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REGULATION OF INTERACTIONS BETWEEN THE NERVOUS AND THE IMMUNE SYSTEMS AND THE HYPOTHALAMO-PITUITARY-ADRENOCORTICAL AXIS ACTIVITY BY HEMORPHINS

N.H. BARKHUDARYAN, H.H. ZAKARYAN

H. Buniatian Institute of Biochemistry NAS RA
nbarkh@yahoo.com

Hemorphins, a family of endogenous nonclassical opioid peptides derived from hemoglobin (Hb), exert a wide spectrum of biological activity by affecting different receptors function. Hemorphins play an important role in the regulation of nervous and immune systems and hypothalamo-pituitary-adrenocortical (HPA) axis activity. Molecular mechanisms underlying the actions of hemorphins involve the integration of Ca^{2+}/calmodulin/calcineurin/NFAT signaling pathway with μ-opioid receptors (MOR) function and other metabolic pathways.

Hemorphin – Ca^{2+}/calmodulin(CaM)/calcineurin/NFAT signaling pathway – MOR – HPA axis – endotoxin-induced stress

Հեմորֆինները հեմոգլոբինից (Hb) առաջացած ոչ դասական էնդոգեն օպիոիդ պեպտիդների ընտանիք են, որոնք օժտված են կենսաբանական ակտիվության լայն սպեկտրով, ազդելով տարբեր ռեցեպտորների ֆունկցիայի վրա: Հեմորֆինները կարևոր դեր են կատարում նյարդային, իմունային և հիպոթալամո-հիպոֆիզար-մակերիկամային (HPA) համակարգերի ակտիվության կարգավորման գործում: Հեմորֆինների ազդման մոլեկուլային մեխանիզմները ներառում են Ca^{2+}/ կալմոդուլին/կալցինեյրին/NFAT ազդանշանային ուղու ինտեգրացումը μ-օպիոիդ ռեցեպտորների (MOR) ֆունկցիայի և այլ նյութափոխանակային ուղիների հետ:

Հեմորֆին – Ca^{2+}/ կալմոդուլին/կալցինեյրին/NFAT ազդանշանային ուղի – MOR – HPA համակարգ – էնդոտոքսինով-խթանված ստրես

Геморфины являются семейством эндогенных неклассических опиоидных пептидов, предшественником которых является гемоглобин (Hb). Они обладают широким спектром биологической активности, воздействуя на функции различных рецепторов. Геморфины играют важную роль в регуляции нервной и иммунной систем, а также гипоталамо-гипофизарно-надпочечниковой (HPA) оси. Молекулярные механизмы действия геморфинов включают интеграцию Ca^{2+}/ кальмодулин/кальцинейрин/NFAT сигнального пути с функцией μ-опиоидных рецепторов (MOR) и других метаболических путей.

Геморфин – Ca^{2+}/ кальмодулин/кальцинейрин/NFAT сигнальные пути – MOR – HPA ось – эндотоксин-индуцируемый стресс.

It is well known that biologically active peptides are power instrument for subtle regulation of metabolic processes in the organism in physiology and pathophysiology (stress, infection, inflammation) and this is one of the ways providing the homeostasis of the organism.

Hemorphins are nonclassical opioid peptides derived from hemoglobin (Hb) presented in CNS [25, 10-11, 31], peripheral organs [59] and body fluid [for review see Ref. 41]. They appeared to be relatively stable in tissue extracts and blood plasma, suggesting a physiological significance of these peptides [41]. Hemorphins demonstrate a wide spectrum of biological activity by affecting different receptors function (e.g. μ-, δ- and κ-opioid receptors [60], angiotensin (Ang) IV receptor (AT4) [39], bombesin receptor subtype 3 (hBRS-3) [33] and corticotropin-releasing factor (CRF) receptor(s) [5]. It should be noted, that hemorphins among opioid receptors demonstrate a higher affinity to μ-opioid receptors (MOR) with IC50 in the μM range [60].

All hemorphins, whatever their source, originated from the same region of the β-chain of Hb (residues 31-40 of bovine and residues 32-41 of human Hb), named LVV-hemorphin-7 [41]. It was shown the small, but significant passage of hemorphin-7 across the blood-brain-barrier [41], however α- and β- globin mRNAs were identified in mouse brain, implying the synthesis of globin in the central nervous system CNS [43].

Accumulating evidence was obtained for the involvement of hemorphins in the regulation of nervous and immune systems function. Hemorphins modulate Ca^{2+}/calmodulin (CaM)-dependent enzymes activity, including calcineurin [11, 8, 14] and inhibit enkephalin-degrading enzymes (e.g. neutral endopeptidase (NEP), aminopeptidase N (APN), dipeptidyl peptidase (DPP) DPP IV [41, 21], and angiotensin-converting enzyme activities [34]. All these enzymes were reported to play an important role in the regulation of nervous and immune system functions [23, 27, 50, 56, 29, 57, 37, 42, 49].

Functional interactions have been described between hemorphins on the one hand and β-endorphin, growth hormone, prolactin [41], substance P (SP) [46], neuropeptide Y (NPY), Met-ENK-Arg-Phe [40] and CRF [5] on the other hand. It should be underscored that hemorphins inhibit the acute inflammatory response to SP by binding with MOR [46], share the pressor activity with NPY and Met-ENK-Arg-Phe [40], which results from the activation of sympathetic nervous system. Furthermore, intracerebroventricular (icv) administration of hemorphin-7 was shown to induce significant enhancement of plasma level of GH and PRL [41]. It has been found out that central CRF and opioid pathways are involved in the interaction between LVV-hemorphin-7 and brain serotonergic system [5]. Because CRF integrates brain multi-system responses to stress [18], the latter finding indicates that hemorphins being present in the hypothalamus [11], pituitary gland [25] and adrenal gland [20], may also be implicated in brain multi-system response to stress. In addition, hemorphin-4 and hemorphin-7 have a capacity to release β-endorphin from pituitary tissue [41]. It should be noted that lymphocytes can synthesize and secrete mentioned neuropeptides. Receptors for these peptides have been found on lymphocytes as well [19].

All of mentioned neuropeptides modulate hypothalamo-pituitary-adrenocortical (HPA) axis activity and participate in the interactions between the immune and the nervous systems [24, 17]. It seems very likely that hemorphins may rank to classical opioid peptides and other mentioned neuropeptides, and share their properties to realize the bidirectional communication between the nervous and the immune system and contribute to the regulation of HPA axis activity. This view is supported by the finding that hemorphins modulate the activity of brain and lymphocytes Ca2+/CaM -dependent protein phosphatase 2B (calcineurin) activity by binding to CaM, exhibiting a concentration-dependent biphasic response on enzyme activity [8, 14]. Calcineurin is known as a key enzyme in the signal transduction cascade leading to T cell activation. This enzyme controls gene expression of several cytokines, including IL-2, tumor necrosis factor α (TNFα) and others via dephosphorylation and nuclear translocation of NFATc (nuclear

factor of activated T cell) family members [50]. In the brain calcineurin regulates synaptic plasticity and synaptic development and participates in neurotransmitters (serotonin (5-HT), noradrenaline (NA), dopamine (DA), glutamate), neuropeptides and neurohormones (e.g. adrenocorticotropic hormone (ACTH) release [27, 56, 23]. Thus, calcineurin may regulate imune-neuro-endocrine interactions and hemorphins, by modulation of calcineurin activity, may also be involved in the regulation of HPA axis activity in physiological and pathophysiological conditions of the organism.

Relationship between the processing of hemorphins and their mechanism of action on the brain and immune system. Earlier we proposed that hemorphins could be formed in the organism during physiological or pathophysiological conditions as a result of limited proteolysis of blood Hb or globin synthesized in nervous tissue [9]. Indeed, brain high molecular weight (HMW) aspartic proteinase was shown to generate LVV-hemorphin-7 from the β-chain of Hb by cleavage of Leu30-Leu31 and Phe40-Phe41 bonds [9]. The same enzyme, presented in erythrocytes membrane, was identified as a cathepsin E [30]. It has been shown that cathepsin D is also a good candidate for generation of stable VV-hemorphin-7 [22]. It is to be noted that brain catepsin B participates in the generation of hemorphin-7, LVV-hemorphin-5 and hemorphin-5 from LVV-hemorphin-7 in vitro, acting both as dipeptidyl carboxypeptidase and endopeptidase [4]. By using in vivo microdialysis in combination with electrospray mass spectrometry in vivo processing of LVV-hemorphin-7 in rat brain and blood was studied. Several hemorphins were formed, including hemorphins-7, in both brain and blood [47].

Obviously, there is a relationship between the processing of hemorphins and their mechanism of action. The network of molecules introduced in Fig.1 is presented both in brain and in the immune system. It involves hemorphins, which modulate both brain and lymphocytes calcineurin activity by binding to CaM. Hemorphins, via the modulation of Ca^{2+}/CaM/calcineurin signalling pathway can participate in the regulation of different cytokines production, such as IL-2, IL-6, TNF α and etc. genes expression [50]. IL-1 is also involved in that network. Since IL-1 exerts its activities often in synergy with TNF α and IL-6, and, moreover, each of these three cytokines is capable of inducing others, it is proposed that calcineurin, by participation in the production of TNFα and IL-6 [for review see Ref. 24; 3], can indirectly affect IL-1 production as well. In addition, β-endorphin was reported to regulate the production of IL-1 [19]. Because hemorphins have a capacity to induce the release of β-endorphin [41], so that they may indirectly affect the IL-1 production by release of β-endorphin as well.

Cytokines, namely IL-2, IL-1, TNFα and IL-6, in turn, demonstrate bi-directional interactions with proteinases [28, 1], involved both in hemorphins processing and in the metabolism of cytokines [32]. Furthemore, cathepsins D, E, B were shown to play an important role in the regulation of the immune system function by implication in the antigen processing in the class II major histocompatibility complex pathway [16].

Alzheimers disease (AD) and brain ischemia [45, 51] are examples of CNS pathologies, associated with cytokine dysfunction [7], where the signaling network is involved as presented in fig.1. In these pathologies high levels of hemorphins and their precursor (β-globin fragments, containing hemorphin sequence) [45, 51] were observed.

This was correlated with increased activity of cathepsins D, E, B [36, 51] during mentioned diseases. The observed raise in CaM level also was consistent with a tissue region undergoing insult associated with degeneration [51]. Because hemorphins modulate activity of Ca^{2+}/CaM dependent enzymes [11, 8, 14] by binding with CaM (Kd 2-10 nM) [12], latter finding points to the possible involvement of hemorphins in pathophysiology of AD and brain insult. This was confirmed by involvement of calcineurin in pathophysiology of the same diseases [35, 45, 51].

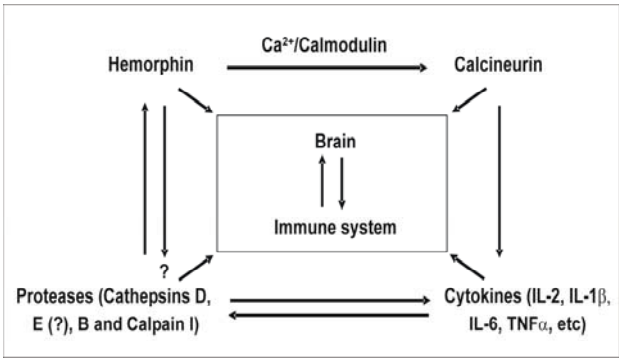

Fig. 1. Potential interactions between the hemorphins processing and their mechanism of action in the brain and immune system [4].

Calpains were reported to be involved in the same CNS pathologies (AD, and brain ischemia) as cathepsins D, E, B [52]. Moreover, it was shown that a calpain-induced cathepsin B release is crucial for the development of the ischemic neuronal death [58]. Because hemorphins regulate μ-calpain activity [6] , it is likely that they may indirectly affect cathepsin B activity, being both substrate and regulators of cathepsin B.

Interactions between the messengers within the nervous and the immune system: *cytokines, neuropeptides/hormones and neurotransmitters.* Accumulated evidence suggests that bidirectional communication existing between the nervous and the immune systems strongly depends on the interactions between the messengers within those systems: cytokines, neuropeptides/hormones and neurotransmitters. Moreover, it has been proposed, that biologically active peptide are important for the manifestation of cytokines functions [7, 17, 24]. It has been reported that one of the mechanisms by which interleukin (IL)-1 stimulates HPA axis on the level of brain is via the stimulation of CRF secretion in the hypothalamus and potential mediators for IL-1 induced CRF secretion are NA and 5-HT [for Review see Ref. 17]. IL-6 and TNFα also activate HPA axis, although they are less potent that IL-1. It has been proposed, that cytokines released from activated immune cells may act as neurotransmitters affecting CNS function [24].

As mentioned above, hemorphins affect the production of Ang II by inhibiting ACE activity [34]; and LVV-H7, which is the most potent in inhibition of ACE activity, is equipotent with Ang IV for AT4 receptor binding [39]. Because pro-inflammatory neuropeptides Ang II [42, 49] and SP [38] are involved in the production of IL-1, TNFα, IL-6, it is suggested that hemorphins by inhibition of Ang II production and SP function may negatively affect the synthesis of these pro-inflammatory cytokines, demonstrating anti-inflammatory properties.

It is necessary to emphasize, that neuropeptides, exert multiple functions in both CNS and periphery either by direct binding with different receptors or by inducing/ inhibiting the release of other neuropeptides and, thus, indirectly affecting those neuropeptides receptors function. The existence of reciprocal synaptic relationships between different peptidergic neurons [26], co-localization of variety of neuropeptides (classical opioid peptides, SP, Ang II, and etc.) and their receptors in certain neurohormone/neurotransmitter (e.g.CRF, oxytocin, vasopressin and etc.) synthesizing neurons [44, 54], and coexistence of neuropeptides and neurotransmitters, as costransmitters [55], in axon terminals in different brain regions provide evidence for functional interactions of their receptors.

Hemorphins may fulfill their role as a modulators between the immune and the nervous system by hemorphin-neuropeptide (β-endorphin, CRF, SP, NPY, Ang II, Ang IV [41, 5, 46, 34, 40, 39]), hemorphin-cytokine (IL-2, TNFα [8, 13]) and hemorphin-neurotransmitter (5-HT, DA, glutamate [5, 48]) interactions and it is suggested that molecular mechanisms underlying the hemorphin function involve the integrated effects of Ca^{2+}/CaM/calcineurin/NFAT signaling pathway with MOR and other receptors function (e.g. CRF receptor(s), [5], NMDA glutamate receptors, serotonin 5-HT2A receptors [48]).

Very recently, it has been shown that LVV-hemorphin-7 and hemorphin-7 act as homeostatic agents in response to endotoxin-induced stress [13]. It is well known that many of the physiological effects associated with LPS are mediated by cytokines, including TNFα, IL-1 and IL-6, the levels of all being elevated as a result of LPS administration [15]. LPS administration activates HPA axis by increasing circulating concentration of adrenocorticotropic hormone (ACTH), which, in turn, induces downstream release of glucocorticoids from the adrenal cortex [15]. It should be noted, that LPS administration was reported to activate calcineurin as well [53]. It has been shown that LVV-hemorphin-7 and hemorphin-7 are able to regulate HPA axis activity by decreasing in corticosterone and TNFα levels in plasma of rats received ip administration of LPS. Increased activity of calcineurin in both plasma and brain of rats, received ip LPS, was recovered by treatment with hemorphins [13]. Down regulatory effect of hemorphins on increased plasma levels of corticosterone in response to LPS, indicate that hemorphins may have a significant therapeutic potential. It is well established that physiological stress responses are generally considered adaptive. However, under chronic stress most physiological systems are negatively affected by prolonged exposure to glucocorticoids and cathecholamines [2] . There is clinical and experimenthal evidence indicating that stress hormones affect tumor pathogenesis at multiple levels (initiation, tumor growth, and methastasis). Therefore pharmacological interventions targeting immune-neuro-endocrine function at the level of the central nervous system and HPA axis represent a novel strategy for protecting cancer patients.

It is to be underscored that hemorphins have a capacity to modulate the HPA axis activity on the level of brain. The presence of hemorphins in hypothalamus, pituitary gland and adrenal gland, their ability to release β-endorphin from pituitary, and the contribution of central opioid and CRF receptors to stimulatory effect of LVV-hemorphin-7 on serotonergic system support our suggestion. Nevertheless, the presence of MOR on immune cells, peripheral neurons, and detection of hemorphins in adrenal gland cortex and medulla indicate that peripheral impact of hemorphins on HPA axis activity have to be considered.

It is necessary to emphasize that hemorphins, as other members of the endogenous protective system of the organism, come into play mainly in response to pathophysiological conditions (e.g. stress, inflammation, cancer and etc.). In that case hemorphins, like other pleiotropic neuropeptides, serve as one of homeostatic factors that switch on the compensatory systems in the organism. This is based on several mechanisms and by implication of different signaling pathways in order to recover the homeostatic disturbance.

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