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The endocannabinoid system in invertebrates

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Summary What is the role of the cannabinoid system in invertebrates and can it tell us something about the human system? We discuss in this review the possible presence of the cannabinoid system in invertebrates. Endocannabinoid processes, i.e., enzymatic hydrolysis, as well as cannabinoid receptors and endocannabinoids, have been identified in various species of invertebrates. These signal molecules appear to have multiple roles in invertebrates; diminishing sensory input, control of reproduction, feeding behavior, neurotransmission and antiinflammatory actions. We propose that since this system worked so well, it was retained during evolution, and that invertebrates can serve as a model to study endogenous cannabinoid signaling.

INTRODUCTION

In recent years many studies have emerged demonstrating that particular signaling molecules and their corresponding receptors, i.e., the opioid system, have been conserved during evolution.[1,2] We are now extending this concept to include cannabinoid-signaling molecules.

In the mammalian brain, cannabinoid 1 receptor (CB1-R) was cloned and sequenced in 1990,[3] and evidence for a second CB2-R was obtained in 1993.[4] Several studies suggest that anandamide is the endogenous agonist of CB1-R.[5–7] Anandamide mimics most of the pharmacological and behavioral actions associated with cannabinoids, i.e., Δ^9-tetrahydrocannabinol (THC).[5] Furthermore, enzymes for the synthesis and degradation of this signaling molecule have been found in mammals.[8] Studies on anandamide amidohydrolase (anandamide amidase, fatty acid hydralase (FAAH)), responsible for the hydrolysis of anandamide,[8,9] have shown that the enzyme may catalyze the hydrolysis of another putative neuroactive amide, the sleep-inducing oleoyl-amide.[5] CB1-R also has been found in immune cells,[4] which also synthesize anandamide.[10,11] Like THC, the active ingredient of marijuana,[6] endogenous anandamide inhibits several aspects of immunocyte function; it down-regulates macrophage contact-dependent cytolysis of tumor cells,[12] alters antigen processing[13] and modulates the expression of select macrophage-associated proteins.[11,14]

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A CANNABINOID SYSTEM IN INVERTEBRATES

In invertebrates, the first example of an effect by cannabinoids was reported by Acosta-Urquidi and Chase.[15] They showed that in isolated buccal and parieto-visceral ganglia of *Aplysia californica*, THC causes a depression in nerve cell excitability that is consistent with its reported effects in mammals. In 1976, McClean and Zimmerman[16] showed in *Tetrahymena pyriformis* GL that THC elicited a dose-dependent effect on form/movement, cellular growth and division in log growth phase. This effect was accompanied by up-regulation of cAMP levels in synchronously dividing *Tetrahymena*.[17]

In the sea urchin *Strongylocentrotus purpuratus* cannabinoids (THC and cannabidiol (CBD)) reduce the fertilizing capacity of sperm cells.[18,19] Pretreatment of sperm with THC prevents, in a dose and time-dependent manner, the triggering of the acrosome reaction by solubilized egg jelly. The motility of THC-treated sperm is not reduced compared to control sperm in seawater or vehicle dissolved in seawater. The inhibitory effects of THC on the acrosome reaction and sperm-fertilizing

capacity are reversible. THC blocks the acrosome reaction by affecting event(s) in the sperm stimulation–secretion coupling mechanism that precedes the opening of ion channels. THC, CBD and cannabinol (CBN) also block the membrane fusion reaction between the sperm plasma membrane and the acrosomal membrane that normally is elicited in response to stimulation by the egg jelly. However, lipid deposits are found in the subacrosomal and centriolar fossae of cannabinoid-treated sperm. The nuclear envelope is fragmented in close proximity to the lipid deposits within the subacrosomal fossa. These data suggested that cannabinoids may activate phospholipase(s) within the sperm. This hypothesis was confirmed by biochemical studies which demonstrate that THC activates phospholipase A2 activity in sperm homogenates.[18–20] Finally in 1993, Schuel et al. provided additional evidence that a cannabinoid receptor and its natural ligand anandamide in sperm play a role in blocking the acrosome reaction. Binding of anandamide to the cannabinoid receptor modulates the stimulus–secretion coupling in sperm by affecting an event prior to ion channel opening.[18,19] Further evidence for the existence in sea urchins of an endogenous cannabinoid system was provided by Bisogno et al.,[21] who showed the presence of anandamide and two related acyl-ethanolamides (palmitoyl- and stearoyl-ethanolamides), as well as enzymatic activities potentially responsible for their biosynthesis and degradation, in ovaries of *Paracentrotus lividus* (Table 1). This tissue contains lipid components with the same chromatographic behavior as the *N*-acyl-phosphatidyl-ethanolamines, the phospholipid precursors of acyl-ethanolamides in mammalian tissues that are capable of releasing anandamide, palmitoyl- and stearoyl-ethanolamides upon digestion with *S. chromofuscus* phospholipase D. Accordingly, whole homogenates from *P. lividus* contained an enzymatic activity capable of converting synthetic [^{3}H]*N*-arachidonoyl-phosphatidyl-ethanolamine into [^{3}H]anandamide. Moreover, mature ovaries of *P. lividus* were also shown to contain an amidohydrolase activity which catalyzes the hydrolysis of anandamide and palmitoylethanolamide to ethanolamine and the corresponding fatty acids.[21] This enzyme displayed subcellular distribution, pH/temperature dependency profiles and sensitivity to inhibitors similar but not identical to those of the 'anandamide amidohydrolase' (later named 'fatty acid amide hydrolase') previously described in mammalian tissues. These data support the hypothesis that anandamide or related substances may be oocyte-derived cannabimimetic regulators of sea urchin fertility (see ref. 4). Further support to this hypothesis was recently provided by Berdyshev,[2] who found that also oleoyl- and linoleoyl-ethanolamide, but not palmitoylethanolamide, inhibit sea urchin sperm fertilizing capacity. The effect was also exerted by THC and other cannabinoid agonists but could not be blocked by the CB1-R antagonist SR141716A, suggesting that the sea urchin sperm cannabinoid receptor is different from CB1-R.

Several long-chain acylethanolamides, including endogenous anandamide and palmitoylethanolamide (as well as some of their putative biosynthetic precursors, the *N*-acyl-phosphatidylethanolamines), were found in lipid extracts of bivalve mollusks.[22] Analogous to observations in mammalian brain,[23] the amounts of these metabolites, the most abundant being palmitoylethanolamide and stearoylethanolamide, appeared to increase considerably when mussels were extracted 24 h after post-mortem. Supporting the presence of cannabinoid signaling in invertebrates is the demonstration, in particulate fractions of membrane homogenates from *Mytilus edulis*, of a highly selective cannabinoid receptor with an immunomodulatory function. In fact, a CBR-1-like cannabinoid receptor is present on immunocytes and microglia from mussels that is coupled to nitric oxide (NO) release[24] (Table 1) and whose actions are inhibited by the nitric oxide synthase (NOS) inhibitor *N*-omega-nitro-L-arginine methyl ester (L-NAME[25]). Furthermore, neural tissues from *Mytilus* also contain high-affinity anandamide receptors that are coupled to NO release.[26] It has been demonstrated in these tissues that another cannabinoid agonist, CP55940, can stimulate NO release whereas SR141716A, a selective CB1-R antagonist, blocks NO release in invertebrate and human tissues.[27–29] These findings suggest that anandamide may be a physiological modulator of NO release in invertebrate ganglia via cannabinoid receptors. 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 for *Mytilus*.[30] The enzyme K_m and V_{max} for anandamide were 29.6 μM and 73 pmol/mg protein/min, respectively. Interestingly, in *Mytilus*, usually inactive concentrations of anandamide (10^{-8} and 10^{-7} M) became effective in releasing a substantial amount of NO in the presence of the specific anandamide amidase inhibitor MAFP (2 nM). The inhibitor caused a three-fold increase in NO from *Mytilus* tissues (3.2 ± 1.3–9.6 ± 1.1 nM).[26]

By using gas chromatography-mass spectrometry, we demonstrated the presence of the endocannabinoids anandamide (*N*-arachidonoylethanolamine, 21.5 ± 0.7 pmol/g) and 2-arachidonoyl-glycerol (147.4 ± 42.7 pmol/g), and of the biosynthetic precursor of anandamide, *N*-arachidonylphosphatidyl-ethanolamine (16.5 ± 3.3 pmol/g), in the leech central nervous system (CNS). Anandamide-related molecules such as *N*-palmitoylethanolamine (32.4 ± 1.6 pmol/g) and *N*-linolenoylethanolamine (5.8 pmol/g) were also detected.[31] We also found an anandamide amidase activity in the leech CNS cytosolic

Table 1 Endocannabinoids in invertebrates (MAPF: Methyl arachidonyl fluorophosphonate)

Phyla	Species	Ligand	IAAH	CB-like receptors	Biological function	Ref.
Sea urchins	*Strongylocentratus purpuratus* *Paracentrotus lividus*	Anandamide Anandamide *N*-arachidonoyl phosphatidyl acylethanolamide Palmitoyl ethanolamide Stearoyl ethanolamide			Regulator of fertility	20 18 19
Mollusks	*Mytilus edulis*	2-AG Anandamide (K_d: 34 nM) *N*-arachidonoyl phosphatidyl ethanolamine Palmitoyl Ethanolamide Stearoyl ethanolamide	K_m 29.6 μM 73 pmol/mgprotein/min MAPF (2 nM) inhibits NO release after anandamide application	K_d of 34.3 nM B_{max} of 441 fmol/mg protein for anandamide	50% excitation concentration (EC 50) for 2 AG on immunocyte cell NO release 80.5 nM 50% excitation concentration (EC 50) for anadamide on ganglia NO release 80.3 nM 50% excitation concentration (EC 50) for anadamide-induced invertebrate immunocyte rounding 80.4 nM anadamide levels increased post-mortem	22 24 26 44 47
Annelids	*Hirudo medicinalis*	2-AG: 147.4 pmol/g Anandamide: 21.5 pmol/g *N*-arachidonoyl phosphatidyl acylethanolamine: 16.4 pmol/g *N*-palmitoylphosphatidylethanolamine: 349 pmol/g *N*-palmitoyl ethanolamide: 32.3 pmol/g linolenoylethanolamide: 5.8 pmol/g Anandamide (K_d: 32 nM) ganglia	14.573 pmol/min/mg 46 kDa No inhibition by MAPF	Stimulates NO release cDNA fragment (47% sequence homology with vertebrates)	Immune response modulation	11 26 29 31
Cnidarian	*Theromyzon tessulatum* *Hydra vulgaris*	2-AG: 112 pmol/g Anandamide: 15.6 pmol/g *N*-arachidonoyl phosphatidyl acylethanolamine: 32.4 pmol/g Palmitoyl ethanolamide Stearoyl ethanolamide	K_m: 400 μm V_{max}: 3.4 nmol/min/mg 66 kDa Inhibition by MAPF	SR141716A (50–100 nM), K_d:1.87 nM B_{max}: 26.7 fmol/mgprotein	Feeding behavior	7

fraction with a maximal activity at pH 7 and little sensitivity to typical fatty acid amide hydrolase (FAAH) inhibitors. Using an antiserum directed against the amidase signature sequence, we described the identification and the localization of the leech amidase. Firstly, leech nervous system protein extract was subjected to Western blot analysis, which showed three immunoreactive bands at ca. ~42, ~46 and ~66 kDa. The former and latter bands were very faint and were also detected in whole homogenates from the coelenterate *Hydra vulgaris*, where the presence of CBlR-like endocannabinoids and an FAAH-like activity was reported previously.[32] Secondly, amidase immunohistochemical detection revealed numerous immunoreactive neurons in the CNS of three species of leeches. In addition, some of the amidase-like immunoreactive neurons were shown to be also immunoreactive for CB1R. Finally, we also found that stimulation by anandamide of this receptor leads, as in mammals, to inhibition of cAMP formation, although this effect appeared to be occurring through the previously described anandamide-induced and CB1R-mediated activation of nitric oxide release. Moreover, the gene for the cannabinoid receptor from leech has been partially cloned and sequenced.[29] The cDNA sequence is similar to those obtained from human (49%)[24] and rat (47%) CBlR.[3] According to Elphick,[33,34] this cDNA fragment would be a chimeric CB/melanocortin receptor. Its deduced sequence, expresses all the amino acids deemed critical for CBlR function in the transmembrane helix 3 region.[35] Besides, the CBIR-like receptor, we also have some evidence for a CB2R-like receptor (Salzet, unpublished data). Taken together, these results suggest the existence of a complete and functional cannabinoid system in leeches, which, we surmise, may be involved in the host–parasite relationship.[36]

Finally, evidence for a functional endogenous cannabinoid system was recently obtained in the first animal organism to have evolved a neural network, i.e. the coelenterate *Hydra vulgaris*.[32] Anandamide (1 nM–1 μM) potently inhibited (up to 45%) the glutathione-induced feeding response by accelerating *Hydra vulgaris* mouth closure. The effect was maximal at 100 nM anandamide and was reversed by the selective antagonist of the CBl subtype of mammalian cannabinoid receptors, SR141716A (50–100 nM). Specific cannabinoid binding sites were detected in membranes from *Hydra* polyps using [^{3}H]SR141716A (K_d = 1.87 nM, B_{max} = 26.7 fmol/mg protein), and increasing anandamide concentrations were found to displace the binding of [^{3}H]SR141716A to these membranes (K_i = 0.505 nM). *Hydra* polyps were also found to contain amounts of anandamide (15.6 pmol/g) and *N*-arachidonoylphosphatidylethanolamine (32.4 pmol/g), as well as 2-arachidonoylglycerol (11.2 nmol/g), similar to those described for the mammalian brain. Finally, a fatty acid amide hydrolase activity (V_{max} = 3.4 nmol/min/mg protein), with subcellular distribution, pH dependency and sensitivity to inhibitors similar to those reported for the mammalian enzyme, but with a lower affinity for anandamide (K_m = 400 μM), also was detected in *Hydra* polyps. These data suggest that the endocannabinoid signaling system plays a physiological role in *Hydra*, i.e., feeding response.

Interestingly, the genomes of the protostomian invertebrates *Drosophila melanogaster* and *Caenorhabditis elegans* do not contain CB1R nor FAAH orthologues.[33,34] According to McPartland et al.[35] BLAST 2.0 sequence alignment with a *D. melanogoster* sequence, CG9753, exhibits 26%, alignment with CBlR amino acid residues 112–412 (64% of the CB1R sequence). In leeches, the isolated fragment (153 amino acid residues) presents in average 48% homology with vertebrate CB1R type.[29] For FAAH, the screening of *Drosophila* and *C. elegans* genomic banks has revealed that these animals expressed a non-FAAH amidase.[35] In leeches such a type of enzyme has also been demonstrated.[31] These enzymes coded three substitutions in the catalytic core as GGSSGGEG-*CIQ* (in *D. melanogaster*) instead of GGSSGGE*GALI* (in human). Moreover, and surprisingly, no specific binding of the synthetic CB ligands [^{3}H]CP55,940 and [^{3}H]SR141716A in a panel of insects (*Apis mellifera, Drosophila melanogaster, Gerris marginatus, Spodoptera frugiperda*, and *Zophobas atratus*) was detected.[35] A lack of functional CB receptors was confirmed by.the inability of THC and HU210 to activate G-proteins in insect tissues, utilizing a guanosine-5′-O-(3-[(35)]thio)-triphosphate (GTPgammaS) binding assay. This lack of CB receptors appears to be unique in the field of comparative neurobiology. However, it must be noted that only a few insect species were examined. If this observation holds true, it may be due to the fact that endogenous CB ligands are metabolites of arachidonic acid, and insects produce little or no arachidonic acid.[35]

Taken together, the results reported in several invertebrate species tend to suggest the very ancient origin of this system, ever since the archeametazoans (Fig. 1; Table 1). The fact that the genomes of the protostomian invertebrates *D. melanogaster* and *C. elegans* do not contain CB1 orthologues may indicate that CB1-like cannabinoid receptors may have evolved after the divergence of deuterostomes (e.g. vertebrates and echinoderms) and protostomes.[33] However, in the light of the new molecular phylogenetic analyses, it appears that these two organisms and other molting invertebrates (ecdysozoans) are closer to mollusks and annelids (lophotrochozoans)[37] than previously believed. Recent studies on neurotrophins involved in the development of the nervous system in vertebrates have shown their existence in the molluskan genome but not in

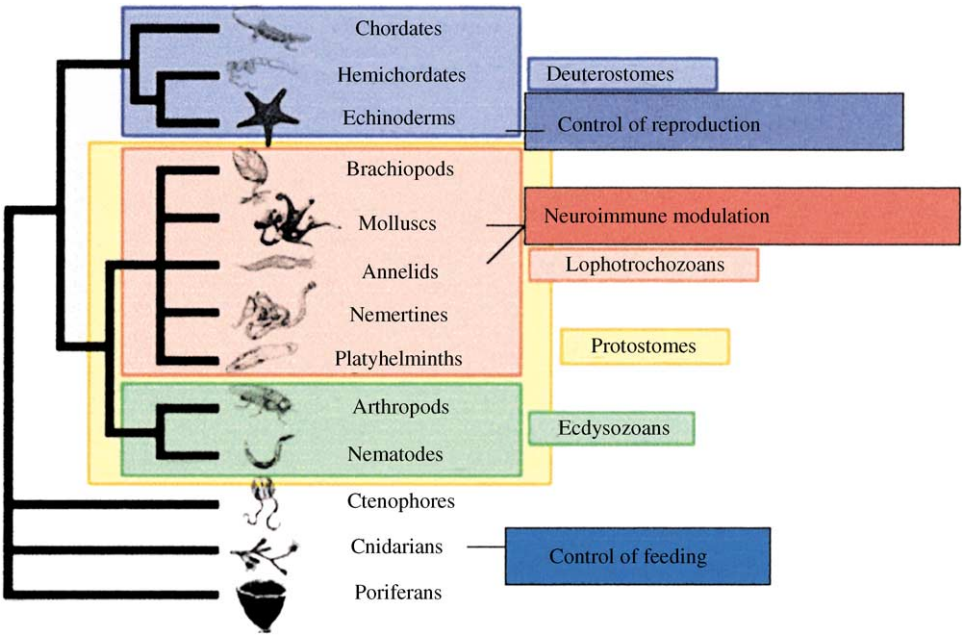

Fig. 1 Evolution of the endocannabinoid system in invertebrates. The possible role of endocannabinoids in invertebrates is highlighted. This scheme takes into consideration the novel classification according Adoutte et al.[59]

D. melanogaster and *C. elegans*.[37] Same data obtained with beta-thymosin support the existence of an arthropod-nematode clade.[38] The whole of these data tend to demonstrate that studies performed in invertebrates must not focus only on insect and nematode models, and should avoid farfetched generalizations.

MOLECULAR MECHANISMS OF THE IMMUNE RESPONSE BY CANNABINOIDS IN INVERTEBRATES

Cellular conformational changes induced by exposure of inflammatory cells to diverse signal molecules have been extensively documented.[39–42] Specifically, opioid peptides have been demonstrated to promote cell morphological changes that can be characterized as being amoeboid,[41] whereas opiate alkaloids induce rounding of cells having an ameboid-like shape for human and invertebrate immunocytes.[53] The evaluation of cellular morphological changes in response to signaling molecules has led to the discovery of cannabinoid and opiate receptors in immunocytes of invertebrates and man.[23–26,39–45]

We recently revisited an earlier observation noted in invertebrates, namely, that in the presence of sodium nitroprusside, a substance that liberates nitric oxide (NO), invertebrate immunocytes become round,[46] and we extended this observation to leech immunocytes. Recent reports indicate that anandamide can induce NO production in human macrophages and human endothelial cells as well as in *Mytilus* immunocytes and leech neural tissues.[31,44,45] Moreover, we also demonstrated that 2-AG acts on *Mytilus edulis* immunocytes,[45] which become round and immobile in correlation with decreased production of cytokines and adhesion molecules, i.e. with an immunosuppressive response. In addition, exposure of these cells to 2-AG results in nitric oxide (NO) release, which is blocked by the NOS inhibitor, L-NAME and by a CB1-R antagonist, but not by a CB2-R antagonist.

We have also demonstrated that both anandamide and *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) can cause previously activated and ameboid immunocytes to become round.[47] Additionally, concomitant administration of anandamide and the NOS inhibitor, L-NAME, significantly inhibited the cannabinoid-induced morphological change, suggesting that anandamide-induced cell conformational changes may be mediated, at least in part, by NO. Further studies using an NO-specific amperometric probe demonstrated an increased production of NO by anandamide-stimulated immunocytes, as noted earlier. The observation that anandamide-induced cell rounding and NO release from these cells are abrogated in the presence of SR14174 as well as L-NAME is consistent with a role for NO in the regulation of cell conformation. In addition, NO may modulate cellular physiological activity since SNAP or anandamide treatment of diverse cell types resulted in rounding and immobilization of the cells. These data suggest that NO that is released following anandamide receptor

activation may mediate the immunosuppressive actions of anandamide on immunocytes.

With regard to the possible molecular mechanism involved in the immobilization and rounding of immunocytes we can only speculate.[48] NO is extremely labile and reacts rapidly (within ms) with proteins and molecular oxygen. Once inside the cytosol it activates guanyl cyclase, thereby increasing the levels of cGMP, which triggers a reduction in the intracellular calcium concentration by enhancing its cellular extrusion and intracellular sequestration.[49] A mechanism that can account for the inhibition of specific immunocyte adherence, aggregation and chemotaxis, as well as for the immunocyte rounding effect, is the direct effect of NO on actin polymerization.[2] Moreover, in order for leukocytes to spread (become ameboid) intracellular free calcium must be present. Clearly, once the intracellular calcium level is reduced and adherence is blocked, the cell will assume a rounded conformation due to lack of contact with a surface. Indeed, given the large number of intracellular mechanisms that can be influenced by NO, any combination of its actions could also inhibit select immunocyte, microglia and endothelial cell behaviors. These actions include autoribosylation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of mitochondrial Fe-S enzymes and alteration of cellular adhesion proteins.[2] Thus, the molecular actions of NO on cellular function may explain the rounding effect observed.

It was demonstrated that within 3 s of anandamide exposure, calcium transients are stimulated in invertebrate immunocytes and this effect lasts for about 120 s.[49] Forty seconds following anandamide exposure NO release occurs. Thus, no release is due to anandamide-induced calcium transients. The fact that opioid peptides do not increase $[Ca^{2+}]_i$, indicates the significance of Ca^{2+} in this process since opioid peptides do not release invertebrate immunocyte NO.[44]

By analogy with mammalian organisms, the final step of this cascade might be the inhibition/stabilization of the cytoplasmic IkB/NFkB-like complex and of the NFkB-like nuclear translocation linked to gene activation with subsequent inhibition of inflammation and cytotoxicity. This hypothesis has recently been strengthened by recent findings obtained in invertebrates. Escoubas' group has described an IkB-like kinase named oIKK in oyster.[50] Hoffmann's team fully described a mammalian NF-κB signaling pathway similar to that controlled by the dorsoventral regulatory gene cassette: Spätzle-Toll-Cactus/Dorsal. This system is implicated in antifungal response in drosophila immunocytes.[51,52]

We and others have also noted that cannabinoid actions in both man and invertebrates resemble those of opiate alkaloids.[27,28,53] In this regard, we demonstrated that these signaling systems utilize the same effector system, i.e., NO release, by separate and distinct receptors. However, we also noted that morphine is more potent than anandamide in this regard.[27,28]

The possible role of the endogenous cannabinoid system in the down-regulation of the immune response was also suggested by studies mimicking bacterial infection and septic shock in invertebrate tissues. Stimulation of leeches with lipopolysaccharide (LPS), a potent immune and neural stimulating agent derived from bacteria, results in the release of anandamide from ganglia after a prolonged latency period of 24 h in a concentration and time-dependent manner (from 2.4 ± 1.1 to 78 ± 12.3 pmol/mg at 1 μg/ml, $P < 0.005$).[11,31] This release might result in the inhibition of immunocytes activation through adenyl cyclase inhibition.

IMMUNE RESPONSE MODULATION BY CANNABINOIDS IN EXO-PARASITES

A good part of parasites 'stealth' depends on their ability to communicate with the host. In this regard, we and others have found that in some cases parasites use the same signaling molecules that are found in their hosts. We surmise that these common signaling molecules are critical for parasitic–host interaction since such parasites have the ability to modify host processes. Recently, these molecules common to some host and parasites have been increased to include the enkephalins, morphine and endocannabinoids.[36,44,53]

Leeches are exo-parasite that have developed a very effective bite, which is associated with the release of chemicals that inhibit the host's immune response.[1,27,26,54–56] Opioid neuropeptides are important for 'awakening' the immune and neuroendocrine systems.[55] However, they are for the most part 'trapped' in their respective precursors, whose processing is initiated upon immunocyte activation.[53,54] We surmise that, when taking its blood meal, the leech injects immunomodulators, i.e., endocannabinoids, into the host to prevent immune opioid peptide processing, thus eliminating the 'early' alert system and simultaneously preventing a general neuroendocrine stress response.[33] This hypothesis also takes into account the ability of this strategy to down-regulate the host endothelial surface, which also responds to these signaling molecules.[56] Based on recent research, we suggest that blockade of leukocytes recruitment is performed by means of opiate alkaloids, i.e., morphine, as well as of endocannabinoids signaling. Furthermore, given the presence of POMC (pro-opiomelanocortin)-derived peptides (Fig. 2) that can be coupled to elements of renin-angiotensin[53,54,56] system, the complexity and dynamic nature of this process becomes all the more apparent.[1,36,53,54] In support of this early 'short-circuit' of the host's immune system by the parasite, we

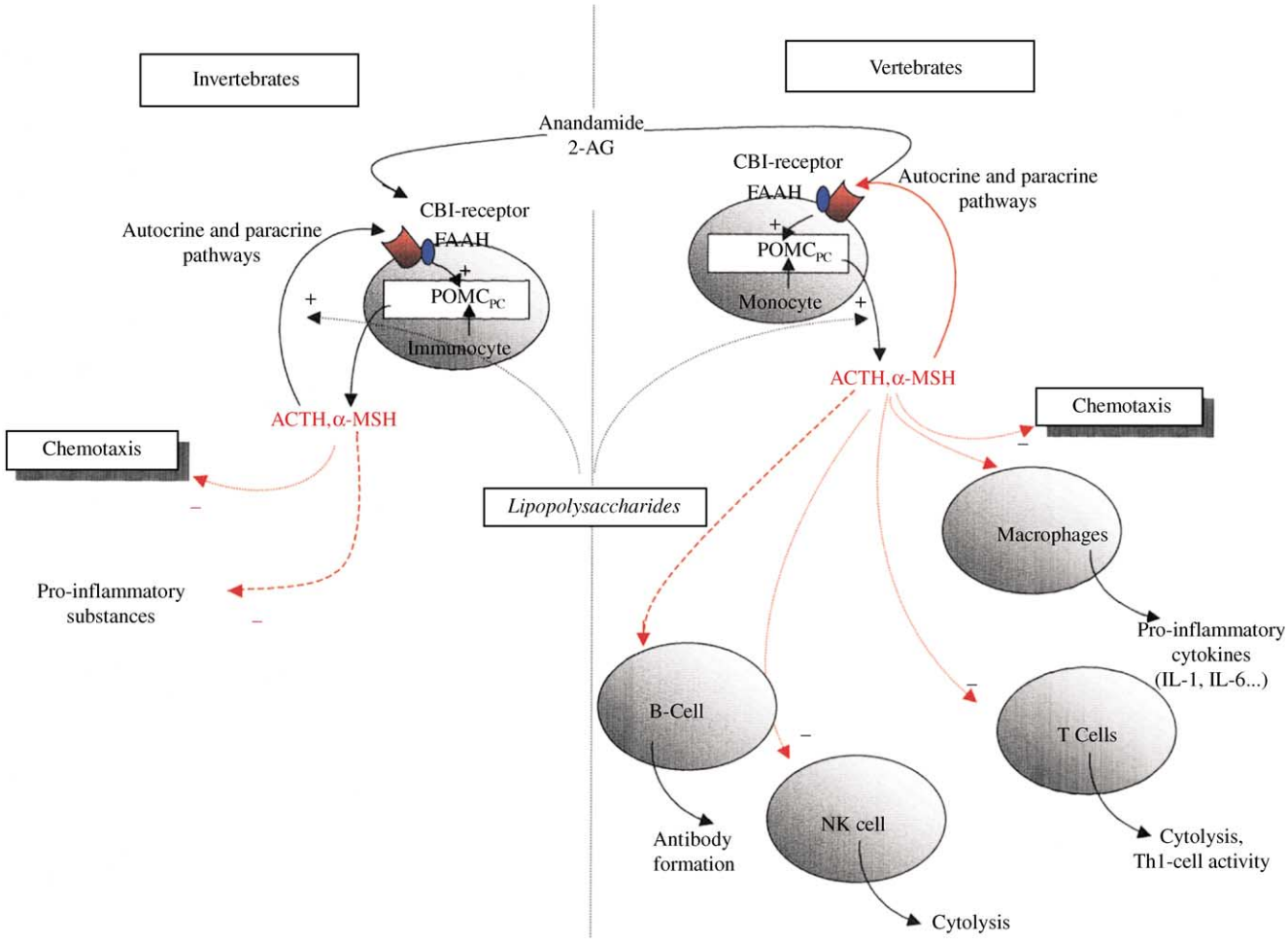

Fig. 2 Comparison between immune response modulation in vertebrates and in mammals: implication of opioid/POMC-derived peptides. In mammals, THC and anandamide inhibit T lymphocyte proliferation and Thl activity. They also stimulate IgE production but inhibit IgG production. THC and anandamide block cytolysis and phagocytosis of natural killer cells and macrophages, respectively. Moreover, both monocytes and invertebrate immunocytes contain POMC, prohormone convertase genes and cannabinoid receptors. After either cognitive stress or pathogen infections, and through autocrine, paracrine or endocrine pathways, these cascade events lead to adrenocorticotropic hormone (ACTH) and α-melanocyte-stimulating hormone (α-MSH) release in both animal kingdoms. These peptides, likeTHC and anandamide, are known to inhibit T cell proliferation, IgG production, macrophage phagocytosis and NK cell-mediated cytolysis. Hence, these substances inhibit in synergy the immune response. IL, interleukin; TNF-α, tumor necrosis factor-α (with permission from Medecine et Sciences).

have observed that these molecules are stored in the leech salivary glands. These glands form the anatomical structures most needed when the leech bites the host. Thus, during the bite, leeches might also inject substances to block the host pain. In fact, a nociceptive stress due to the injury will lead to an inflammatory response with a greater mobilization of leucocytes to the blood meal.[53,54] Leeches avoid this response, which would otherwise affect negatively the digestion during the long period of time used to digest the blood meal. The challenge is to block the peripheral nociception immediately during the bite. In this context, the production of endocannabinoids, known to be antinociceptive,[35,57] might be a survival strategy to escape host-immune defense.

Finally, endocannabinoids might also participate on prey–predator relationships. This hypothesis is in line with the recent investigations in an aquatic beetle, *Agabus affinis*. This insect contains 2-AG in its defense glands,[58] which discharge their content when the beetle is seized by a fish.[58] 2-AG thereby released could exert an adverse effect on the predator.

CONCLUSIONS

In conclusion, given the wealth of information now emerging on the mammalian-like neuroendocrine processes found in invertebrates, and especially in some parasites, it would appear that the cannabinoid system in all probability originated in 'simple' and primitive animals

to control physiopathological responses either similar or entirely different from those that are modulated by endocannabinoids in higher vertebrates and mammals.

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