PHARMACOLOGY AND TOXICOLOGY

Effect of Dynorphin A (1-17) on Proliferation and IL-2, IL-4, IFN-γ Production by Peripheral Blood Mononuclears

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Dynorphin A (1-17) in concentrations of 10^{-8}-10^{-9} M inhibits phytohemagglutinin (2.5 μg/ml)-induced proliferative response of mononuclear fraction lymphocytes. In mitogen-stimulated cultures, 10^{-8} M dynorphin A (1-17) stimulates the production of IL-4, inhibits the production of IL-2, and does not modify the production of IFN-γ. Nonselective κ-receptor antagonist naloxone and selective antagonist binaltorphimine hydrochloride abolish the inhibitory effects of both dynorphin A concentrations on the lymphocyte proliferative response. On the other hand, evaluation of the effect of κ-receptor blockade on the production of IL-2 and IL-4 showed that this effect depends on peptide concentration and antagonist type. Hence, the results attest to an important role of κ-receptors in modulation of functional activity of immune cells.

Key Words: *dynorphin A; IL-2; IL-4; proliferation; opiate receptors*

Dynorphins are a family of endogenous opioid peptides forming as a result of cleavage of a large precursor molecule, prodynorphin (proenkephalin B). The structure of prodynorphin includes amino acid sequences of α- and β-neoendorphins, dynorphin A (1-17), and dynorphin B (rimorphin) [3]. Prodynorphin processing can result in the formation of shorter dynorphin A fragments, for example (1-13) and (1-8). The dynorphin group peptides regulate generalized and local neurogenic processes, digestion, reproduction, lactation, development of the common adaptation syndrome, and congenital and acquired immunity reactions [7]. Prodynorphin products are present (in high concentrations) in the hypothalamus, posterior pituitary lobe, and spinal ganglia [6]. In addition, dynorphins are formed in cells of the gastrointestinal tract, adrenals, sex glands, placenta, and in immune system cells [15]. Dynorphin synthesis by the immune system cells is induced by their contact with a foreign antigen leading to elevation of proinflammatory cytokine content (IL-1, TNF-α, IL-6) [14]. Dynorphins released by lymphocytes and macrophage monocytes modulate (along with endorphins and enkephalins) the immune system functions through their endocrine, neurocrine, paracrine, and autocrine effects on target cells. All peptides of the dynorphin family contain a leu-enkephalin sequence on their N-terminal sites and are characterized by selective affinity for κ-opiate receptors.

The immune system cells express κ-opiate receptors similar to those in the CNS [13]. However, the immunological effects of κ-receptor stimulation are little studied in comparison with those of μ, δ-receptors. κ-Receptors are expressed at a high density on thymocyte membranes, which indicates their involvement in the regulation of functional activity of maturing T cell. Later, the density of κ-receptors on the surface of maturing of T cells significantly decreases [10]. Mitogen

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stimulation enhances the expression of κ-opiate receptor on $CD4^+$ and $CD8^+$ cells. Endogenous (dynorphins A and B) and exogenous (U50.488H and U69.593) κ-agonists modulate proliferation, antibody production, absorption and secretory activity of natural immune cells, exhibiting pro- and anti-inflammatory activities [5]. On the other hand, some aspects of this problem remain not studied, for example, dynorphin regulation of the production of cytokines responsible for Th1/Th2 polarization of T-helpers (Th1 cells produce mainly IFN-γ and IL-2, leading to predomination of cell-mediated response, while Th2 cells produce mainly IL-4 and mediate the humoral immune response). It is sometimes not clear which receptor type is involved in the realization of these effects, because the capacity to bind other opiate receptors (though with far lower affinity) in addition to their own one is characteristic of all endogenous opioid receptors [11].

We studied the effect of dynorphin A (1-17) on the proliferative response and production of IL-2, IL-4, and IFN-γ by mononuclears under conditions of κ-receptor blockade.

MATERIALS AND METHODS

Peripheral venous blood leukocytes from healthy volunteers (n=7-9) were studied. The mononuclear fraction was isolated in Ficoll-verografin density gradient (ρ=1.077). The cell suspension was then washed twice, suspended in medium 199, and incubated for 1 h at 4°C in order to eliminate activation caused by isolation. The mononuclears were cultured with phytohemagglutinin P (PHA; 2.5 μg/ml, Sigma) in 96-well round-bottom plates for 72 h. Each culture contained 2×10^5 cells/0.2 ml complete nutrient medium prepared on medium 199 with 10 mM HEPES (Sigma), 2 mM L-glutamine (Sigma), 100 μg/ml gentamicin, and 10% fetal calf serum (Biolot). ^{3}H-methylthymidine (2 μCi) was added to the cultures 18 h before the end of culturing. Radioactivity of the samples was measured on a Guardian scintillation counter (Wallac). Selective κ-receptor agonist dynorphin A (1-17) was used in concentrations of 10^{-7}-10^{-10} M, nonselective μ,δ,κ-antagonist naloxone hydrochloride (Warsaw Pharmaceutical Plants) and selective κ-antagonist binaltorphimine hydrochloride (nor-BNI) in a concentration of 10^{-6} M.

Supernatants were collected after 24 h (IL-2) and 48 h (IL-4 and IFN-γ) of cell culturing, centrifuged, and stored at -20°C. Cytokine concentrations were measured by solid phase EIA with Biosource (IL-4) and Vector-Best (IL-2, IFN-γ) kits according to the instruction. Analytical sensitivity of the test systems was 2 pg/ml (lower threshold sensitivity).

Statistical analysis of the results was carried out using paired unifactorial analysis of dispersions in order to evaluate the dose–effect relationship and using the next paired LSD test (post-hoc comparison) for evaluating combined effects (Student's t test) of dynorphin A and opiate receptors. All data in the figures are presented as the means and their standard errors ($M\pm m$).

RESULTS

Dynorphin A (1-17) in concentrations of 10^{-8}-10^{-9} M inhibits the PHA-induced proliferative response of mononuclear lymphocytes and does not modify the spontaneous response (Fig. 1). The production of the main Th1/Th2-polarizing cytokines is changed as follows: the peptide increases the production of IL-4, inhibits the production of IL-2, and does not modify the synthesis of IFN-γ in PHA-stimulated cultures. The concentrations of the studied cytokines virtually do not change in cultures without stimulation.

The effects of dynorphin A (1-17) under conditions of κ-opiate receptor blockade were studied using two most active concentrations of the peptide (10^{-8}-10^{-9} M) and nonselective (naloxone hydrochloride) and selective (binaltorphimine hydrochloride) antagonists. The effect of κ-receptor blocking was evaluated only in PHA-stimulated cultures (Fig. 2). Naloxone and nor-BNI abolished the inhibitory effects of both concentrations of dynorphin A on the proliferative response of lymphocytes. No statistically significant effects of opiate antagonists on proliferative activity of mononuclears were detected.

Evaluation of the effect of κ-receptor blocking on the production of IL-2 and IL-4 revealed that the effect depends on the peptide concentrations and antagonist type (Fig. 2). The inhibitory effect of 10^{-8} M dynorphin A on the production of IL-2 was leveled by naloxone, but did not change in the presence of nor-NBI. By contrast, the inhibitory effect of the concentration of 10^{-9} M was arrested by nor-BNI, but not naloxone. A similar picture was observed for IL-4 production. The stimulatory effect of the concentration of 10^{-8} M was abolished by naloxone, but not nor-BNI. On the other hand, the stimulatory effect of the concentration of 10^{-9} M was slightly neutralized by naloxone and more markedly by nor-BNI. No statistically significant effects of opiate receptors on secretory activity of mononuclears were found; just a trend to IL-2 suppression in the presence of naloxone can be traced.

Hence, dynorphin A (1-17) inhibits the PHA-induced proliferative response of mononuclears, reduces the production of IL-2, does not modify the secretion of IFN-γ, and stimulates the production of IL-4. Evaluating the spectrum of the cytokines produced, one can speak about the Th2-polarizing effect of endogenous κ-agonists. On the other hand, the direction of the pro-

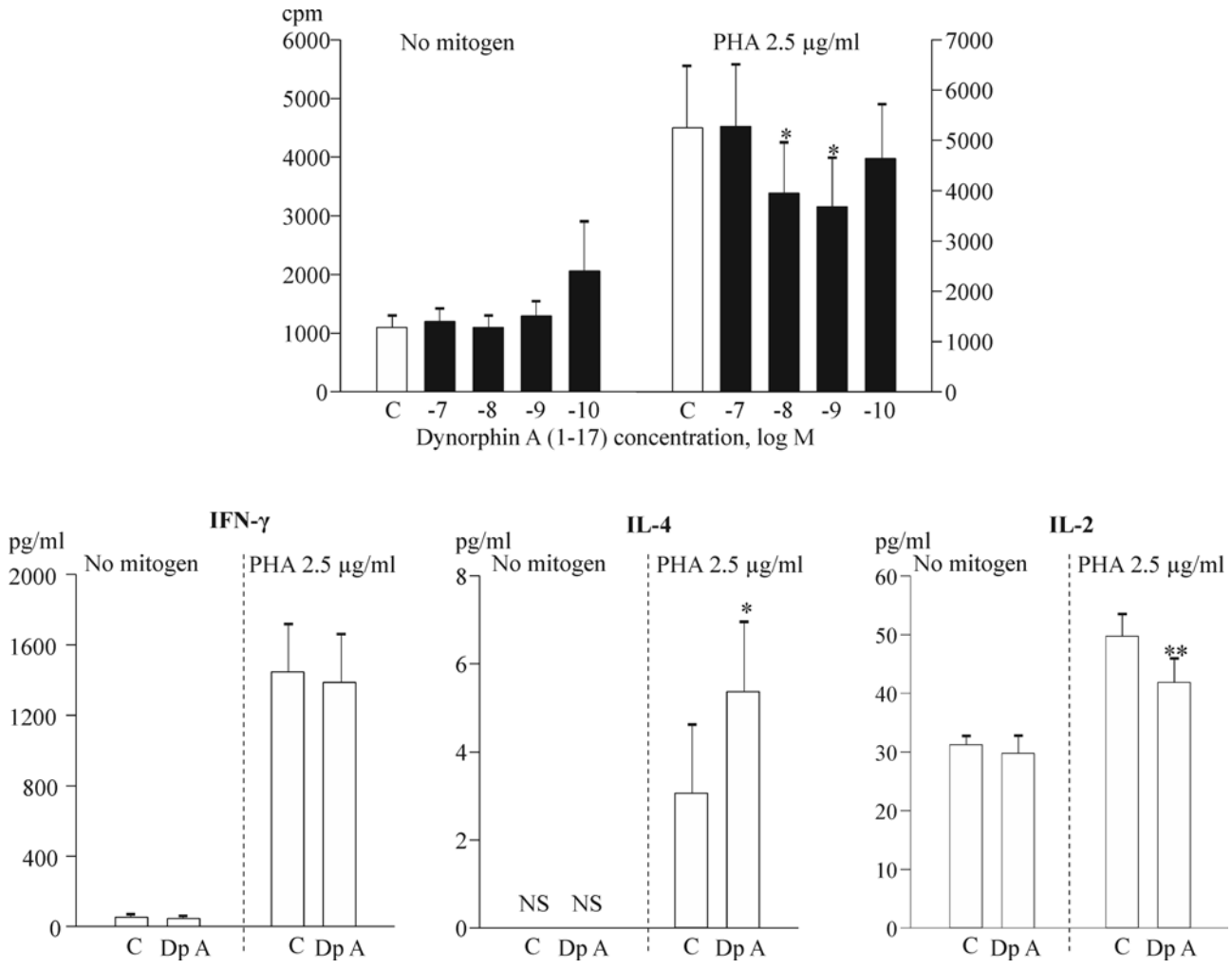

Fig. 1. Effects of dynorphin A (1-17) (Dp A) on the proliferative response and production of IFN-γ, IL-2, and IL-4 (n=9). *p<0.05; **p<0.01 compared to the control (C). NS: cytokine concentration was below the analytical sensitivity of the test system.

dynorphin fragments effects on cell proliferation and IL-2 production can vary greatly depending on cell fraction composition and object of the study. We previously showed opposite effects of dynorphin A (1-13) on lymphocyte proliferation in a purified lymphocytic fraction and mixed leukocyte suspension [2]. Stimulatory effect of dynorphin A on mouse splenocyte proliferative response and IL-2 and IL-1β production were shown [12]. The stimulatory effect of dynorphin A on mononuclear proliferation depends on the time of peptide addition to the culture [4]. Dynorphin added to the culture 48 h after suboptimal concentrations of PHA stimulated cell proliferation and the effect was not abolished by naloxone. Addition of dynorphin A simultaneously with or before the mitogen caused no appreciable effects on mononuclear proliferation [4]. On the other hand, dynorphin B suppressed proliferation of rat fetal brain cells and this effect was blocked by nor-BNI [9]. Low-molecular-weight κ-agonists (U50.488H and U69.593) are characterized by exclusively suppressive effect on the intensity of immune reactions [5] in comparison with dynorphins, this in turn attests to a relationship between effects of κ-agonist and their chemical nature.

Changes in the production of cytokines under conditions of κ-receptor blockade differed from those in evaluation of the intensity of proliferative response. The intensity of IL-4 and IL-2 production depended on the concentration of dynorphin and selective or nonselective type of the antagonist. The effect of a higher concentration of dynorphin A was neutralized by naloxone, that of the lower one by naloxone and nor-BNI. Failure of naloxone to abolish dynorphin A (10^{-9} M) inhibition of IL-2 production can be due to its own agonistic effect, which were previously found during evaluation of combined effects of naloxone and β-endorphin. The stimulatory effect of β-endorphin on the lymphocyte proliferative response is amplified in the presence of naloxone and the intensity of this effect depended on naloxone concentration [1].

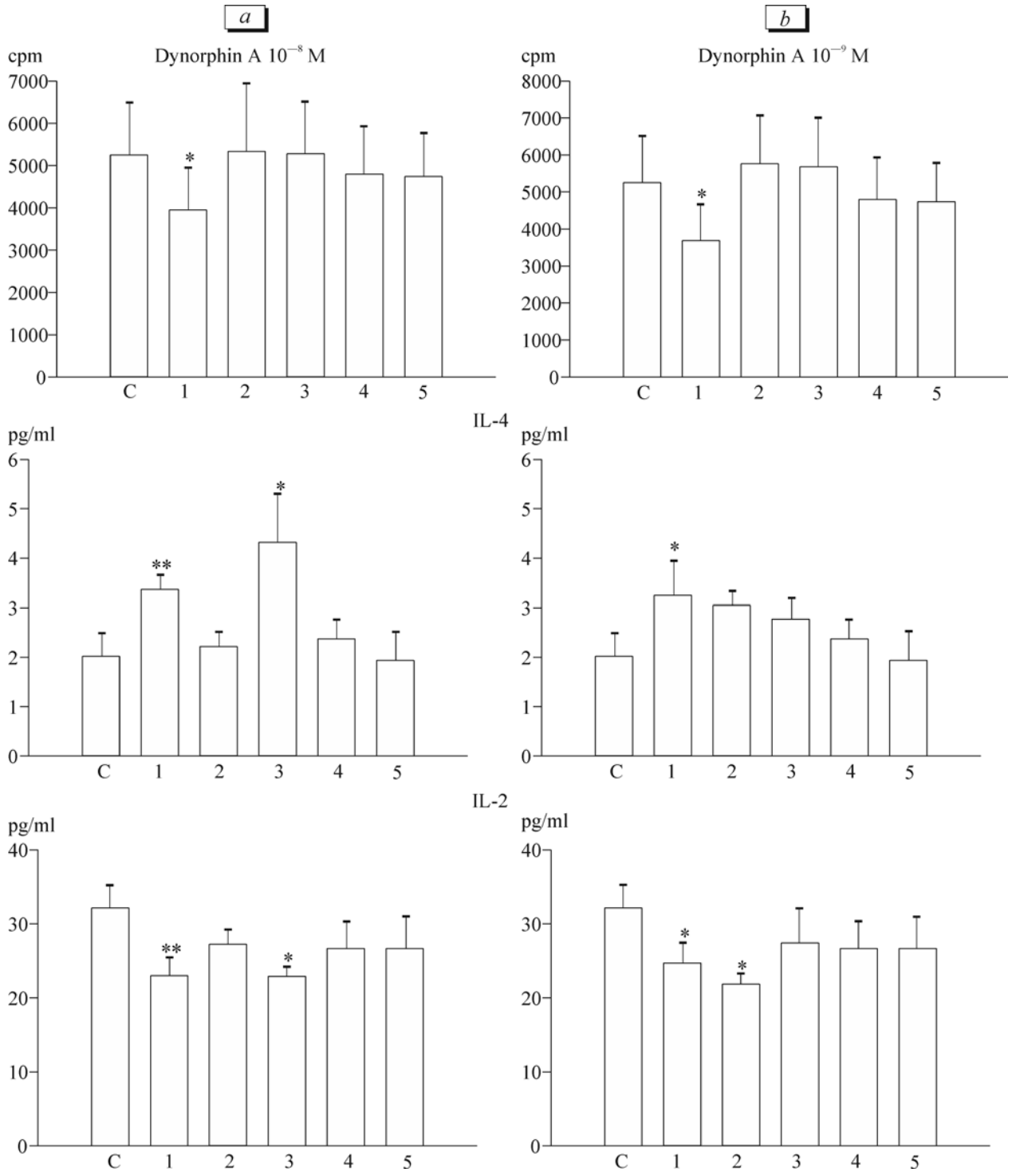

Fig. 2. Effects of dynorphin A (1-17) in concentrations of 10^{-8} M (*a*) and 10^{-9} M (*b*) on PHA-induced proliferation and production of IFN-γ, IL-2, IL-4 under conditions of κ-receptor blockade. Abscissa: C: control; *1*: dynorphin A; *2*: dynorphin A+naloxone; *3*: dynorphin A+norBNI; *4*: naloxone; *5*: nor-BNI. **p*<0.05, ***p*<0.01 compared to the control.

Stimulation of IL-1β production in the presence of β-endorphin was not abolished by naloxone and was amplified by δ-antagonist naltrindole [8]. Hence, the presence of certain concentrations of the agonist and antagonist in the culture in some cases leads not to discontinuation, but to amplification of the final effect. This phenomenon can be explained by an intricate system of interactions between endogenous opioids and a wide spectrum of their specific binding sites on cell surface, and by the presence of different domains

for peptides and low-molecular ligands in one and the same receptor [11].

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