中国药理学报 *Acta Pharmacologica Sinica* 1989 May; **10** (3) : 266-270

Enhancement of interleukin-1 production in mouse peritoneal macrophages by methionine-enkephalin

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Abstract Methionine-enkephalin, an endogenous opioid, has been reported to have some effects on immune responses. By thymocyte proliferation method, we herein report that methionine-enkephalin over a wide range of concentrations (1 pmol-0.1 μmol/L) significantly increases both extracellular interleukin-1 release and intracellular interleukin-1 production from peritoneal macrophages induced by lipopolysaccharide in mice. Naloxone, having no effect *per se* on interleukin-1 production, does not block the enhancing effect of the neuropeptide. Interleukin-1 production was also elevated following ip methionine-enkephalin into mice. The results suggest that methionine-enkephalin mediates the enhancement of interleukin-1 synthesis and release as well, and that the effect is not mediated through classical opioid receptors. The results also provide the further links between immune and nervous systems.

Key words methionine- enkephalin; naloxone; macrophages; lipopolysaccharides; interleukin 1

The endogenous opioid peptide methionine-enkephalin (Met-Enk), a five-amino acid peptide, is presesnt in plasma at concentrations ranging from 10-100 pmol/L[1]. Various types of stress, such as footshock, physical exercise and immobilization, may result in increasing the levels of circulating endorphins and Met-Enk. The concomitant stress-induced release of β-endorphin from the adenohypophysis suggests that a peripheral hormonal role for β-endorphin. Recently, opioid receptors[2] and specific nonopioid receptors[3] have been detected on some immunocompetent cells, indicating that these cells may be the targets for circulating peripheral opioid peptides.

Met-Enk has been shown to enhance mouse lymphocyte proliferation induced by phytohemagglutinin (PHA)[4] and concanavalin A (Con A) (our unpublished data) to generate cytotoxic T cells[5]. It is also found that Met-Enk can prolong the survival time of mice challenged with tumor cells[4]. In addition, the pentapeptide inhibits phagocytosis of peritoneal macrophage in concentrations ranged from 1-100 nmol/L, but increases the phagocytosis at higher concentrations[6]. Moreover, it suppresses T lymphocyte chemotactic factor production[7].

In the view of the fact that interleukin-1 (IL-1) plays an important role in immune functions and nervous activities, such as increasing interleukin-2 (IL-2) production and inducing slow-wave sleep (SWS), it is very interesting to investigate the effect of Met-Enk on IL-1 production from peritoneal macrophages induced by lipopolysaccharides (LPS), both *in vitro* and *in vivo*.

Materials and methods

Inbred strain C 57 BL mice (♀, 19± SD 2 g, for IL-1 production) and BALB/cA(♀, 8-10 wk old, for IL-1 assay) were obtained from Shanghai Animal Center, Shanghai Branch of Chinese Academy of Sciences.

Met-Enk, Con A and LPS were purchased from Sigma Chemical Company. Naloxone hydrochloride was obtained from Shanghai Medical University.

RPMI 1640 media was purchased from

Received 1988 Jul 29 Accepted 1988 Nov 28

Gibco Lab. All RPMI 1640 containing media were supplemented with HEPES buffer (Merck) 10 mmol/L, penicillin 100 IU/ml, streptomycin 100 μg/ml, *L*-glutamine 2 mmol/L, 2-mercaptoethanol (Buchs, packed in Switzerland) 50 μmol/L and 10% inactivated newbown bovine serum. pH adjusted to 7.2.

Production of IL-1 by peritoneal macrophages

1 *In vitro* study The method used for IL-1 production was based on that described by Gearing[8] with some modifications. In brief, peritoneal macrophages (PMϕ) were obtained from the normal mice or the mice injected ip with 10% thioglycollate broth (Difco, 1 ml/mouse, 4 d later). The peritoneal cavities were lavaged with RPMI 1640 media. The cells were washed 3 times in RPMI 1640 media and plated in 24-well plates (Costar) at 2×10^6 cells/well in 1 ml, then allowed to adhere for 1 h at 37℃ in air + 5% CO_2. Nonadherent cells were washed out prior to the addition of Met-Enk or LPS. Macrophages were treated with LPS (5 μg/ml) and various doses of Met-Enk at 37℃ in air + 5% CO_2 for 24 h. The viability of PMϕ at the end of incubation was greater than 85% measured with trypan blue cxclusuin. There is no difference in viability between control and experimental groups. After incubation, the supernatant (extracellular IL-1) was collected and clarified by centrifugation. The remaining adherent cells were added with 1 ml RPMI 1640 media and frozen-thawed 3 times. This supernatant (intracellular IL-1) was obtained by centrifugation, dialysed for 24 h at 4℃ against phosphate-buffered saline (PBS) in volume of 1:200 to remove some small molecules, then passed through a millipor filter (0.22 μm) and stored at −25℃ until assayed.

2 *In vivo* study Mice were treated with ip daily Met-Enk 0.1, 0.6 mg/kg twice. Peritoneal cells were harvested on the third day and cultured with LPS (5 μg/ml) for IL-1 (extracellular) production.

IL-1 assay IL-1 activity was evaluated by thymocyte proliferation assay[8]. Thymoctes (1.5×10^8 cells/well) isolated from BALB/cA mice, were co-cultured with PMϕ cultural supernatant in 96-well microtiter plates (Costar) for 72 h in the presence of 1.5 μg/ml of Con A. The cultures were pulsed with [^{3}H]-TdR (46.25 kBq/well) for the final 6 h of incubation period and were harvested then onto glass fiber filter paper. IL-1 activity was evaluated by [^{3}H]TdR uptake. Each experiment repeated at least 3 times.

Results

Effect of Met-Enk on extracellular IL-1 production from PMϕ *in vitro* LPS is a well-known IL-1 triggering agent. When Met-Enk (1 pmol-1 μmol/L) was added to LPS-stimulated PMϕ cultures for 24 h, IL-1 production evaluated by thymocyte proliferation was significantly enhanced (Tab 1). Met-Enk has no effect on nonstimulated PMϕ IL-1 production or thymocyte proliferation (data are not shown).

Tab 1. Effect of methionine-enkephalin (Met-Enk) on extracellular and intracellular IL-1 production from peritoneal macrophages induced by lipopolysaccharide 5 μg/ml. IL-1 activity is expressed as cpm/1.5×10^6 thymocytes. n = 5, $\bar{x}\pm$ SD. $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Met-Enk (pmol/L)	$10^{-2}\times$IL-1 activity (cpm) Extracellular	Intracellular
0	105±18	70±16
10^0	168±20***	96±9**
10^2	164±34***	121±31**
10^4	152±21***	115±14***
10^6	150±18***	135±14***

Effect of Met-Enk on intracellular IL-1 production from PMϕ In order to differentiate whether the observed enhance-

ment of IL-1 by Met-Enk is due to the increase of synthesis or release of the monokine, the effect of the peptide on intracellular IL-1 was determined. The results in Tab 1 also demonstrate that intracellular IL-1 is enhanced proportionally by the pentapeptide (1 pmol-1 μmol/L).

Influence of naloxone on the enhancement of IL-1 production from PMϕ *in vitro* As both opioid and non-opioid receptors were detected on immune competent cells, the characterization of the receptor on PMϕ was tested with naloxone, a specific opioid receptor antagonist. The results in Tab 2 showed that naloxone (50 nmol/L), which *per se* has no effect on IL-1 production, does not block the enhancing effect of IL-1 production caused by Met-Enk.

Tab 2. Influence of naloxone (50 nmol/L) on the enhancement of extracellular IL-1 production by methionine-enkephalin (Met-Enk) from the adherent peritoneal macrophages induced with lipopolysaccharide (5 μg/ml) *in vitro*. IL-1 activity is expressed as cpm/1.5×10^6 thymocytes. n=5, $\bar{x}\pm$SD. ***$P<0.01$ *vs* control; †$P>0.05$ *vs* without naloxone.

Met-Enk (pmol/L)	$10^{-2}\times$IL-1 activity (cpm) Without naloxone	With naloxone
Control	129±20	159±28†
10^0	217±23***	259±63***
10^2	290±41***	251±42***
10^4	355±42***	306±40***
10^6	270±37***	246±41***
10^8	323±30***	361±49***

Effect of Met-Enk on IL-1 production by PMϕ following ip Met-Enk into mice To investigate whether Met-Enk has any effect on extracellular IL-1 production *in vivo*, the mice were treated daily with ip Met-Enk 0.1, 0.6 mg/kg for 2 d. Extracellular IL-1 is markedly elevated from PMϕ induced by LPS (5 μg/ml) in dose dependent manner. IL-1 activity of control group is 18 657±3213, for 0.1 mg/kg group is 25 359±2639 ($P<0.01$) and for 0.6 mg/kg group is 41 655±4866 ($P<0.01$) cpm/1.5×10^6 thymocytes.

Discussion

The present studies demonstrated that Met-Enk significantly enhanced IL-1 production in mouse PMϕ induced by LPS. Comparison of the levels of intracellular IL-1 with extracellular IL-1 indicated that both synthesis and release of IL-1 were increased. Naloxone, the specific opioid receptor antagonist, did not affect the increase of IL-1 production and naloxone alone had no effect on IL-1 production induced by LPS, suggesting that enhancing effect of IL-1 synthesis by Met-Enk was not mediated through classical opioid receptors. In addition, in the absence of mitogen, Met-Enk alone did not stimulate IL-1 production, indicating a preference for activated cells. This suggests that only activated macrophages express functional receptors for Met-Enk.

It is unlikely that Met-Enk affects the enhancement of Con A-induced thymocyte proliferation by IL-1 instead of increasing IL-1 production, because the direct addition of Met-Enk to suboptimal Con A-stimulated thymocytes does not affect their proliferative response to LPS-induced macrophage supernatants (containing IL-1) (data not shown). Thus, residual Met-Enk in macrophage supernatants is not responsible for the observed enhancement.

It was reported that some stressors, such as treating animals with LPS[9], injuries of mammalian brain[10,11], induced IL-1 like factor production from glia cells in brain. Furthermore, isolated astrocytes treated with LPS *in vitro* released significant amount of IL-1 like factor[12,13]. No distinction is apparent when comparing macrophage derived IL-1 with the IL-1 like mediator produced by astrocytes[12]. On the other hand, IL-1 from both macro-

phages and astrocytes induced fever[9,13], SWS[13-15] and astrocyte proliferation[10,11] as well. These activities mediated by IL-1 are beneficial to the defense mechanisms against invaders and repair from brain injuries. In view of the fact that the level of opioid peptides increaed significantly in the brain during stress, the observation that Met-Enk enhanced IL-1 production from LPS treated macrophages both *in vitro* and *in vivo*, suggested that Met-Enk might contribute to both immunoprotective and immunopathological responses in the brain.

Since IL-1 not only regulates immune responses, but also affects the functions of nervous system, the regulation of IL-1 by the opioid peptides provides further links between immune and nervous systems. Our results also suggest that Met-Enk is an immunopotentiator and may have important implications in host response to infections and neoplasms.

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甲硫氨酸脑啡肽对小鼠腹腔巨噬细胞产生白细胞介素1的增强作用

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提要　应用胸腺细胞增殖法检测腹腔巨噬细胞产生的IL-1.本文发现甲硫氨酸脑啡肽(Met-Enk)1 pmol-1 μmol/L，显著增强LPS诱导的腹腔巨噬细胞合成及释放IL-1，阿片受体阻断剂纳洛酮50 nmol/L不能阻断Met-Enk的这一作用．Met-Enk 0.1-0.6mg/(kg·d)ip也显著提高LPS在体外诱导的腹腔巨噬细胞产生IL-1.结果提示Met-Enk介导的IL-1产生不是通过经典的阿片受体起作用的．实验结果为神经与免疫系统之间的联系提供了又一证据．

关键词　甲硫氨酸脑啡肽；纳洛酮；巨噬细胞；脂多糖；白细胞介素1

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