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MINIREVIEW

Biologically active peptides in molluscs

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Abstract

The immune and neuroendocrine systems of invertebrates, as well as vertebrates, share a common pool of molecules that have been conserved throughout evolution. Now we add a new interface to this bidirectional interaction, demonstrating the involvement of the gut system, showing that these three systems use the same biologically active peptides.

Key Words: neuropeptides; hormones; molluscs; immunocytochemistry; evolution

Introduction

Homeostasis, coined by Claude Bernard (1965), is a healthy state that is maintained by the constant adjustment of biochemical and physiological pathways. The immune and neuroendocrine systems play a fundamental role in this complex mechanism that thanks to the fluid compartment wetting/covering the cells of the body, transfers biologically active peptides (BAP), such as neuropeptides and hormones, to all the cells of the body. More importantly, the immune and neuroendocrine system of vertebrates and invertebrates produce the same BAP and data in the literature show a bidirectional correlation between them (Weigent and Blalock, 1987; Ottaviani and Franceschi, 1997; Ottaviani *et al*., 1997). Here we review the presence and the possible roles of BAP in molluscs.

Presence of biologically active peptides

In molluscs the synthesis of BAP was detected in the neuroendocrine, immune and gut system. Moreover, in gastropods the entire periphery of the nervous system, including nerves, connectives and commissure is used for hormone release. The central nervous system (CNS) of the freshwater snails *Lymnaea stagnalis* and *Planorbarius corneus* have been the most studied models for the presence of BAP using immunocytochemical approaches (Grimm-Jørgensen, 1978; Boer *et al*., 1979; Schot *et al.,* 1981; Sonetti *et al*., 1994, 2005).

Especially interesting are *P. corneus* immunocytes which exhibit immunoreactivity for several vertebrate BAP, thus presenting a framework comparable to that observed in the CNS (Schot *et al*., 1981). These BAP, include bombesin, calcitonin, cholecystokinin (CCK)-8, CCK-39, gastrin, glucagon, growth hormone, insulin, met-enkephalin, neurotensin, oxytocin, pancreatic polypeptide (PP), somatostatin, substance P, secretin, serotonin, substance P, thyroglobulin, vasopressin and vasointestinal P (VIP) (Ottaviani and Cossarizza, 1990; Ottaviani *et al*., 1992), adrenocorticotropin hormone (ACTH), β-endorphin (Ottaviani *et al*., 1990), α-melanocyte-stimulating hormone (α-MSH) (Franchini *et al.,* 1994) and corticotropin-releasing hormone (CRH) (Ottaviani *et al.,* 1998). Other pro-opiomelanocortin (POMC)-derived peptides were also confirmed in immunocytes by the expression of POMC-mRNA (Ottaviani *et al.,* 1995). Furthermore, thyrotropin-releasing factor-like peptides were found in the hemolymph of *L. stagnalis* (Grimm-Jørgensen, 1978).

The CNS of *P. corneus* and other gastropods is characterized by three pairs of ganglia (cerebral, pleural and pedal) located around the esophagus. ACTH-like molecules are present in a relatively small number of neurons in all ganglia (Sonetti *et al.,* 2005). Moreover, in *P. corneus*, ACTH immunopositivity is detected in a distinctive class of neuroglial cells, comparable to vertebrate microglia. However, ACTH was not found in the neuroglial cells of the bivalve *Mytilus edulis*, indicating that there are species-specific differences in expression (Sonetti *et al*., 1994). Furthermore, immunoreactive responses have been observed in the snail CNS for some vertebrate neuropeptides such as substance P, neuropeptide Y, calcitonin gene-peptide (CGRP) and CCK (Sonetti *et al*., 1990). Previous studies demonstrate different neurosecretory cells in the CNS of *L. stagnalis* and *Helicella virgata* using the Alcian blue/Alcian yellow method (Wendelaar Bonga, 1970; Franchini and Ottaviani, 1982). The comparison

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between histochemical and immunohistochemical techniques showed a low overlap between the classically evidenced neurosecretory cells and the BAPs detected by immunocytochemical procedures. However, in *P. corneus* some overlap is present, for example, CGRP-like peptide is observed in one cluster of Yellow cells and CCK8-like has been detected in three different neurosecretory cells (Sonetti *et al.,* 1990). Other BAPs such as thyrotropin-releasing factor-like- (Sonetti *et al.,* 1994), dopamine-like-, serotonin-like- and vasotocin-like peptides (Boer *et al*., 1984), were observed in the CNS of *L. stagnalis*, somatostatin-like peptides in CNS of *Limax maximus* (Marchand *et al.*, 1984) and in the hepatopancreas, mantle edge and hemolymph of *Helix aspersa* (Marchand *et al*., 1989).

Likewise, steroids have been identified in ganglionic tissue as well. The presence of 17beta-estradiol, involved in the stimulation of the release of nitric oxide, has been detected by Stefano *et al.* (2003a), while Canesi *et al.* (2006) described this molecule in hemocytes that have a known immunomodulation effect. Furthermore, studies in the pedal ganglia of mussel have allowed to isolate a 266 bp fragment with 100 % identity to the human 17beta-estradiol gene (2003b).

Furthermore, BAPs have been detected in the gut system. Many mammalian gastroenteropancreatic (GEP) hormones have their counterparts in invertebrates (molluscs, annelids and arthropods), which are mainly localized in the nervous system (Van Noorden and Falkmer, 1980). These GEP hormone-like substances include insulin, somatostatin, glucagon, gastrin, secretin, VIP, PP, substance P and enkephalin.

Role of biologically active peptides

BAP are involved in fundamental mechanisms that allow the survival of every form of life, such as, reproduction, growth, defense and homeostasis. With regard to reproduction, functional androgen and estrogen receptors, have been detected in gastropods, but their genetic basis has yet to be clarified (Köhler *et al*., 2007). A different behavior with respect to *Octopus vulgaris* where endogenous estrogens are involved in the reproduction (Di Cosmo *et al*., 2001).

On the whole, although the literature suggests the presence of steroid-like molecules in molluscan tissues, their functional role is far from clear. In this context, some authors hypothesize the possibility of the biosynthesis of steroids in molluscs (Fernandes *et al.,* 2011), while others are doubtful that this can be achieved (Scott, 2012). Furthermore, although in *Aplysia californica* Gonadotropin-releasing hormone (GnRH)-like molecules have been identified, these do not seem to be involved in reproduction (Tsai *et al*., 2010).

Also, Insulin-like peptide was found in cerebral light-green cells of the CNS *of L. stagnalis* (Smit *et al*., 1988). These neurosecretory cells contain and release immunoreactive insulin that regulate various processes related to the growth of the snail body, such as, soft body parts and the shell (Geraerts, 1976; Joosse and Geraerts, 1983; Ebberink and Joosse, 1985; Ebberink *et al*., 1987).

As far as defense is concerned, the ACTH fragment (1-24) has been suggested to play an important role. Indeed, it is involved in cell shape changes (cell motility), chemotaxis (non-random locomotion) and phagocytosis. This immunocyte motility is activated by an adenylate cyclase/cAMP/protein kinase A-dependent pathway, as well as through the activation of protein kinase C (Sassi *et al*., 1980). Chemotaxis and phagocytosis display different behaviors, indeed in contrast to the conventional paradigm, there is no direct correlation between the two processes (Genedani *et al*., 1994; Ottaviani *et al*., 1994). Furthermore, ACTH is involved in the stress response and as in mammals, the key mediator molecules are the same with the cascade of events following the same order and pattern: CRH-ACTH-biogenic amines. In contrast, in invertebrates, unlike in mammals, there are no organs involved in the stress response, such as the hypothalamus, pituitary and adrenal glands; in invertebrates all the stress response related molecules are concentrated in immunocytes (Ottaviani and Franceschi, 1996).

Unity of living beings and the "game" theory of evolution

At all times, biologists have been fascinated by the extraordinary variety of living forms. From the time when Lucretius asked: "How can there be this infinite variety of things and what is behind this incredible variety of shapes and sizes?", philosophers asked themselves this important question over and over again, searching for more general unifying principles. One of the solutions that has emerged since the time of Epicurus and Lucretius has been to assume that it is the arrangement of the constituent elements and the relationships among them that generates different and new forms of living.

In the case of biology the unifying principle was initially given by the cell: all living organisms are composed of cells that work basically in the same way, at least with regard to the main functions and properties, such as the production of protein and energy, proliferation, etc. Today, however, we have come to the realization that the unification of living organisms is based on precise molecular mechanisms, that is, fundamental information making up all living beings, is contained in the nucleic acids. How then can we reconcile the unity of the living and the variety of forms of the species? Such a result is obtained from Nature in accordance with laws and extremely economic strategies that are revealed to us by what is the fundamental approach to the study of the living, *i.e.,* evolution.

Traditionally, the study of evolution is presented as the study of changes that have gone through the taxa and different species in their story on earth. This approach emphasizes what changes and, at the molecular level, what mechanisms allow the accumulation of different molecular variants fundamental for the selection of mechanisms that govern them. However, the enormous mass of molecular data available today, suggests we should adopt a rather opposite approach supporting conservation compared to changes (Ottaviani *et al*., 2001). In order to understand the most strategically

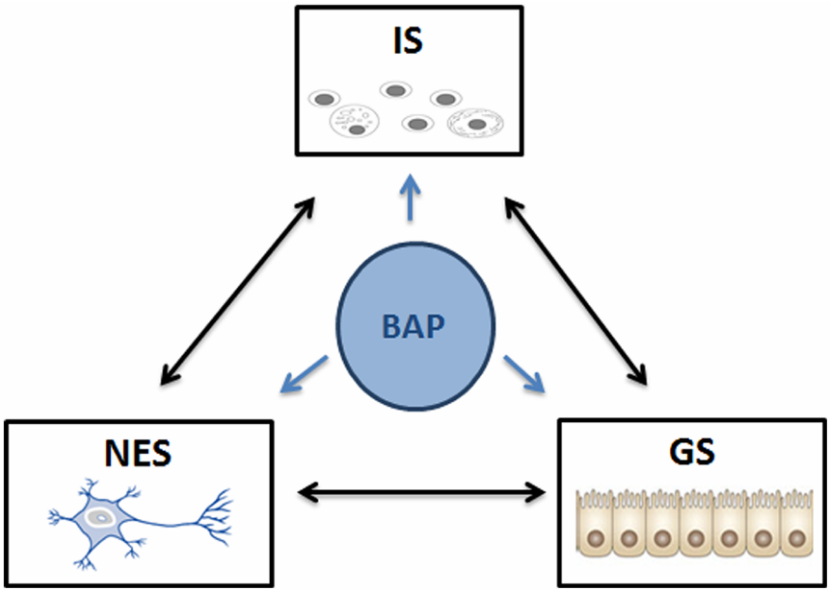

Fig. 1 The Biologically active peptides (BAP) mediate the interactions among the immune (IS), the neuroendocrine (NES) and gut (GS) systems.

important biological functions, it is thus more interesting to search for what has been preserved rather than what has changed, which is true, for example, for most of the different proteins. Overall, the data available today, as reported in this manuscript, seem to indicate that the major signaling molecules (neuropeptides, hormones, cytokines) have been preserved in an extraordinary way in the course of evolution. This suggests that, despite the progressive complication of organs and systems, the mechanisms that govern the exchange of information between cells, remained essentially unchanged. In other words, the flow of information among the different cell types that make up higher organisms, such as vertebrates, is exchanged using the same pool of signaling molecules. More importantly, animals apparently much less complex, such as the molluscs, use the same type of signaling molecules to exchange information.

Finally, Lucretius' statement still holds true: "What happens with the words and verbs is also true for the elements of matter: it is the combinatorial change of their movement, order and position which explains the very essence of beings and species".

Concluding remarks

From this brief survey of BAPs emerges that a common pool of BAPs have been employed since the beginning of evolution to set up an integrated type of response capable of maintaining body homeostasis. Alternative use of the same molecules for different functions is a widespread, economical strategy used throughout evolution. Normally this hypothesis is supported by research studying the immune and neuroendocrine systems, but as reported in this review, another system, that is, the gut system, has activated the same strategy (Fig. 1).

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