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REVIEW

ACTH in invertebrates: a molecule for all seasons

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Abstract

In vertebrate and invertebrate models, adrenocorticotropic hormone (ACTH) belongs to the melanocortin group of related peptides, which share a common precursor, pro-opiomelanocortin (POMC). Functional experiments indicate that in invertebrates, ACTH plays a major role in several biological functions. ACTH, whose effects have been conserved during evolution more than its amino acidic sequence, is, directly or indirectly, able to contrast agents that perturb a body's homeostasis. Here we review evidence highlighting the involvement of ACTH and ACTH-like molecules in the response of invertebrate models versus immune, environmental and parasitic challenges.

Key Words: ACTH; invertebrates; immunomodulation; stress response; parasitization

Introduction

Adrenocorticotropic hormone (ACTH) is a small, bioactive peptide derived from the pro-opiomelanocortin (POMC) gene that was first found in the pituitary gland and later also found in other organs in both vertebrates and invertebrates (Ottaviani *et al*., 1997). POMC is the common precursor for both the melanocortin-related peptides (ACTH/α-MSH, β-MSH and γ-MSH) and the opioid β-endorphin, whereas the other members of the opioid/orphanin gene family, *i.e.,* proenkephalin, prodynorphin and proorphanin, are mainly precursors of opioid peptides (Dores and Baron, 2011).

It has been herein summarized and updated the information about investigations on the presence and role of ACTH and ACTH-like molecules in invertebrates.

The presence of POMC-products in invertebrates

In invertebrates, information on POMC peptides and their sequences have been provided for the flatworm *Schistosoma mansoni* (Duvaux-Miret *et al*., 1990), the leech *Theromyzon tessulatum* (Salzet *et al*., 1997) and in two species of mussels, *Mytilus galloprovincialis* and *Mytilus edulis* (Franchini *et al*., 1994; Ottaviani *et al.,* 1995; Stefano *et al.,* 1999). The analysis of POMC peptides in *M. edulis* indicated that there was poor conservation of the overall sequence but a higher level of similarity in the peptides contained within POMC (Malagoli *et al.,* 2011). Among the fragments of POMC, ACTH has been found using several techniques, including immunocytochemistry, flow cytometry and radioimmunoassays in different tissue of various species examined (Table 1). Moreover, *in situ* hybridization experiments using a bovine ACTH receptor cDNA probe evidenced that *M. galloprovincialis* immunocytes express an mRNA encoding for a molecule similar to ACTH receptor (Ottaviani *et al*., 1998).

Several studies, based on mammalian-derived ACTH or anti-ACTH polyclonal antibodies, were performed on the mollusc gastropod, *Planorbarius corneus*, where ACTH-like molecules have been found in both the immune and nervous systems. The hemolymph is characterized by two cell types, and ACTH-like molecules were detected in the cells endowed with phagocytic activity, *i.e*., the so-called immunocytes (Ottaviani, 1983, 2011; Ottaviani and Franchini, 1988). The central nervous system (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 were found in a relatively small number of neurons in all the ganglia (Sonetti *et al*., 2005). Moreover, in *P. corneus,* ACTH immunopositivity was also detected in a distinctive class of glial cells that are comparable to vertebrate microglia. It should be stressed that ACTH-like molecules were not found in the neuroglial cells in the bivalve *M. edulis*, indicating that there are species-specific differences in expression, or that the expression may be transient and related to factors presently unknown (Sonetti *et al*., 1994).

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Table 1 Presence of ACTH-like molecules in invertebrates

	immunocytes	neurons	neuroglial cells	intestinal cells	CC/CA	Refs
Protostomia						
Mollusca						
Planorbarius corneus	+	+	+			1-3
Viviparus ater	+					4
Lymnaea stagnalis	+	+				5-7
Achatina fulica		+				8
Helix aspersa		+		+		9,10
Mytilus edulis	+		-			11,12
Mytilus galloprovincialis	+					13
Insecta						
Periplaneta americana				+		14
Leucophaea maderae	+				+	15,16
Calliphora vomitoria	+					17
Heliothis virescens	+					18
Annelida						
Eisenia foetida	+					
Theromyzon tessulatum	+					19 20
Nematoda						
Goodeys ulmi	+					21
Trichinella spiralis	+					20
Trematoda						
Schistosoma mansoni	+					20
Deuterostoma						
	neurons	neural gland	gonads			
Tunicata						
Ciona intestinalis	+			+		22,23
Stiela plicata		+	+			24,25
Halocynthia roretzi		+				26

corpus cardiacum/corpus allatum = CC/CA

Refs
1- 3 = Ottaviani, 1983; Sonetti *et al*., 1994, 2005
4 = Ottaviani *et al*., 1995
5 - 7 = Boer *et al*., 1979; Leung *et al*., 1990; Ottaviani *et al*., 1991
8 = Van Noorden *et al*., 1980
9, 10 = Marchand and Dubois, 1982; Marchand and Colard, 1991
11, 12 = Smith *et al*., 1990; Sonetti *et al*., 1994
13 = Franchini *et al*., 1994
14 = Schols *et al*., 1987
15, 16 = Hansen *et al*., 1986; Smith *et al*., 1991
17 = Franchini et *al*., 1996
18 = Grimaldi *et al*., 2012
19 = Cooper *et al*., 1995
20 = Pryor and Elizee, 2000
21 = Leach *et al*., 1987
22 = Georges and Dubois, 1979
23 = Fritsch *et al*., 1985
24 = Pestarino, 1985
25 = Pestarino and Facchinetti, 1995
26 = Kawahara *et al*., 2002

The different distribution of ACTH-like molecules in the various regions of the cellular body of invertebrates implies the presence of several biological functions, such as immunomodulation, stress response and parasitization.

The role of ACTH in immunomodulation

ACTH affects cell shape changes (cell motility), chemotaxis (non-random locomotion) and phagocytosis. Using a computer-assisted microscopy image analysis, it was observed that mammalian ACTH (1-24) induces the motility of molluscan immunocytes via an adenylate cyclase/cAMP/protein kinase A-dependent pathway, as well as through the activation of protein kinase C (Sassi *et al*., 1998).

With regard to chemotaxis, it has been detected that the various fragments of mammalian ACTH have different effects on the migration of immunocytes. Indeed, stimulatory effects were

observed for fragments (1-4), (1-13), (1-17), (1-24), (4-9), and (11-24), while an inhibitory effect was detected for fragment (4-11) and for the whole molecule (1-39) (Genedani *et al.,* 1994). The incubation of immunocytes with ACTH (1-24) induces cytoskeletal changes the freshwater snail *Viviparus ater* (Franchini and Ottaviani, 1994). The non-activated immunocytes typically have a nucleus in the central position and a cytoplasm characterized by thin pseudopods. Furthermore, bundles of microfilaments are distributed radially from the nucleus to the cell periphery, where they enter into cytoplasmatic protrusions. The incubation with ACTH (1-24) provokes morphological changes that become more evident after 30 min. The cells then show a polarized morphology, most of the cytoplasmic protrusions are retracted, and the elongated cells extend lamellipodia. The microfilaments are reorganized, with thin bundles surrounding the nucleus, and are seen under the plasma membrane, while phalloidin-positive areas are evident at the cell periphery on the lamellipodia. With regard to the distribution of microtubules, immunofluorescent positivity is observed in control cells but not in cell protrusions. After incubation with ACTH (1-24), the fibronectin is more abundant in areas where the cell contacts the substrate, whereas in control cells, it is predominantly localized at the cell periphery. In treated cells, the adenylate cyclase activity increases, while no modification in cyclic 3'-5'-nucleotide phosphodiesterase activity has been detected.

Phagocytosis shows a different behavior with respect to chemotaxis (Ottaviani *et al*., 1994), and in contrast to the conventional paradigm, there is no direct correlation between the two processes in molluscan immunocytes. For example, ACTH (1-24) increases phagocytic activity, while it has no effect on chemotaxis. Moreover, for both chemotaxis and phagocytosis, it has been observed that the effect of a single peptide is species-and dose-dependent.

ACTH in stress response

In vertebrates, ACTH has traditionally been associated with stress*, i.e*., the complex series of responses that the body generates when its balance and internal composition are threatened. The stress response starts with the production of corticotropin-releasing hormone (CRH) by the hypothalamus, which induces the production of ACTH by the anterior pituitary cells. In turn, ACTH stimulates the synthesis and release of glucocorticoid hormones by the cells of the adrenal cortex. These hormones regulate the activity of the enzyme that catalyzes the final step of epinephrine biosynthesis from the adrenal medulla, whose activity is basically under the control of the sympathetic nervous system.

What meaning could the presence of ACTH-like molecules have in animals that do not possess any of these sophisticated organs, such as the hypothalamus, pituitary and adrenal glands. A first point is that ACTH is found, together with CRH (Ottaviani *et al.,* 1990), in cells endowed with phagocytic activity in invertebrates and human as well. This could suggest that ACTH was already present in vertebrate progenitors and that it was retained also in lymphocytes, an immune-related cell type retrieved exclusively in vertebrates. A second point is that antigenic stimuli cause in *P. corneus* a release of ACTH-like material (Ottaviani *et al*., 1992), suggesting the existence of an interconnection between stress and immune responses also in invertebrates. In this respect, the initial studies assessed organisms for the presence of enzymes such as tyrosine hydroxylase and dopamine β-hydroxylase, which are responsible for the synthesis of biogenic amines (epinephrine, norepinephrine and dopamine), well-known mediators of the stress response (Ottaviani *et al.,* 1993).

In order to understand the mechanism underlying the stress response, the *P. corneus* hemolymph was incubated with stress-related peptides, such as human ACTH and CRH, and the levels of biogenic amines produced in both serum and immunocytes within 45 min were determined by HPLC. It was observed an increase in biogenic amines in the serum and a concomitant decrease of both ACTH- and CRH-like molecules into immunocytes (Ottaviani *et al.,* 1992). This rapid increase of free biogenic amines in the serum is likely due to the release of biogenic amines by immunocytes. Time-lapse experiments based on HPLC have demonstrated that the addition of human CRH to the *P. corneus* hemolymph increases the concentration of ACTH-like material in immunocytes. These results allowed to assume that the release of biogenic amines might be the final step of the sequential activation of CRH and ACTH receptors. However, the pre-incubation of *P. corneus* hemolymph with an anti-ACTH antibody did not abolish the release of biogenic amines, suggesting that human CRH could directly mediate the release of biogenic amines, and that the contribution of ACTH was not indispensable (Ottaviani *et al*., 1992).

On the whole, it appears that in an invertebrate model, a stress response axis is concentrated in a single cell type, *i.e*., the immunocyte, while the key mediator molecules are the same as in vertebrates.

ACTH in parasitization

In insects, the host/parasitoid interaction results in several events including the production of amyloid fibrils (Falabella *et al.,* 2012). This process called amyloidogenesis**,** involves the conversion of a soluble protein into insoluble and fibrillar protein aggregates known as amyloid fibrils (Bhak *et al*., 2009). In humans, amyloidogenesis may lead to pathological events such as neurodegenerative or cardiovascular diseases (Catafau and Bullich, 2015), but in invertebrates and humans as well, it may also play physiological roles in melanin synthesis (Grimaldi *et al*., 2012, 2014), in detoxifying events, and in storing peptide/protein hormones within endocrine secretory granules (Maji *et al*., 2009).

The inoculation of parasitic eggs in the tobacco budworm *Heliothis virescens* larvae by the endophagous parasitoid *Toxoneuron nigriceps* (Hymenoptera) induces the production of amyloid fibrils. These fibrils are involved in the immune response, serving as a necessary scaffold and providing a template for melanin deposition

(Tettamanti *et al*., 2008; Bhak *et al*., 2009). In insects, melanin is massively produced in the hemolymph by the pro-phenoloxidase pathway. Amyloid fibrils develop in consequence of exocytotic activity of circulating cells, which release amyloid fibrils that adhere to the non-self, driving the pigment accumulation close to the invaders (Tettamanti *et al*., 2008; Falabella *et al*., 2012; Grimaldi *et al.,* 2012). The exocytosis of amyloid fibril precursors by circulating cells suggest that immunocytes activation may play a pivotal role in contrasting parasite development. Accordingly, in addition to the formation of amyloid fibrils, the parasitization triggers host immunocytes to release ACTH-like and melanocyte-stimulating hormone-like (α-MSH-like) molecules and neutral endopeptidase (NEP). As stated above, ACTH-like mediators are involved in the migration and activation of circulating cells, and α-MSH-like molecules promote melanin synthesis and do not affect cell motility. The ACTH/α-MSH loop is modulated by the newly synthesized NEP localized on the cell surface of activated immunocytes. This enzyme is able to abolish the excitatory effect of ACTH-like molecules by converting them into α-MSH-like molecules. Besides, NEP can also hydrolyze the amyloid fibrils (Grimaldi *et al*., 2012). Several aspects of this complex relationship wait for further elucidation, but present evidence indicate in the ACTH/α-MSH loop a possible controller of immunocyte activation.

This loop has been exploited and subverted towards immunosuppression by the parasite *Schistosoma mansoni* (Duvaux-Miret *et al.,* 1992). The presence of POMC-derived peptides and their release from *S. mansoni* has been detected. Co-incubation of adult worms with human polymorphonuclear leukocytes (PMN) or immunocytes from the mollusc *Biomphalaria glabrata* led to the appearance of α-MSH in the medium that was derived from the conversion of the parasitic ACTH by the host NEP. In this way, the worm can escape immune reactions in humans and molluscs because α-MSH inhibits the adherence and locomotor activity of both PMN and invertebrate immunocytes (Duvaux-Miret *et al*., 1992).

Concluding remarks

In invertebrates, ACTH-like molecules are present in different tissues. Exogenous ACTH exerts various biological functions, being involved in immune-neuroendocrine responses and parasitism.

There is a deep correlation between the lymphocytes of vertebrates and immunocytes of invertebrates (Scapigliati, 2013) because both contain several molecules, such as POMC-derived peptides and cytokines. As suggested by Blalock and Smith (1985) for mammalian lymphocytes, the immunocytes may also be described as an "immune-mobile brain". Indeed, lymphocytes are able to recognize a variety of stimuli and to set up a correspondingly complex response in which primitive but very efficient forms of immune and neuroendocrine responses are intermixed. In invertebrates, there is no lymphocytes and immune and stress phenomena are triggered concomitantly in the same cell (Malagoli *et al*., 2015), suggesting that antigens and stress were indistinguishable from the outset. This is probably the reason why antigens and stress evoke an overlapping set of responses in mammals that are mediated by the same pool of conserved signaling molecules (Ottaviani and Franceschi, 1997). The consequences of this hypothesis are potentially far-reaching, particularly for the interpretation of pathological conditions involving inflammation and cytotoxic phenomena, such as most infectious diseases, neuropathological conditions and aging, where biological and psycho-emotional stimuli are concomitantly present.

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