The evolution of proopiomelanocortin: looking for the invertebrate fingerprints

DavideMalagoli, Alice Accorsi, EnzoOttaviani*

Department of Biology, University of Modena and Reggio Emilia, Via Campi 213/D, 41125 Modena, Italy

∗ Corresponding author. Tel.: +39 059 205 5536; fax: +39 059 205 5548.

E-mail address: enzo.ottaviani@unimore.it (E. Ottaviani).

ABSTRACT

The presence and role taxa of the pro-opiomelanocortin (POMC) gene and encoded peptides in invertebratesare here summarized and discussed. Some of the POMC-derived peptides show a significant similarity regarding their functions, suggesting their appearance before the split of protostomian-deuterostomian lineages and their maintenance during evolution. The basic mechanisms that govern the exchange of information between cells are usually well conserved, and this could have also been for POMC-derived peptides, that are mainly involved in fundamental functions such as immune and neuroendocrine responses. However, the presence and functions that POMC-derived peptides exhibited in taxonomically distant models, are not always reflected by the expected gene homology, leaving the problem of POMC evolution in invertebrates IN NEED OF ADDITIONAL STUDY????? open to debate.

Keywords:

proopiomelanocortin (POMC)

POMC-products

immune response

stress response

evolution

Abbreviations: Proopiomelanocortin (POMC); g-melanocyte-stimulating hormone (g-MSH); adrenocorticotropin hormone (ACTH); b-lipotropin (b-LPH); corticotropin-like intermediate lobe peptide (CLIP); Human ACTH (1-24) (hACTH); neutral endopeptidase 24.11 (NEP)

1. Introduction

The proopiomelanocortin (POMC) gene has been described more than thirty years ago in different mammalian species including humans [2]. Post-translational processes undergone by the precursor POMC protein have given rise toa set of structurally and functionally different peptides [5,8]. The vertebrate POMC gene is composed of three exons separated by two large introns,and except in the mouse, appears to be present as a single copy per haploid genome [18,43]. Exon 3, which is the biggest, encodes for all the biologically active peptides, i.e., g-melanocyte-stimulating hormone (g-MSH), adrenocorticotropin hormone (ACTH) and b-lipotropin (b-LPH). ACTH can be further cleaved to a-MSH and corticotropin-like intermediate lobe peptide (CLIP), while b-LPH can be further processed to g-LPH and b-endorphin [8,16].

In addition to their well-known and varied rolesin neuro-endocrine functions, POMC-derived peptides also are involved in mammalian immune responses.The presence and functions ofACTH in human leukocytes have been well-documented [1]. The POMC-derived peptides found in leukocytes show the same structure of those present in the pituitary [36]. ACTH and b-endorphin stimulate the chemotaxis of human peripheral blood mononuclear cells [13,28,44], and the ACTH (1-24) fragment increases the phagocytic activity of human monocytes [23]. The overlap between immune and neuroendocrine functions of POMC-derived molecules is further evidenced by corticotrophin-releasing hormone (CRH), the main inducer of the release of ACTH from pituitary [31], stimulates the production and the release of ACTH from leukocytes [38].

Recently, Dores and Baron [5] analyzed the evolution of the POMC gene in the principle vertebrate lineages and concluded that the gene probably does not represent the common ancestor of the opioid/orphanin gene familiy. A point raised by the authors concerns the obscure origin of the melanocortin component in the vertebrate POMC gene, despite the fact thatMSH/ACTH (1-24) sequences are highly conserved through all the gnathostomes. The evolution of opioid genes, including POMC gene, is in general accepted to have started with whole genome duplication events with the diversification of chordate and jawed vertebrates [41]. Accordingly, the reports concerning opioid genes outside vertebrates are not supported by gene sequencing [41] and no POMC genes have been observed in non-vertebrate chordates (e.g., tunicates and lancets) [5]. Despite several questions concerning the evolution of POMC gene outside gnathostomes, there is some evidence to suggest the presence of POMC-derived peptides in numerous invertebrate lineages.

The intent of this overview was to comparatively analyze published reports dealing with the molecular, immunocytochemical and functional data that was used to address the proposed roles of POMC-products in invertebrate models, and thus obtain abetter understanding of the evolution of POMC-related peptides

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2. Invertebrate POMC gene and related peptides

2.1. Presence EARLY REPORTS OF ????

The first demonstration of the presence of a POMC gene in invertebrates was made by Duvaux-Miret et al. [6] in the parasitic flat worm Schistosomamansoni (platelmints). The presence of the POMC gene, as well as that of the POMC-derived peptides, (e.g., ACTH, g- and a-MSH, CLIP, g-LPH, b-endorphin and MET-enkephalin), were reported in the annelid Theromyzontessulatum [32] ThePOMC molecule presented an overall moderate (i.e., <40%) degree of sequence similarity with vertebrate POMC sequences, whereas the POMC-derived peptides were highly similar. Leech ACTH, a-MSH and MET-enkephalin displayed a percentage of sequence identity of 75.5, 84.6 and 100%, respectively, with the corresponding human sequences. Unlike ACTH, a-MSH and MET-enkephalin, there was no sequence in the annelid showing high similarity with vertebrate b-endorphin [32]. The high level of similarity between the POMC-derived peptides of the two invertebrates and those of vertebrates raised reasonable concerns, especially in consideration of the phylogenic distance existing among annelids (i.e., protostomian) and vertebrates (i.e., deuterostomian). The transfer of genetic material from host to parasite [30] cannot be ruled out for explainingthe presence of the POMC gene in the two invertebrate species [6].

These possibility of genetic transfer from parasite to host was addressed in studies of the bivalve Mytilusedulis [40]. In the hemolymph of the mollusk a POMC protein of 20 kDa was found. The six peptides retrievable within this protein exhibit the following sequence identity with the mammalian counterpart: ACTH (74%), g- and a-MSH (80%, 85%), g-LPH (10%), b-endorphin (25%) and MET-enkephalin (100%). As observed for leech, the ACTH, a-MSH and MET-enkephalin peptides appear to be well conserved also in molluscs, suggesting an ancient origin of their sequence. The comparison between the POMC found in leech and mussel confirms that the POMC sequence is conserved only in correspondence of specific peptides, and especially in correspondence of ACTH, a-MSH and MET-enkephalin. While the global percentage of identity between leech and mussel POMC is 51.3%, leech and mussel ACTH molecules display 91.2% identity, while the identity between leech and mussel a-MSH molecules corresponds to 80%. Notably, b-endorphin molecules do not appear to be present, or at least to be conserved, in invertebrates. *T. tessulatum* and M. edulis b-endorphin peptides display just the 25% of identity, and this value derives form the total identity (100%) registered for the MET-enkephalin fragment. The same is true when leech or mussel b-endorphin and MET-enkephalin are individually compared to their human counterparts [32,40]. The MET-enkephalin sequence observed in invertebrates in not flanked by basic amino acidic residues or amidationsignalling sequences, as it is the case for leech and mussel a-MSH [40]. The processing of POMC-products in leech and mussel has been observed to proceed

similarly to vertebrates [32,40], and as a consequence the actual role of invertebrate b-endorphin and MET-enkephalin is unclear. On the other side, the presence of both melanocortin and opioid fragments in annelid and molluscan POMC proteins does not support the hypothesis that POMC gene emerged after the chordate first whole genome duplication event [5].

Even though no other sequences for POMC genes and peptides have been retrieved in invertebrates, several studies provide indirect evidence for their presence. It has been shown by *in situ* hybridization that immunocytes of three molluscs, the bivalve Mytilusgalloprovincialis, and the freshwater pulmonatesPlanorbariuscorneus and Viviparusater contain POMC mRNA [9,19,23]. Using a probe designed on a bovine ACTH receptor, it has been observed that mussel immunocytes and human peripheral blood mononuclear cells both express an ACTH receptor-like mRNA [25].

Beside evidences on gene expression, the presence of POMC-like peptides was detected in different invertebrate taxa, including protochordate, by radioimmunoassay, flow cytometry and immunocytochemistry [10,12,21,23,27,29,34,37,39]. Indirect evidences have intrinsic limits and can be misleading [40], but they should not be overlooked as they have represented [7] and may still represent a useful indication for more informative approaches. On the whole, while direct evidences of POMC peptides in invertebrates are scanty, indirect evidences of their presence have been retrieved by several research groups in different taxa (Fig. 1). As described hereafter, functional experiments suggested that POMC-derived peptides also have a conserved immune-neuroendocrine role in metazoans.

2.2. Immune response

Cell motility, chemotaxis and phagocytosis are the cornerstones of immunity, and together with stress and behavioural responses, guarantee body homeostasis and survival. Human ACTH (1-24) (hACTH) influences molluscanimmunocytes cell motility [33] and migration [24]. hACTH induces motile events in molluscanimmunocytes [11] promoting cytoskeletal rearrangements through the adenylatecyclase/cAMP/protein kinase A pathway, and the activation of a calphostin C-sensitive protein kinase C [33]. hACTH also increases phagocytic activity of molluscanimmunocytes, whereas human b-endorphin promotes their migration but not phagocytosis [24].

As observed in vertebrates, POMC-derived peptides in evolutionary distant invertebrates also influence the activity of immune-related cells [1]. Control of their activity seems to have evolved at the time of theprotostomian-deuterostomian split [35]. The neutral endopeptidase 24.11 (NEP), also known as enkephalinase is a cell surface zinc metallopeptidase of 749 amino acids, which displays a degradative activity over several neuropeptides [42]. More in detail, NEP cleaves peptide bonds involving the a-amino groups of many peptides, including ACTH, a-MSH and enkephalins [4,7,17]. The immunocytes of the molluscs*M. edulis* [35], P*. corneus*and

V. ater [20] as well as the synaptic membrane of the insect *Schistocercagregaria* [15] present a NEP-like enzymatic activity. The inhibition of NEP-like activity reduces the amount of Met-enkephalin required for the activation of M. edulisimmunocytes [35]. The addition of hACTH to the hemolymph of freshwater snails increase the endogenous NEP-like activity suggesting a possible control in the activation of immunocytes. Moreover, the endogenous NEP-like activity of molluscanimmunocytes is blocked by phosphoramidon, a potent inhibitor of mammalian NEP [20]. It has been proposed [7] that NEP-like activity may be used by the parasitic trematode S. mansoni to escape the immune responses of the intermediate and definitive hosts, the freshwater snail *Biomphalariaglabrata* and human, respectively. According to this view, the ACTH produced by the parasite could be converted in the host tissue to a-MSH, a molecule that inhibits the adherence and locomotory activity of *B. glabrata*immunocytes as well as that of human polymorphonuclear cells and monocytes [7].

2.3. Stress response

Stress responses can be depicted as a coordinate series of metabolic events that allowsthe body to cope with a variety of life-threatening agents. Evidence from several studies conducted during the 80's and the 90's suggestes that the series of stress-related events occurring in invertebrates is based on the same mediators described for vertebrates [22]. In vertebrates, the stress response involves CRH, ACTH and biogenic amines, molecules secreted by hypothalamus, pituitary and adrenal gland, respectively. The three organs are not present in invertebrates, but the stress response axis can be retrieved in the circulating immunocytes, where the CRH-like, ACTH-like and biogenic amines are present [22].

3. Concluding remarks

On the basis of the data reviewed here, more than one aspect of the evolution of metazoan POMC-derived peptides still awaits to be elucidated. The absence of molecular data in deuterostomian invertebrates [5] is in contrast with the observations performed in flat worms [6], annelids [32] and molluscs [40] (Fig. 1). The studies of POMC-derived peptides in protostomes indicate a very ancient origin for these molecules, and an extremely high level of conservation. Indeed, the available invertebrate sequences of POMC-derived fragments are highly similar to those collected in the sarcopterygian lineage of vertebrates [41]. Unfortunately, this relativelyeasy conclusion does not explain the absence of POMC genes in basal chordates or echinoderms (Fig. 1). A further hypothesis may be advanced if also the immunocytochemical and functional evidences are considered.

The immune-reactivity observed with mammalian antibodies and the effects that human ACTH and b-endorphin exert on immunocytes, allow to arguesuggest that some domains of POMC peptides are widely conserved among extant metazoans. The domains could have a relatively low similarity in terms of sequences, but could still display a structure that would allow the ligand-receptor interaction. Salzet et al

[32] observed that both leech and human a-MSH, similarly inhibit the activity of human granulocytes and mussel immunocytes. Moreover, the effect of both the peptides was annulled when the target cells werepre-incubated with a rabbit polyclonal anti-a-MSH antibody. Based on these data, the authors concluded that despite the fact that leech and human a-MSH are not completely identical (84,6% identity), they are both specifically bound to the same receptors. In this context, a certain degree of promiscuity for the receptors of POMC-derived melanocortins has been ascertained in vertebrates [5]. This view would justify the discrepancies existing between the numerous morpho-functional observations and the few molecular evidences collected so far. Accordingly, all the invertebrate POMC-peptides have been discovered through peptide isolation and sequencing, rather then by mean of database searching or gene expression screening. Also for other immune-neuroendocrine mediators such as cytokines it has been demonstrated a high degree of sequence divergence accompanied by a tight conservation of molecular structure [14,26] and functions [3]. Despite recent advances in our understanding of POMC-derived peptides, much remains to be learned evolutionary history of the important molecules in both vertebrate and invertebrate species.

Conflict of interest

None.

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