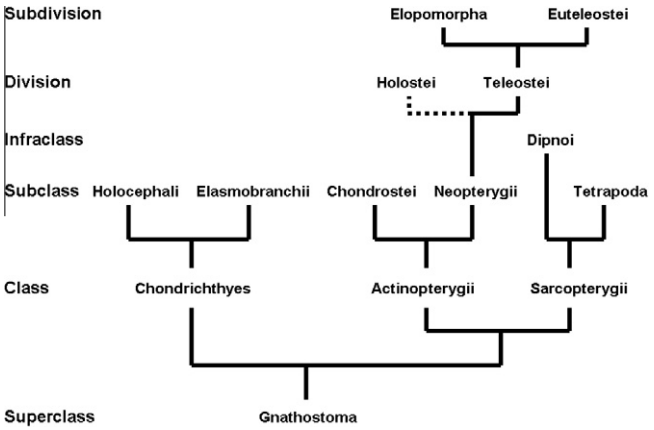

Fig. 1. Phylogeny of the Gnathostome. Fishes The hierarchy of the gnathostome fishes presented in this review is presented in this figure modified from Nelson [38]. The chondrichthian fishes commented on in this review were either from subclass Holocephali or subclass Elasmobranchii. The actinopterygian fishes were from subclass Chondrostei and subclass Neopterygii. Given the diversity within division Teleostei (subclass Neopterygii) it seemed appropriate to distinguish whether a species was in subdivision Elopmorpha (*Anguilla anguilla*) or subdivision Euteleostei (all other teleost fishes cited in this review). "Holostei" is listed as a division in the subclass Neopterygii that consists of two distantly related extant orders: Semionotiformes (the gars) and Amiiformes (*Amia calva*).

and the holocephalian ratfish *Hydrolagus colliei* [58,62,63]. Most of these studies used leu-enkephalin-specific antisera to identify cell groups homologous to brain regions in other vertebrates. In these studies both leu-enkephalin-immunoreactive cells and fibers have been localized in all the brain subdivisions, with some variations depending on the species. The distribution of both met-enkephalin and leu-enkephalin immunoreactive cell bodies and fibers in the whole brain of a cartilaginous fish was reported by Vallarino et al. [70].

In the telencephalon, leu-enkephalin-immunoreactive neurons are widely distributed in both the pallium and the subpallium. In the spiny dogfish *S. acanthias*, leu-enkephalin-positive elements are distributed in several regions: the granular cells of the olfactory bulbs, the pars superficialis of the dorsal pallium, the medial pallium, the septal nuclei, the periventricularis ventrolateralis area, the entopeduncular nucleus, the interstitial nucleus, the nucleus Q, the suprapeduncular nucleus and the nucleus M [40]. The finding of leu-enkephalin-immunoreactive neurons in the dorsal and medial pallium, and in the suprapeduncular nucleus has been confirmed in the brain of the small spotted dogfish *S. canicula* [70].

In the diencephalon, leu-enkephalin positive elements are strictly restricted to the hypothalamus [58–61,70]. Leu-enkephalin cell bodies and fibers have been found in the preoptic nucleus, the nucleus lateralis tuberis and the nucleus lobi lateralis [70], whereas in *H. colliei* and *S. acanthias* leu-enkephalin-positive cells have been detected in the nucleus periventricularis hypothalami, the nucleus of the posterior commissure and the pretectal areas [58,60]. In the hypothalamus of *H. francisci*, leu-enkephalin has been localized on the ventrolateral lip of the third ventricle and in the infundibulum [60].

In the brainstem, leu-enkephalin-immunoreactive neurons are densely distributed in the metencephalon and myelencephalon [59,60,62,63,70]. In the mesencephalon, leu-enkephalin-positive cells and fibers have been described only in the caudal part of the ventral tegmentum and in the nucleus raphe linearis of *H. francisci* [60], *H. colliei* [58], and *S. acanthias* [59,63]. The mesencephalon of *R. binoculata*, *R. rhina* and *M. californica* contain a high density of leu-enkephalin-immunoreactive cells in the ventral tegmental area bordering the red nucleus [61]. In the metencephalon and myelencephalon leu-enkephalin is widely distributed within