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Hemorphins Stimulate the Expression Level of NFκB Transcription Factor in Rats Brain in Response to Endotoxin-Induced Stress

(Submitted by academician M. A. Davtyan 13/XII 2012)

Keywords: *hemorphin, calcineurin, NFκB transcription factor, endotoxin-induced stress.*

Introduction. LVV-hemorphin-7 (LVVH7) and hemorphin-7 (H7) are members of the hemorphins family, an endogenoius nonclassical opioid peptides, derived from hemoglobin (Hb) and exhibiting a multiple functions (For Review see Ref.[1]).

Earlier it has been established that Ca^{2+}/ calmodulin (CaM)-dependent protein phosphatase calcineurin is a key enzyme underlying the molecular mechanism of hemorphins action in the brain and immune system [2]. Hemorphins *in vitro* were shown to modulate calcineurin activity by binding with CaM, exhibitting a concentration-dependent biphasic response on enzyme activity.

Recently, it has been shown that hemorphins act as homeostatic agents in response to endotoxin-induced stress [3]. It has been reported, that endotoxin ((lipopolysaccaride, LPS) administration activates calcineurin [4]. Increased activity of calcineurin in both plasma and brain of rats, received ip LPS, was recovered by treatment with hemorphins [3]. Calcineurin 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 [5]. NFAT transcription factors cooperate with AP-1 (activator protein-1), and NFκB transcription factor, which plays a critical role in the expression of IL-2, TNFα and other cytokines on gene transcription level [5]. The involvement of calcineurin and transcription factor NFκB in immunological synapse formation has also been reported [6]. In its inactive form NFκB consists of three-subunit complex, consisting of 50 kDa (p50) and 65 kDa (p65; RelA), and inhibitory subunit, called IkB (IkBα or IkBβ). The NFkB complex is located in the cytosol and is activated when IkB is induced to dissociate from the complex. The p50-

p65 dimer then translocates to the nucleus and binds to 5′ regulatory elements consisting of the decameric sequence in genes responsive to NFκB [7-8]. NFkB/Rel proteins share a highly conserved 300 amino acid long N-terminal Rel homology domain (RHD) responsible for DNA binding, dimerization, and association with inhibitory proteins. In resting cells most NFkB/Rel dimmers are bound to IkB and retained in cytoplasm. It has been shown, that calcineurin mediates NFκB activation [9] in LPS-stimulated mouse peritoneal macrophages. The involvement of calcineurin and protein kinase A (PKA) in the regulation of NFκB activity was found in human monocytes as well [10]. Moreover, μ-opioid receptors (MOR) agonist β-endorphin induced hyperphosphorylation
and rapid degradation of IkBα in human monocytes was reversed by inhibitors of calcineurin and PKA [10]. It has been reported that hemorphins have a capacity to induce β-endorphin release from pituitary into circulation [1]. It addition, we have shown that H7 induces activation of DNA binding ability of NFκB in stimulated Jurkat T cells [11]. Because the action of hemorphins are more pronounced in pathophysiology, so that it is considered advisable to study DNA binding activity of NFκB in brain nuclear fraction of endotoxin-induced stress, by using NFkB/p65 Active ELISA Kit.

Materials and methods. In the experiments were used rats, Wistar line both sexes, weighing 180-220g at the time of experiment. Rats, provided by the Animal House of the Institute of Biochemistry of NAS RA, were caged in groups of 5 with food and water given ad libitum. The animals were kept at 22° C on a 12 h light-dark cycle. Experiments were conducted between 09:00 and 14:00 h. Stress was induced by single intraperitoneal (ip) injection of LPS (0.5 mg/kg). Rats were randomly divided into 4 groups (n=10 per group): the first control group received ip injection of an equivalent volume (0.5 ml) of 0.9% w/v saline; the second group received ip injection of LPS; the third and fourth groups received simultaneously ip injection of LPS with either LVV-hemorphins-7 or hemorphin-7 (1mg/kg). Animals were sacrificed at 4 h after ip injection of saline, LPS and LPS+hemorphin. The brains were rapidly removes frozen and stored at -70° C until use.

The brain tissue lysate preparation, nuclear fraction collection and the measurement of NFκB (free p65) in the brain tissue nuclear fraction were done using NFκB/p65 Active ELISA^{TM} Kit (IMGENEX Corporation, San Diego, CA) according to the manufacturer's recommendation.

Statistical analysis. The results were expressed as the means ± SEM. Data were analysed statistically by one-way ANOVA, using GraphPad Prism 4 software. Statistical significance indicates $p < 0.05$.

Results and discussion. As one can see from the Fig. 1, ip injection of LPS (0.5mg/kg) in combination with H7 or LVVH7 (1 mg/kg) induces the increase in expression level of NFκB (2.3 fold for LPS+H7 and 2.2 fold for LPS+LVVH7 respectively) in brain tissue nuclear fraction, in compare with the level of NFκB in the brain of rats in control group, received ip injection of saline. LPS administration alone induces increase in NFκB level 1.52 fold only.

Interestingly, MOR agonist morphine was reported to modulate NFκB activation in macrophages [12].

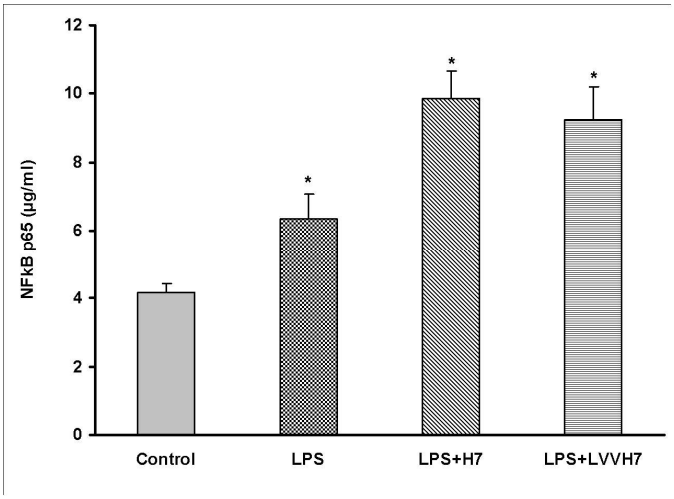

Fig.1. The regulatory influence of LVVH7 and H7 on the expression level of NFkBin rat brain tissue nuclear fraction in response to endotoxin-induced stress.

It seems very likely, that hemorphins, likewise β-endorphin[10], have capacity to activate NFκB-dependent transcriptional processes in response to stress. It is well known an antiapoptotic function of NFκB [13]. It should be emphasized, that very recently, our data obtained give us reason to suggest, that hemorphins may induce apoptosis of cancer cells [14]. In addition β-endorphin has also been recorded to increase apoptotis in lung cancer [15] as well. Moreover, it is suggested, that the role of calcineurin in NFkB activation could be cell type- and/or stimulus specific in LPS-stimulated macrophages [9]. It is proposed that different effect of hemorphins and β-endorphin in LPS-induced stress and in pathophysiology of cancer may depend from type of disease as well. Both hemorphins and β-endorphin are members of endogenous protective system of the organism and in pathophysiological conditions (e.g. stress, infection, inflammation, cancer and etc.) they serve as one of factors that switch on the compensatory systems in the organism, in order to recover homeostatic disturbance.

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Առնետների ուղեղում NFκB տրանսկրիպցիոն գործոնի էքսպրեսիայի խթանումը հեմորֆինների կողմից ի պատասխան էնդոտոքսինով խթանված սթրեսի

Ցույց է տրված, որ LPS-ի (0.5 մգ/կգ) համատեղ ներորովայնային ներարկումը հեմորֆինի (1 մգ/կգ) հետ խթանում է NFκB-ի էքսպրեսիան ուղեղի կորիզային ֆրակցիայում (2.3 անգամ LPS +H7-ի համար և 2.2 անգամ LPS +LVVH7-ի համար, համապատասխանաբար) ի համեմատ NFκB-ի քանակի հետ ստուգիչ խմբի առնետների ուղեղում, որոնք ստացել էին 0.9%-ոց NaCl: Միայն LPS-ի ներարկում ստացած առնետների մոտ NFκB-ի քանակը ավելանում էր 1.52 անգամ: Ստացված տվյալները վկայում են այն մասին, որ հեմորֆինները ունակ են խթանել NFκB-կախյալ տրանսկրիպցիոն պրոցեսները ի պատասխան սթրեսի:

Э. А. Закарян

Стимуляция геморфинами экспрессии NFκB транскрипционного фактора в мозге крыс в ответ на эндотоксин-индуцируемый стресс

Показано, что совместная внутрибрюшинная инъекция LPS (0.5 мг/кг) с геморфином (1 мг/кг) вызывает значительное повышение экспрессии NFκB (в 2.3 раза для LPS +H7 и 2.2 раза для LPS +LVVH7, соответственно) в ядерной фракции мозга крыс с LPS-индуцированным стрессом по сравнению с контрольной группой крыс, получивших 0.9%-ный NaCl. Инъекция LPS в отдельности вызывала повышение экспрессии NFκB в 1.52 раза. Полученные данные свидетельствуют о способности геморфинов стимулировать активацию NFκB-зависимых процессов транскрипции в ответ на стресс.

H. H. Zakaryan

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It has been shown that intraperitoneal (ip) injection of endotoxin ((lipopolysaccaride, LPS) (0.5mg/kg) in combination with H7 or LVVH7 (1 mg/kg) induced the increase in expression level of NFκB (2.3 fold for LPS+H7 and 2.2 fold for LPS+LVVH7 respectively) in nuclear fraction of brain tissue, in compare with the level of NFκB in the brain of rats in control group, received ip injection of 0.9% w/v saline. LPS injection induced the increase of NFκB level at 1.52 fold only. Data obtained indicate that hemorphins have ability to activate NFκB-dependent transcriptional processes in response to stress.

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