Brain Research Reviews 34 (2000) 69–79

Full-length review

Invertebrate molecular neuroimmune processes

Michel Salzet*,[1]

Laboratoire d'Endocrinologie des Annélides, UPRES-A CNRS 8017, SN3, Université des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq Cédex, France

Accepted 15 August 2000

Abstract

During the course of evolution, invertbrates and vertebrates have kept in common similar signaling molecules e.g. neuropeptides, opiates etc . . . Complete hormonal–enzymatic systems such as the opioid–opiate–cannabinoid systems have been found in both nervous central and immune systems of these animals. These signaling molecules can be found free in blood circulation and act as immunomodulators. The present review is focused on peptides derived from the opioid proopiomelanocortin precursor, the opiates and the endocannabinoids, which are very powerful immunosuppressors, and example models of the bidirectional communications between the endocrine and the immune systems. Parasites use these immunosuppressors with magnificence in their crosstalk with their host.

Theme: Endocrine and autonomic regulation

Topic: Neural–immune interactions

Keywords: Invertebrates; Neuroimmunity; POMC derived peptides; Endocannabinoinoids; Morphine; Parasites

Contents

Abbreviations: NT, neurotransmitters; NO, nitric oxide; POMC, proopiomelanocortin; ACTH, adrenocorticoptropin hormone; MSH, melanostimulating hormone; IL-1, interleukin 1; PACE, prohormone convertase; ACE, angiotensin converting enzyme; NEP, neutral endopeptidase; FAAH, fatty acid amino hydrolase

*Tel.: +33-3-2043-6839; fax: +33-3-2004-1130.

E-mail address: michel.salzet@univ-lille1.fr (M. Salzet).

[1]Membre de l'Institut Universitaire de France.

1. Introduction

A new understanding gained in invertebrates as well as vertebrates, is that substances involved in chemical communication among immunoactive cells are the same, or closely related to those used in the bidirectional exchange

of information between the immune system and the neuroendocrine apparatus [3,4,33,68,85,86]. Recently, this concept has been expanded to include parasites since they also appear to use the same signal molecules to escape immunodetection [16,44].

1.1. Opioid peptides and opiate alkaloids

Stefano's group demonstrates with Deltorphin I, a naturally occurring opioid peptide isolated from amphibian skin, the ability of this substance to modulate both human and invertebrate immunoregulatory activities in a manner quite similar to Met-enkephalin [69]. Its binding and pharmacological studies also have provided evidence for a special subtype of delta opioid receptor δ2, sensitive to naltrindole antagonist on human and invertebrate immune cells [66]. It is also of interest to note that both the invertebrate immunocytes and human granulocytes thus have a δ1 and δ2 receptor [66]. It is clear within the context of this review that host immunocytes having these receptors, and those to be mentioned below, have the potential to respond to similar peptides secreted by the parasite within the host body, thus effectively 'taking' over the local communication in their vicinity, without altering global host function.

Opiate alkaloids, e.g., morphine, deserve special attention within the context of this report for several reasons. First, unlike antinociceptive mechanisms, opiate alkaloids and opioid peptides initiate different immunocyte behaviors [66]. Opioid peptides may be generally regarded as immunocyte stimulatory and/or activating ligands whereas morphine, as first noted by Wybran et al. [87], is inhibitory [66]. Secondly, confusion exists in the scientific literature as to the proper terminology for these ligands, opioid alkaloid and opiate peptide. Thirdly a novel opiate alkaloid sensitive and opioid peptide insensitive receptor, namely μ3, has been demonstrated which does not recognize μ-opioid ligands [66]. Lastly, opiate alkaloids appear to be naturally occurring substances found both in mammals and invertebrates [24,63,66].

In this regard, the immunosuppressive effect of opiate material expresses itself in a lowering of chemotactic activity, cellular velocity and adherence as well as making active immunocytes inactive (rounded; [66]). These pharmacological effects of morphine on immunocytes are consistent with those actions attributed to opiates reported in the literature [66]. Indeed, it has been surmised that morphinergic transmission may regulate the downregulation of immune activation [66]. Recent studies demonstrate that human granulocytes also contain the μ3 subtype opiate receptor mediating inhibition by morphine and other opiates of cytokine-induced activation and chemotaxis [31]. The discovery of this receptor site mediating opiate effects were first found in an invertebrate and then in man, again demonstrating the value of the comparative approach [66,73,74]. This also suggested that these processes might be operational in parasites as well.

1.2. Adrenocorticotropin

Proopiomelanocortin (POMC), i.e., adrenocorticotropin (ACTH) and β-endorphin (βE), is expressed in the cells of the immune system [5,29,30,55–57]. These observations were then extended to mouse splenocytes [29,30]. It was only in 1988 that the presence of a mRNA species hybridizing a POMC probe was demonstrated in human B and T lymphocytes [34]. The identity of the peptides produced in lymphocytes with those originally described in the pituitary was definitively shown in a very elegant study by Smith et al. [59]. These authors isolated and microsequenced ACTH produced by LPS-activated mouse splenocytes. The purified peptide was found to be identical to mouse ACTH 1–25. In the same study, reverse transcription of murine lymphocyte RNA, followed by a specific amplification of the POMC mRNA by polymerase chain reaction and sequence analysis, demonstrated the identity of pituitary and lymphocyte POMC mRNAs. This work constitutes the definitive demonstration of the transcription, translation and post-translational processing of POMC in cells of the immune system.

The presence of POMC derived peptides in spreading hemocytes from the snail *Planorbarius corneus* also has been demonstrated. In this animal, ACTH and βE appear to modulate chemotaxis and phagocytosis by these hemocytes [27,36,37]. ACTH-like molecules have also been identified in the marine mollusc *M. edulis* [59]. In this model, Stefano and co-workers [71,79] have demonstrated that both α- and β-MSH can inactivate mollusc and insect hemocytes by inhibiting their motility. This was studied by a technique of computer-assisted analysis of cellular conformation. Using the same method, MSH was found to exert the same activity on human granulocytes and monocytes, in agreement with the results of Van Epps and coworkers [49,68,71,82,83].

Recently, Salzet, Stefano and co-workers in a series of reports [44–49,64], have demonstrated that heamatophagous invertebrates (leeches) contain the major opioid peptide precursors, i.e., prodynorphin, POMC and proenkephalin, that contain mammalian-like opioid peptides exhibiting high sequence identity with their mammalian counterparts. These studies corroborated the earlier works of Duvaux-Miret, Capron and co-workers [6–9,16–19], suggesting that parasites may communicate with their hosts via common signaling molecules.

1.3. Cytokine-like molecules

The above data also suggest that neuroimmune interactions emerged early in evolution. In this context, the question arises as to whether cytokines exist in invertebrates. Stefano and co-workers first demonstrated that

cytokine-like molecules are synthesized in the nervous and immune systems of invertebrates, and that neuropeptides can regulate their production in both tissues [22,23,40,72]. However, real proof from the molecular point of view has not been yet given in invertebrates. Some indirect evidence (immunological) has recently been provided [36]. Moreover, Hoek et al. [21] have found a new Ig superfamily member in the mollusc *Lymnaea stagnalis*. This molluscan defense protein is down regulated during parasitosis from *Trichobilharzia ocellata*. Furthermore, with the discovery in Drosophila of a receptor which is a mammalian homolog of the interleukin-1 type 1 receptor containing a highly conserved region in its cytosolic domain, so-called Toll [35], a novel receptor superfamily present in invertebrates and mammals, the IL-1R/toll-like receptor (TLR) superfamily has been defined.

In summary, there is today growing evidence that the nervous and the immune systems can exchange information, mainly through small molecules, either cytokines or neuropeptides. Furthermore, it appears that some so-called neurotransmitters like neuropeptides can function as endogenous messengers of the immune system, and that they most likely play an important part in the regulation of the various components of the immune response. Parasite infections are a very attractive model in immunology since the immune response fails to kill the parasite but still continues to function. We have been interested in the implications of neuroimmunology in models of host–parasite interactions.

2. Vascular neuroimmunology

An exciting new finding by Stefano et al. [78] demonstrates that the distribution of opiate receptors has been broadened to several cell types other than the nervous and immune systems. Based on the well-established hypotensive effect of morphine, they hypothesized in 1996 that endothelial cells may represent a target for this opiate substance. Endothelial cells (human arterial and rat microvascular) contain a high-affinity, saturable opiate binding site presumed to mediate the morphine effects, that is stereoselectively and characteristically antagonized by naloxone [78]. This opiate alkaloid-specific binding site is insensitive to opioid peptides. It is therefore considered to be the same subtype of opiate receptor (designated μ_3) used in the mediation of morphine in other cell types exhibiting the same binding profile [12,31,74]. Experiments with endothelial tissue and aortic ring of rats cultured in vitro demonstrate that morphine exerts direct modulatory control over the activities of endothelial cells which leads to vasodilation. It induces the production of nitric oxide, a process that is sensitive to naloxone antagonism and nitric oxide synthase inhibition [78]. In contrast with that of opiates, the administration of opioid peptides does not induce nitric oxide production by endothelial cells.

In conclusion, the data presented above reveal a novel site of morphine action, endothelial cells, where a μ_3 receptor is coupled to nitric oxide release and vasodilation. In other reports we demonstrate that this induced endothelial downregulation also manifests itself in lowering immunocyte activity, i.e. adhesive actions see [66,74], demonstrating a dynamic communication of immune cells with the endothelium. We surmise this interaction is also of critical significance in host–parasite relationships, since the parasite must not stimulate vascular elements as they in turn will stimulate immune activation.

3. Neuroimmunology and parasitology

Most parasites share their life cycle between several hosts, these being vertebrates, insects or molluscs. It was therefore of interest to investigate the possible commonalities implied in the concept of neuroimmunology and autoimmunoregulation.

Parasitism implies a very precise equilibrium between the parasite and the various microenvironments where each stage of the life cycle takes place. In each biotope the parasite is able to achieve its survival, growth and/or maturation by using a whole set of external and/or self molecules. In the immunocompetent hosts, it has to evade the immune response, mainly through two mechanisms: (i) expression of appropriate antigens, either by changing the expressed antigens fast enough to prevent any efficient immune response (antigenic variation), or by expressing epitopes similar, if not identical, to host molecules (antigen mimicry); (ii) modification of the host immune response (via autoimmunoregulatory similarities), either directly by its own molecules, or indirectly by disregulating the host effector cells [6,9,43,61,84].

In this review, we have focused our attention on the latter mechanism, which has been experimentally demonstrated in parasite models, although the molecular characterization of the active molecules has seldom been achieved. Considering the immunomodulatory properties of POMC-derived peptides and opiate elements, and their phylogenetic conservation, we have postulated and demonstrated their existence in endoparasites, *Schistosoma mansoni*, and in leeches, representing ectoparasites, and suggested their possible implication in the interactions with both hosts.

3.1. Proopiomelanocortin in parasites

3.1.1. In endoparasites: Schistosoma mansoni

In *S. mansoni*, Capron and co-workers identified POMC-related peptides in the main stages of the parasite life cycle (cercariae, schistosomula, adult worms and miracidia). Radioimmunoassays detected molecules related to ACTH,

αMSH and βE in crude extracts of all stages [13,16]. The anti-ACTH antiserum was directed against the ACTH 11–24 region, presenting no cross-reactivity with αMSH. Antibodies anti-βE were mainly directed against the middle portion of the molecule (βLPH 66–91), exhibiting no cross-reactivity with met-enkephalin. The anti-αMSH did not cross-react with ACTH. These specificities suggest that some biological functions of the human molecules might be conserved in the parasite peptides.

Reversed-phase HPLC analysis was performed on a partially purified adult worm extract to analyze the homology of parasite and human βE. Prior the first HPLC run, a perfect co-elution of the two peptides was obtained [15]. In a further experiment, the authors used a shallower gradient allowing the separation of three derivatives of human βE found in the pituitary: βE 1–31, βE 1–27 and N-acetyl βE 1–31 [62]. Under these conditions, the main parasite βE-like molecule was eluted one fraction ahead of βE 1–31. These results allowed the authors to speculate that *S. mansoni* βE is highly homologous to human, although slight differences might lead to different neuroimmunological effects [14]. The presence of three main POMC-derived peptides led to hypothesize the existence of a POMC gene in the parasite. Therefore, using oligonucleotide probes specific for the regions of the genes encoding POMC conserved amino-acid motifs; they demonstrated that related sequences are present in the parasite genome.

In order to assess the implication of the peptides in *S. mansoni* immune evasion, a series of in vitro incubations with the different life stages of the parasite were performed. In the case of cercariae or in vitro transformed schistosomula, no detection of any release of the three considered peptides (ACTH, α MSH and βE) was obtained. The absence of detectable peptide levels might be due to the release of proteases and peptidases from the cercariae. In vitro incubations of adult worms resulted, consistently, in detectable amounts of ACTH and βE in the medium. Comparison of the levels measured inside worms prior to incubation or in the medium indicated that the released peptides resulted most likely from a de novo synthesis [14].

In order to study the interactions with the intermediate host, incubations with miracidia in diluted Eagle's minimum essential medium, a medium known to trigger morphological modifications similar to a transformation into mother sporocysts, were carried out and the peptides in the medium assayed. Under these conditions, ACTH and βE were detected in the medium, whereas no peptides were released when miracidia were incubated in water. In a second approach, a series of molluscs were infested on day 0 by miracidia, and ten to fifteen individuals were taken each day, for 24 days, a delay which allows the development of mature cercariae. Hemolymph and hemocytes were withdrawn from the pericardiac area, separated by centrifugation, and the resulting peptides were assayed in the hemolymph. POMC-derived peptides were detected in all samples, whereas none was found in age-matched healthy molluscs. A striking feature of the results was that the main peptide was MSH during the first 10 days, while ACTH became predominant afterwards. On day 24, only ACTH could be detected in the hemolymph [14]. All detectable peptide concentrations were in the picomolar range; this most likely reflects the local concentration of the signal molecule in the vicinity of the parasite. In general, it is quite hard to detect the in vivo levels of the peptides in the parasites local area.

The authors explained that the apparent discrepancy between the results of in vitro incubations of miracidia and assays in hemolymph from infested molluscs may be accounted for by the following: first, within the snail, host factors regulate the liberation, most likely processing, of MSH, which might be triggered by specific factors. A snail factor modifying the miracidium metabolism has already been identified [11]. Second, it should be considered that the peptides might be either from the parasite or from the host, or both. POMC-derived peptides have first been described in molluscs, particularly in the hemocytes of another planorb [38] and the parasite might induce the release of autocrine factors by the host cells or by the central nervous system, which is known to be influenced by parasite infections. The third hypothesis is that the parasite factors might be modified by the host so as to be transformed into another molecule.

Neutral endopeptidase 24–11 (NEP) is known to neurobiologists as 'enkephalinase' and to immunologists as CALLA (the common acute lymphoblastic leukaemia antigen) or CD10. This molecule is present on the surface of human polymorphonuclear leukocytes and on the surface of invertebrate immunocytes (hemocytes from the bivalve *Mytilus edulis*) [53,79,81]. Using computer-assisted video analysis of cell conformation the authors were able to demonstrate that the neuroimmunological effects of enkephalin on these cells are highly dependent on the expression of NEP. The specific NEP inhibitor, phosphoramidon potentiated by five orders of magnitude the effects of enkephalin on cell conformation [53,54]. More recently, Smith et al. showed that NEP present on these cells is implicated in the conversion of ACTH into an MSH-like peptide. Inhibition by phosphoramidon inhibits this conversion, which is slow and time-dependent [58]. Similarly, in leeches immunocytes, we demonstrated that NEP and angiotensin-converting enzyme (ACE) have a synergic effect on opioid peptides inactivation of ACTH cleavage [49].

Their involvement could explain the presence of high levels of MSH within the infested mollusc whereas only ACTH was detected during in vitro incubations. Two questions had to be addressed: (i) can the parasite ACTH-like molecule be a substrate for host neutral endopeptidase? (ii) is there any NEP on the surface of the host cells? The first question was tested by incubating adult worms,

which liberate ACTH, but not MSH, with host cells exhibiting NEP activity (CD10+) [16]. Incubation of adult worms with human polymorphonuclear leukocytes induced the appearance of MSH and the diminution of the amount of ACTH in the medium. The MSH level was strongly reduced by the presence of phosphoramidon in the ACTH containing medium. Bestatin, another peptidase inhibitor (aminopeptidase), did not change the amount of MSH detected in the medium. Incubation of adult worms with human monocytes, which are devoid of NEP, also did not yield MSH in the medium [14]. From these results, it can be deduced that parasite ACTH is most likely cleavable into a MSH-like molecule by NEP from the human host.

For the intermediate host, *B. glabrata* hemocytes were first tested for their ability to respond to MSH like *M. edulis* immunocytes, namely by inhibition of their activation [71]. They appear to respond to the same doses as the marine mollusc hemocytes [14]. In a second experiment, they were incubated in the presence of ACTH and their conformation was analyzed using the same computer-assisted technique. Within several hours, hemocytes in the ACTH medium appeared to become statistically more inactivated than controls. This ACTH-induced inactivation is never seen in short-period incubations. This inactivation or cellular immunosuppression in less than 1 h is typical of an MSH effect [71]. When phosphoramidon was added to the ACTH containing medium the slow inactivation noted for ACTH was not observed and the hemocytes spontaneously became activated. Captopril, an ACE inhibitor, which does not modify NEP activity, did not affect ACTH activity. Moreover, a set of experiments confirms the above results. In fact, hemocytes from 14-day infected snails were withdrawn from the pericardiac area, and analyzed for their conformation. A large number of the cells were found to be inactivated, a finding which had already been observed by several authors using different techniques [1,82]. These cells were then incubated with anti-ACTH and/or anti-MSH antibodies. Both antibodies reversed the inactivation, anti-MSH being effective in a shorter time period. Incubation with both antisera resulted in the synergestic inhibition of inactivation [17]. This experiment constitutes the direct demonstration of the implication of both peptides, most likely through conversion of one into the other by NEP, in this main mechanism of adaptation of the parasite in *B. glabrata* [2]. Several reports have described an increase in the number of circulating hemocytes during infection of the mollusc. Stefano and co-workers were able to show that this phenomenon can be attributed in *M. edulis* to hemocyte inactivation by MSH, which results in an inhibition of margination [69–71,76].

Taken together, the results strongly favor the hypothesis regarding the role of neuropeptides, specifically, ACTH and MSH in partially modulating the 'masking' of the parasite from the host immune system (Fig. 1). In the definitive host, liberated ACTH appears to exert a direct

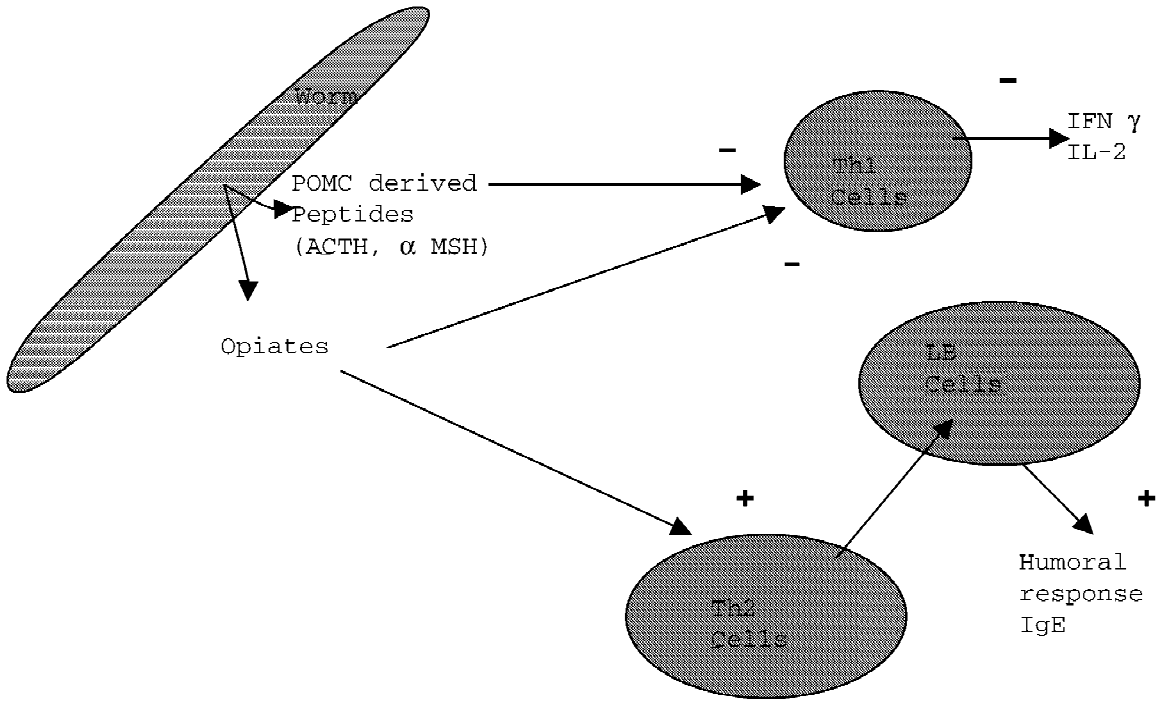

Fig. 1. Host immune modulation by Schistosomes. Production by the schistome of opiates and POMC-derived peptides to escape the host immune response. The transformation of ACTH into an MSH-like molecule would account for the immune evasion of adult worms from many non-specific effector mechanisms. Moreover, during schistosomiasis, after maturation of the worms and oviposition it was observed the decrease of host interferon (IFNγ) and IL-2 by ACTH and MSH worm production. This conducts in the lost of a recruitment of T helper 2 cells (Th2) and a reduction of the Th1 populations.

effect on host cells. In addition, MSH inhibits cells such as monocytes that do not have NEP, thus if they are in the vicinity of a granulocyte and a worm, the immunosuppresory action will be extended thus multiplying the effect. Of particular relevance is the observation of a decrease of IFNγ and IL-2 during schistosomiasis, after maturation of the worms and oviposition [20,41]. It is now suggested that in the mouse this is due to a recruitment of Th2 and a diminution of the Th1 populations [41,42]. Decrease of IFN production by T cells is one of the described effects of ACTH. The transformation of ACTH into an MSH-like molecule, which can exhibit different immunosuppressive properties, could account for the immune evasion of adult worms from many non-specific effector mechanisms.

3.1.2. In exoparasites: leeches (Fig. 2)

In leeches, ectoparasites, we have recently demonstrated that mammalian-like opioid precursors are present [45–49].

Duvaux-Miret et al. [15] first demonstrated the presence of β-endorphin and of a POMC-related gene in *Schistosoma mansoni*. In leeches, we have sequenced a mammalian-like POMC, and six of its derived peptides, including ACTH and MSH, in the immune tissues of the leech *Theromyzon tessulatum*. Of the six peptides, three showed high sequence similarity to their vertebrate counterparts, namely, met-enkephalin, α-MSH and ACTH (100, 84.6 and 70% respectively) whereas γ-MSH, β-endorphin and γ-LPH exhibited only 45, 20 and 10% sequence identity. No dibasic amino acid residues were found at the C-terminus of the γ- and β-MSH peptides. In contrast, the leech α-MSH was flanked at its C-terminus by the Gly–Arg–Lys amidation signal. ACTH and CLIP were also C-terminally flanked by dibasic amino acid residues. The coding region of leech POMC was also reported by RT-PCR using degenerated oligonucleotide primers [49].

Furthermore, their functions, i.e. immune regulatory actions, appear to be conserved as well [66,76,77]. α-MSH from leeches significantly decreased the activity of human granulocytes 20 min after application (2.5±0.8% SEM;

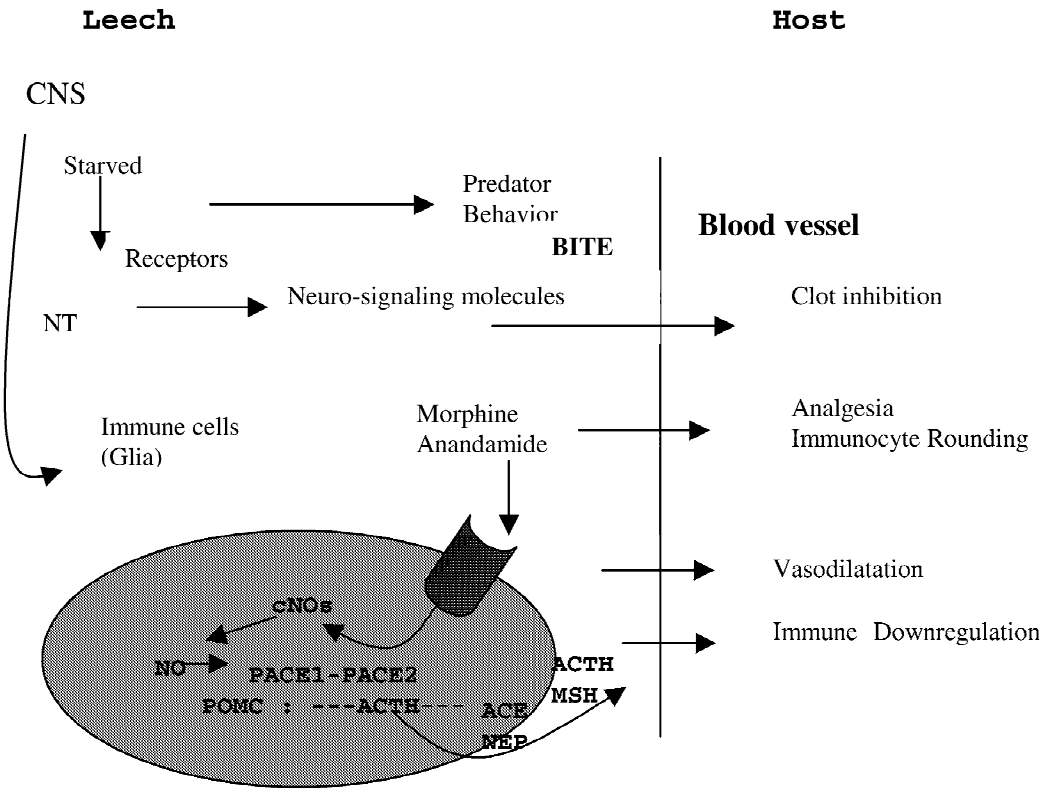

Fig. 2. Possible pathways for host immune response down-regulation by leech chemical signals. We speculate that during the bite, leeches inject substances to block the host pain, inflammation and induce vasodilatation. Normally, a pain stress due to injury will lead to an inflammatory response in the host, with the activation and infiltration of leukocytes. Leeches try to avoid this, as during the months they use to digest their blood meals, it would result in a potentially increased release of blood-degrading enzymes from leukocytes. The challenge for leeches is to block the immediate peripheral nociception and local inflammation during a bite, and to synthesize anticoagulants [50] that maintain the blood in a fluid state during feeding, and subsequently in their digestive tracts. In this regard, a variety of coagulation inhibitors [50], opiates [26], cannabinoids [51], and neuro-signaling molecules (ACTH, MSH) [49] have been isolated. In this context, morphine-like substance or anandamide, after binding to their receptors (μ3 and CB1, respectively) rapidly stimulate NO production by the constitutive NO synthase (cNOS). NO production stimulates POMC, ACE and NEP gene expression at both the transcriptional and translational level and inhibits inducible NOS (iNOS) by inhibiting adenylyl cyclase. This leads to POMC maturation, ACTH release and α-MSH production. By either autocrine or paracrine actions and binding to its receptor (MC-like R), α-MSH down regulates invertebrate immunocytes. Furthermore, in this context, PACE2 and ACE allowed ACTH and α-MSH released from POMC. Moreover, during inflammatory response.

$P<0.01$ vs. $10\pm1.4\%$). Prior treatment of both cell types with anti-MSH for 2 min, followed by MSH, neutralized the leech MSH's inhibitory action [percentage spontaneous activation 10.2 (granulocytes) and 9.4 (immunocytes)] demonstrating that despite the sequence variation from human MSH, it still exhibits specific receptor recognition [48,49].

Taken together, we suggest that leech MSH may be used by the leech internally, and possibly externally, to diminish the host immune response during its blood meal, as is the case for *S. mansoni*.

3.2. Opiate alkaloid and endocannabinoids

Over the last decade, several groups have demonstrated that invertebrates can also, like mammals, make a morphine-like molecule [26,28,52,63]. The Leung [28] study demonstrates that morphine- and codeine-like molecules are present in *S. mansoni* following HPLC separation and identification with an appropriate commercially available antibody. This opiate-like material appears to exist in a conjugated form. Furthermore the endogenous material, corresponding to morphine, mimics authentic morphine in its ability to induce immunocyte rounding and immobility [31,78], an action that is naloxone sensitive. The codeine-like material is not found at high concentrations compared to morphine, indicating, as in mammals and *Mytilus edulis*, its potential rapid conversion to morphine. Coincubation with human leukocytes increases the endogenous level of this material in adult Schistosom worms, indicating the presence of a positive feedback loop. Lastly, EDTA, a chelator of divalent cations, has a strong stimulating effect in the synthesis of morphine-like material by the worm as noted by higher levels of this material in its presence. Taken together, the results suggest that this parasite may utilize this immune downregulating molecule in its effort to escape host immunosurveillance as well as in inhibiting an immune response directed against itself.

Furthermore, naturally occurring morphine can diminish not only immune actions but endothelial as well [66], further reducing the capacity of the host to respond to a parasitic presence. The significance of the worm's ability to make a morphine-like molecule is reinforced by the discovery of a novel opiate receptor designated μ3 [78]. This opiate alkaloid and opioid peptide insensitive receptor is present on both human monocytes and granulocytes and invertebrate immunocytes [31,78] and exerts established morphine-like actions [78]. Thus in the context of the host–parasite relationship we surmise local morphine release would also inhibit an immunocyte response to the worm's presence. It would also serve to keep a limited number of immunocytes in the vicinity of the worm. i.e. granulocyte with NEP. In so doing it would not only passively maintain immunosuppression, but actively reinforce it as well.

In addition to these studies we also surmise that both ecto- and endo-parasites, in addition to controlling the host immune tissues must also control the host vascular endothelium as well, since this may also impact on immune excitation [74]. Interestingly, we have recently demonstrated that human vascular endothelia contain anandamide, a naturally occurring endocannabinoid, also with the CB1 receptor that is coupled do to NO release [10], suggesting vascular downregulation [74].

In leeches, central and immune systems evidence was given using immunocytochemistry, binding experiments, nitric oxide release measurement and molecular biology that μ-type receptors are present [26]. Moreover, evidence was provided by HPLC coupled to electrochemical detection (500 mV and 0.02 Hz), immunoassay assay with anti-morphine, nitric oxide release detection with amperometric system and cellular activity modulation tests, that a morphine-like substance exists endogenously in leeches [26]. Based on the evidence of endogenous morphine-like substance, we speculated that in both the immune and nervous system opiates, after a latency period, acts as a general down regulating signal molecule. This hypothesis was then strengthened by injecting leeches with lipopolysaccharides, a potent immune and neural stimulating agent derived from bacteria. After a prolonged latency period of 24 h ganglionic morphine-like levels statistically increase (from 2.4 ± 1.1 to 78 ± 12.3 pmol/mg ($P<0.005$, LPS injected 1 μg/ml) in a concentration and time-dependent manner demonstrating the involvement of opiates in leech neuroimmunity [26].

Similarly, several long chain acylethanolamides, including anandamide and palmitoyl-ethanolamide (as well as their putative biosynthetic precursors, the *N*-acyl-phosphatidyl-ethanolamines), were found in lipid extracts of leech brain [39]. Moreover, a CB_1-like cannabinoid receptor was identified in both immunocytes and microglia of leeches [Matias, submitted for publication]. As in mammals, the activation of this receptor is coupled to NO release and its effects are inhibited by the nitric oxide synthase (NOS) inhibitor *N*-omega-nitro-L-arginine methyl ester (L-NAME) [51,65]. Furthermore, neural tissues from leeches also contain high affinity anandamide receptors that are coupled to NO release [51,65]. It has been demonstrated in these tissues that another cannabinoid agonist, CP 55940, can stimulate NO release whereas SR 141716A, a selective CB_1 antagonist, blocks this effect [51,65]. These findings suggest that anandamide may be a physiological stimulant of NO release in invertebrate ganglia by acting on cannabinoid receptors, an effect that results in the modulation of neurotransmitter release. An enzymatic activity capable of catalyzing the hydrolysis of anandamide, and displaying pH dependency and inhibitor sensitivity profiles similar to those of mammalian FAAH was also described in leeches [39]. These data indicate that preventing the breakdown of anandamide prolongs its activity in these invertebrate tissues, and, therefore, that FAAH-like amidases may serve to terminate anandamide

action under physiological conditions also in invertebrate ganglia. Recently, two antisera directed against the highly conserved active site of rat FAAH and the extracellular loop of the rat CB_1, respectively, have allowed us to co-localize FAAH and CB_1 immunoreactivity in leech neurons and immunocytes. Furthermore, measurable levels of anandamide, 2-AG and NArPE, and a FAAH-immunoreactive band of 42–44 kDa, were detected in leech ganglia [39]. These data suggest the existence of a complete cannabinoid system in leeches.

Moreover anandamide, like morphine, inhibits cytokines and neurotransmitters though cyclase adenylate inhibition, demonstrating a feedback control of the inflammatory response in leech central nervous system [80]. We have also demonstrated similar effects of morphine and anandamide on ACTH levels; this phenomenon is receptor dependent. They increased proteolytic processing of both the ACTH precursor and of ACTH peptide through nitric oxide release from leech immunocytes [49,80].

Taken together this suggests that these ectoparasites may simultaneously down regulate host immune and vascular processes. Moreover during the bite, leeches inject substances able to block the host pain. In fact, a nociceptive stress due to the injury will lead to an inflammatory response with a great amount of leucocytes. Leeches try to avoid this scenario which will provoke in the intestine, during months used to digest the blood meal, a release of blood-degrading enzymes contained in leucocytes. So, the challenge for leeches is to block the peripheral nociception immediately during the bite. In this context, the production of opiates like morphine and anandamide, known to be antinociceptive [32,66] is a survival strategy to escape host–immune defense. This also true for endoparasites. *S. mansoni* which thrive in the host vasculature; a similar process may also occur there, especially since these worms appear to contain a morphine-like molecule [28], that we have also shown to be coupled to NO release. Thus, using common host signal molecules that have been conserved during evolution, parasites by serving as the source of the signal molecule can intervene in normal host processes involving these signals. Furthermore, given their presence in divergent animals, the parasite requires little new DNA messaging to interact.

3.3. Behavioral evidence

Evidence that *S. mansoni* may release opioid neuropeptides and or opiate alkaloids into its vertebrate host comes from behavorial studies on parasitic infections. Hamsters infected with *S. mansoni* display heightened thresholds to thermal stimulation suggestive of analgesia. This analgesia has been shown to be inhibited by administration of the opioid antagonist naloxone. *S. mansoni* infection also affects locomotor activity levels of hamsters in a bimodal way. This bimodal activity pattern resembles that induced by low and high doses of morphine in hamsters. The schistosome induced enhancement of locomotor activity in hamsters is blocked by injection of the specific antagonist toward κ-opiate receptors, ICI 154,129, while the later decrease in locomotor activity is blocked by naloxone [24,25]. This pattern of antagonism is parallel to that for low and high morphine doses given to hamsters. The effects of these signal substances give supportive evidence that the parasitic effects on behavior may indeed be neuromodulatory and show that schistosome infection can lead to a differential augmentation of endogenous opioid activity. Such an effect has already been observed in the model of rodent infection by the cestode *Spirometra mansonoides*.

Sedation and a general decrease in the responsiveness of host to stimuli is also seen in animals experimentally infected with other parasites. A protozoan infection of *Toxoplasma gondi* causes reduced activity and less response to novel stimuli in mice. The activity levels were also reduced in rats and the mountain vole, *Microtus montanus* by infection with *Trypanosoma gambiense*. Infection with the nematode *Trichinella spiralis* also results in the reduction of locomotory activity and modifications of open field behaviors in mice. Mice experimentally infected with *Eimeria vermiformis* showed behavorial effects similar to hamsters infected with *S. mansoni* which were blocked by the opiate antagonist naloxone [24,25]. From these reports, it is likely that opiate like substances may be secreted by a wide variety of parasites and may play an important role in many parasitic diseases.

4. Conclusion

During the last 2 decades, a number of mammalian-like or identical signal molecules have been identified in different parasite species. The presence of POMC-like derived peptides (such like ACTH, a MSH or bE), morphine-like substances (codeine, morphine sulfate) or endocannabinoids (anandamide, 2 AG) in endo- and ectoparasites demonstrates that the fields of neuroimmunology and parasitology overlap, highlighting the significance of these signal molecules in autoimmunoregulatory processes since parasites are usually found highly localized in a host. It also demonstrates that the immunosuppressive function of these molecules during the course of evolution till human appears to have been retained. Moreover, the dynamic communication of immune cells with the endothelium revealed that such a microenvironment is also of critical significance in the host–parasite relationship, since the parasite must not stimulate vascular elements as they in turn will stimulate immune activation. The number of survival strategies mounted by parasites, for instance, reflects their adaptive plasticity as well as the complexity of their life cycle. These adaptive strategies converge at one point where they bring about a state of tolerance, in which dual modifications in reply both to the host response

and parasite immunogenicity, reduce any disparity between the two partners. Among adaptive strategies which are essential to developing genuine host molecular parasitism are: molecular mimicry, appropriation and capture of signals, lures of cell communication and integrated language [48].

Acknowledgements

This work was in part supported by the Centre National de la Recherche Scientifique, FEDER, Conseil Regional Nord-Pas de Calais and the NIH Fogarty INT 00045 grant.

References

[1] J.M. Abdul-Salam, E.H. Michelson, *Biomphalaria glabrata* amoebocytes: effect of *Schistosoma mansoni* on in vitro phagocytosis, J. Invert. Pathol. 35 (1980) 241–248.
[2] C.J. Bayne, T.P. Yoshino, Determinants of compatibility in mollusc-trematode parasitism, Am. Zool. 29 (1989) 399–407.
[3] J.E. Blalock, E.M. Smith, A complete loop between the immune and neuroendocrine system, Fed. Proc. 44 (1985) 108–111.
[4] J.E. Blalock, A molecular basis for bidirectional communication between the immune and neuroendocrine systems, Physiol. Rev. 69 (1989) 1–32.
[5] J.E. Blalock, E.M. Smith, Human leukocyte interferon: structure and biological relatedness to adrenocorticotropic hormone and endorphins, Proc. Natl. Acad. Sci. USA 77 (1980) 5972–5974.
[6] A. Capron, J.P. Dessaint, Le language moleculaire des parasites, Med. Sci. 15 (1995) 431–439.
[7] A. Capron, J.P. Dessaint, Molecular basis of host–parasite relationship: toward the definition of protective antigens, Immunol. Rev. 112 (1989) 27–48.
[8] A. Capron, J.M. Balloul, D. Grezel, J.M. Grzych, I. Wolowczuk, C. Auriault, D. Boulanger, M. Capron, R.J. Pierce, Progress toward vaccine against schistosomiasis, in: H. Kiyono, E. Jirillo, C. De Simone (Eds.), Molecular Aspects of Immune Response and Infectious Diseases, Raven Press, New York, 1990, pp. 183–191.
[9] A. Capron, J.P. Dessaint, M. Capron, J.H. Ouma, A.E. Butterworth, Immunity towards schistosomes: progress toward vaccine, Science 238 (1987) 1065–1072.
[10] D.G. Deutsch, M.S. Goligorsky, P.C. Schmid, R.J. Krebsbach, H.H. Schmid, S.K. Das, S.K. Dey, G. Arreaza, C. Thorup, G. Stefano, L.C. Moore, Production and physiological actions of anandamide in the vasculature of the rat kidney, J. Clin. Invest. 100 (1997) 1538–1546.
[11] C. Dissous, J.M. Grzych, A. Capron, *Schistosoma mansoni* shares a protective oligosaccharide epitope with freshwater and marine snails, Nature 323 (1986) 443–445.
[12] K. Dobrenis, M.H. Makmann, G.B. Stefano, Occurrence of the opiate alkaloid-selective mu3 receptor in mammalian microglia, astrocytes and Kupffer cells, Brain Res. 686 (2) (1995) 239–248.
[13] O. Duvaux-Miret, A. Capron, Proopiomelanocortin gene and gene products: a comparative study, Adv. Neuroimmunol. 1 (1991) 41–57.
[14] O. Duvaux-Miret, A. Capron, Proopiomelanocortin in the helminth *Schistosoma mansoni*. Synthesis of β-endorphin, ACTH and α-MSH. Existence of POMC-related sequences, Ann. NY Acad. Sci. 650 (1992) 245–250.
[15] O. Duvaux-Miret, C. Dissous, J.P. Gautron, E. Pattou, C. Kordon, A. Capron, The helminth *Schistosoma mansoni* expresses a peptide similar to human β-endorphin and possesses a POMC-related gene, New Biologist 2 (1990) 93–99.
[16] O. Duvaux-Miret, M.K. Leung, A. Capron, G.B. Stefano, An enkephalinergic system in *Schistosoma mansoni* that may participate in intra-and extra-organismic signaling, Exp. Parasit. 76 (1993) 76–84.
[17] O. Duvaux-Miret, G.B. Stefano, E.M. Smith, C. Dissous, A. Capron, Immunosuppression in the definitive and intermediate hosts of the human parasite *Schistosoma mansoni* by release of immunoactive neuropeptides, Proc. Natl. Acad. Sci. USA 89 (1992) 778–781.
[18] O. Duvaux-Miret, G.B. Stefano, E.M. Smith, A. Capron, Neuroimmunology of host–parasite interactions: proopiomelanocortin derived peptides in the infection by *Schistosoma mansoni*, Adv. Neuroimmunol. 2 (1992) 297.
[19] O. Duvaux-Miret, G.B. Stefano, E.M. Smith, L. Mallozzi, A. Capron, Proopiomelanocortin-derived peptides as tools of immune evasion for the human trematode *Schistosoma mansoni*, Acta Biol. Hung. 43 (1992) 281–286.
[20] J.M. Grzych, E.J. Pearce, A. Cheever, Z.A. Caulada, P. Caspar, S. Heiny, F. Lewis, A. Sher, Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis, J. Immunol. 146 (1991) 1322–1327.
[21] R.M. Hoek, A.B. Smit, H. Frings, J.M. Vink, M. de Jong-Brink, W.P. Geraerts, A new Ig superfamily member, molluscan defence molecule (MDM) from Lymnaea stagnalis, is down-regulated during parasitosis, Eur. J. Immunol. 26 (1996) 939–944.
[22] T.K. Hughes, R. Chin, E.M. Smith, M.K. Leung, G.B. Stefano, Similarities of signal systems in vertebrates and invertebrates: detection, action, and interactions of immunoreactive monokines in the mussel, *Mytilus edulis*, Adv. Neuroimmunol. 1 (1991) 59–70.
[23] T.K. Hughes, E.M. Smith, P. Cadet, J. Sinisterra, M.K. Leung, M.A. Shipp, B. Scharrer, G.B. Stefano, Interaction of immunoactive monokines (IL-1 and TNF) in the bivalve mollusc *Mytilus edulis*, Proc. Natl. Acad. Sci. USA 87 (1990) 4426–4429.
[24] M. Kavaliers, R.B. Podesta, M. Hirst, B. Young, Evidence for the activation of the endogenous opiate system in hamsters infected with human blood flukes, *Schistosoma mansoni*, Life Sci. 35 (1984) 2365–2373.
[25] M. Kavaliers, R. Podesta, Opioid involvement in parasite-induced behavioral modifications: evidence from hamsters infected with *Schistosoma mansoni* Can, J. Zool. 66 (1988) 2653–2657.
[26] V. Laurent, B. Salzet, M. Verger-Bocquet, F. Bernet, M. Salzet, Morphine-like substance in leech ganglia: neuroimmune implications, Eur. J. Biochem. 267 (2000) 2354–2362.
[27] D. LeRoith, A.S. Liotta, J. Roth, J. Shiloach, M.E. Lewis, C.B. Pert, D.T. Krieger, Corticotropin and β-endorphin materials are native to unicellular organisms, Proc. Natl. Acad. Sci. USA 79 (1982) 2086–2090.
[28] M.K. Leung, C. Dissous, A. Capron, H. Woldegaber, O. Duvaux-Miret, S. Pryor, G.B. Stefano, Schistosoma mansoni: the presence and potential use of opiate-like substances, Exp. Parasitol. 81 (1995) 208–215.
[29] S.J. Lolait, J.A. Clements, A.J. Marckwick, C. Cheng, M. McNally, A.I. Smith, J.W. Funder, Proopiomelanocortin messenger ribonucleic acid and post- translational processing of β-endorphin in spleen macrophages, J. Clin. Invest. 77 (1986) 1776–1779.
[30] S.J. Lolait, A.T.W. Lim, B.H. Toh, J.W. Funder, Immunoreactive β-endorphin in a subpopulation of spleen macrophages, J. Clin. Invest. 73 (1984) 277–280.
[31] M.H. Makman, K. Dobrenis, S. Downie, W.D. Lyman, B. Dvorkin, Presence of opiate alkaloid-selective mu3 receptors in cultured astrocytes and in brain and retina, Adv. Exp. Med. Biol. 402 (1996) 23–28.
[32] J. Manzanares, J. Corchero, J.A. Fuentes, Opioid and cannabinoid receptor-mediated regulation of the increase in adrenocorticotropin hormone and corticosterone plasma concentrations induced by central administration of δ-(9)-tetrahydrocannabinol in rats, Brain Res. 839 (1999) 173–179.

[33] J.E. Merrill, E.N. Benveniste, Cytokines in inflammatory brain lesions: helpful and harmful, Trends Neurosci. 19 (1996) 331–338.
[34] E.L. Oates, G.P. Allaway, G.R. Armstrong, R.A. Boyajian, J.K. Kehrl, B.S. Prabhakar, Human lymphocytes produce proopiomelanocortin gene-related transcripts, J. Biol. Chem. 263 (1988) 10041–10044.
[35] L.A.J. O'Neill, C.A. Dinarello, The IL-1 receptor/toll-like receptor superfamily: crucial receptors for inflammation and host defense, Immunol. Today 21 (2000) 206–209.
[36] E. Ottaviani, C. Franceschi, The invertebrate phagocytic immunocyte: clues to a common evolution of immune and neuroendocrine systems, Immunol. Today 18 (1997) 169–174.
[37] E. Ottaviani, E. Caselgrandi, M. Bondi, A. Cossarizza, D. Monti, C. Franceschi, The immune mobile brain: evolutionary evidence, Adv. Neuroimmunol. 1 (1991) 27–40.
[38] E. Ottaviani, F. Petraglia, G. Montagnani, A. Cossarizza, D. Monti, C. Franceschi, Presence of ACTH and β-endorphin immunoreactive molecules in the freshwater snail *Planorbarius corneus* (L.) (*Gastropoda pulmonata*) and their possible role in phagocytosis, Regul. Pept. 27 (1990) 1–9.
[39] I. Matias, T. Bisogno, D. Melck, F. Vandenbulcke, C. Breton, V. Di Marzo, M. Salzet, Evidence for a cannabinoid system in the central nervous system of the leech Hirudo medicinalis, Eur. J. Biochem. 2000, submitted for publication.
[40] L.R. Paemen, E. Porchet-Hennere, M. Masson, M.K. Leung, T.K. Hughes, G.B. Stefano, Glial localization of interleukin-1a in invertebrate ganglia, Cell. Mol. Neurobiol. 12 (1992) 463–472.
[41] E.J. Pearce, A. Sher, Functional dichotomy in CD4+T cell response to *Schistosoma mansoni*, Exp. Parasitol. 73 (1991) 110–116.
[42] E.J. Pearce, P. Caspar, J.M. Grzych, F.A. Lewis, A. Sher, Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth *Schistosoma mansoni*, J. Exp. Med. 173 (1991) 159–166.
[43] B. Salzet, M. Verger-Bocquet, G.B. Stefano, M. Salzet, Putative leech dopamine1-like receptor molecular characterization: sequence homologies between dopamine and serotonin leech CNS receptors explain pharmacological cross-reactivities, Mol. Brain Res. 58 (2) (1998) 47–58.
[44] M. Salzet, G.B. Stefano, Evidence for an invertebrate neuroendocrine system: neuropeptide processing in leech–host communication, Trends Comparat. Biochem. Physiol. 5 (1998) 85–98.
[45] M. Salzet, G.B. Stefano, Biochemical evidence for a dynorphin precursor in invertebrates, Mol. Brain Res. 52 (1997) 47–55.
[46] M. Salzet, G.B. Stefano, First biochemical evidence for an enzyme related to mammalian renin in an invertebrate the leech *Theromyzon tessulatum*, Mol. Cell. Endocrinol. 131 (1997) 1–8.
[47] M. Salzet, G.B. Stefano, Isolation and biochemical characterization of proenkephalin from invertebrate immunocytes: δ-opioid binding sites in leech immunocytes, Brain Res. 768 (1997) 224–232.
[48] M. Salzet, A. Capron, G.B. Stefano, Molecular cross talk in host parasite relationships: schistosomes or leeches–host interactions, Parasitol. Today, in press.
[49] M. Salzet, B. Salzet, M. Cocquerelle, Verger-Bocquet, S. Pryor, V. Laurent, G.B. Stefano, Biochemical and molecular characterization of ACTH, its precursor and receptor in the *leech Theromyzon tessulatum*: morphine increases ACTH levels, J. Immunol. 159 (1997) 5400–5411.
[50] M. Salzet, D. Vieau, G.B. Stefano, Serpins: an evolutionarily conserved survival strategy, Immunol. Today 20 (1999) 541–544.
[51] M. Salzet, T. Bisogno, C. Breton, V. Di Marzo, Comparative biology of endogenous Endocannabinoids system: possible role in immune response, Eur. J. Biochem. 267 (2000) 4917–4927.
[52] B. Scharrer, Neuroimmunology: the importance and role of a comparative approach, Adv. Neuroimmunol. 1 (1991) 1–6.
[53] M.A. Shipp, G.B. Stefano, L. D'Adamio, S.N. Switzer, F.D. Howard, J. Sinisterra, B. Scharrer, E. Reinherz, Downregulation of enkephalin-mediated inflammatory responses by CD10/neutral endopeptidase 24.11, Nature 347 (1990) 394–396.
[54] M.A. Shipp, G.B. Stefano, S.N. Switzer, J.D. Griffin, E.L. Reinherz, CD10 (CALLA)/Neutral endopeptidase 24.11 modulates inflammatory peptide-induced changes in neutrophil morphology, migration, and adhesion proteins and is itself regulated by neutrophil activation, Blood 78 (1991) 1834–1841.
[55] N.E. Sibinga, A. Goldstein, Opioid peptides and opioid receptors in cells of the immune system, Ann. Rev. Immunol. 6 (1988) 219–249.
[56] E.M. Smith, J.E. Blalock, Human lymphocyte production of ACTH and endorphin-like substances: association with leukocyte interferon, Proc. Natl. Acad. Sci. USA 78 (1981) 7530–7534.
[57] E.M. Smith, F.S. Galin, R.D. LeBoeuf, D.H. Coppenhaver, D.V. Harbour, J.E. Blalock, Nucleotide and amino acid sequence of lymphocyte-derived corticotropin: endotoxin induction of a truncated peptide, Proc. Natl. Acad. Sci. USA 87 (1990) 1057–1061.
[58] E.M. Smith, T.K. Hughes, F. Hashemi, G.B. Stefano, Immunosuppressive effects of ACTH and MSH and their possible significance in human immunodeficiency virus infection, Proc. Natl. Acad. Sci. USA 89 (1992) 782–786.
[59] E.M. Smith, T.K. Hughes, M.K. Leung, G.B. Stefano, The production and action of ACTH-related peptides in invertebrate hemocytes, Adv. Neuroimmunol. 1 (1991) 7–16.
[61] S.R. Smithers, D.J. McLaren, F.J. Ramalho-Pinto, Immunity to schistosomes: the target, Am. J. Trop. Med. Hyg. (suppl.) 26 (1977) 11.
[62] D. Smyth, D.E. Massey, S. Zakarian, M.D.A. Finnie, Endorphins are stored in biologically active and inactive forms: isolation of α-N-acetylated peptides, Nature 279 (1979) 252–254.
[63] D. Sonetti, L. Mola, F. Casares, E. Bianchi, M. Guarna, G.B. Stefano, Endogenous morphine levels increase in molluscan neural and immune tissues after physical trauma, Brain Res. 835 (1999) 137–147.
[64] G.B. Stefano, M. Salzet, Invertebrate opioid precursors: evolutionary conservation and the significance of enzymatic processing, Int. Rev. Cytol. 187 (1999) 261–285.
[65] G.B. Stefano, B. Salzet-Raveillon, M. Salzet, Leech CNS cannabinoid receptor is coupled to nitric oxide release: high sequence homology with mammals, Brain Res. 753 (1997) 219–224.
[66] G.B. Stefano, B. Scharrer, E.M. Smith, T.K. Hugues, H.I. Magazine, T.V. Bilfinger, A. Hartman, G.L. Fricchione, Y. Liu, M.H. Makman, Opioid and opiate immunoregulatory processes, Crit. Rev. Immunol. 16 (1996) 109–144.
[68] G.B. Stefano, Role of opioid neuropeptides in immunoregulation, Prog. Neurobiol. 33 (1989) 149–159.
[69] G.B. Stefano, P. Cadet, B. Scharrer, Stimulatory effects of opioid neuropeptides on locomotory activity and conformational changes in invertebrate and human immunocytes: evidence for a subtype of δ-receptor, Proc. Natl. Acad. Sci. USA 86 (1989) 6307–6311.
[70] G.B. Stefano, M.K. Leung, X. Zhao, B. Scharrer, Evidence for the involvement of opioid neuropeptides in the adherence and migration of immunocompetent invertebrate hemocytes, Proc. Natl. Acad. Sci. USA 86 (1989) 626–630.
[71] G.B. Stefano, D.E. Smith, D. Smith, T.K. Hughes, MSH can deactivate both TNF stimulated and spontaneously active immunocytes, in: H. Boer, A. ter Maat (Eds.), Molluscan Neurobiology, Elsevier–North Holland, Amsterdam, 1992.
[72] G.B. Stefano, E.M. Smith, T.K. Hughes, Opioid induction of immunoreactive interleukin 1 in *Mytilus edulis* and human immunocytes: an interleukin1 like substance in invertebrate neural tissue, J. Neuroimmunol. 32 (1991) 29–34.
[73] G.B. Stefano, W. Zhao, D. Bailey, M. Metlay, M.K. Leung, High affinity dopamine binding to mouse thymocytes and *Mytilus edulis* (Bivalvia) hemocytes, J. Neuroimmunol. 21 (1989) 67–74.
[74] G.B. Stefano, Autoimmunovascular regulation: morphine and anandamide stimulated nitric oxide release, J. Neuroimmunol. 83 (1999) 70.
[76] G.B. Stefano, Conformational matching: a determining force in maintaining signal molecules, in: G.B. Stefano (Ed.), Comparative

Opioid and Related Neuropeptides Mechanisms, Vol. 2, CRC Press, Boca Raton, FL, 1986, pp. 271–277.
[77] G.B. Stefano, R.M. Kream, R.S. Zukin, Demonstration of stereospecific opiate binding in the nervous tissue of the marine mollusc *Mytilus edulis*, Brain Res. 181 (1980) 445–450.
[78] G.B. Stefano, M.K. Leung, T.V. Bilfinger, B. Scharrer, Effect of prolonged exposure to morphine on responsiveness of human and invertebrate immunocytes to stimulatory molecules, J. Neuroimmunol. 63 (1995) 175–181.
[79] G.B. Stefano, B. Salzet-Raveillon, M. Salzet, *Mytilus edulis* hemolymph contains pro-opiomelanocortin: LPS and morphine stimulate differential processing, Mol. Brain Res. 63 (1998) 340–348.
[80] G.B. Stefano, C.M. Rialas, D.G. Deutsch, M. Salzet, Anandamide amidase inhibition enhances anandamide-stimulated nitric oxide release in invertebrate neural tissues, Brain Res. 793 (2) (1997) 341–345.
[81] A. Turner, M.K. Leung, G.B. Stefano, Peptidases of significance in neuroimmunoregulation, in: B. Scharrer, E.M. Smith, G.B. Stefano (Eds.), Neuropeptides in Neuroimmunology, Springer-Verlag, 1994, pp. 152–169.
[82] W.P.W. Van der Knaap, E.A. Meuleman, T. Sminia, Alterations in the internal defense system of the pond snail *Lymnea stagnalis* induced by infection with the schistosoma *Trichobilharzia ocellata*, Parasitol. Res. 73 (1987) 57–65.
[83] D.E. VanEpps, M.M. Mason, Modulation of leukocyte migration by alpha-melanocyte stimulating hormone, in: E. Florey, G. Stefano (Eds.), Comparative Neuropeptide Pharmacology, Manchester University Press, Manchester, 1990, pp. 335–345.
[84] M.F. Wahab, K.S. Warren, R.P. Levy, Function of the thyroid and the host parasite relation in murine *Schistosomasis mansoni*, J. Inf. Dis. 124 (1971) 161–171.
[85] D.A. Weigent, J.E. Blalock, Production of peptide hormones and neurotransmitters by the immune system, Neuroimmunoendocrinology 69 (1997) 1–30.
[86] D.A. Weigent, J.E. Blalock, Interactions between the neuroendocrine and immune systems: common hormones and receptors, Immunol. Rev. 100 (1987) 79–98.
[87] J. Wybran, T. Appelboom, J.P. Famaey, A. Govaerts, Suggestive evidence for receptors for morphine and methionine-enkephalin on normal human blood T lymphocytes, J. Immunol. 123 (3) (1979) 1068–1070.