Influence of Bone Marrow Hexapeptides (Myelopeptides) on Antibody Production and Algesthesia in Mice and the Dependence of These Effects on Naloxone

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It is shown that synthetic analogs of two bone marrow hexapeptides (myelopeptides 1 and 2), which are identical in structure to the N-terminal peptide fragments of hemoglobin α- and β-chains, are characterized by naloxone-independent antibody-stimulating activity. The antibody-stimulating effect of the myelopeptides becomes naloxone-dependent and stronger against the background of immunosuppression provoked by the hot plate test carried out before immunization. Intraperitoneal administration of both myelopeptides in doses of 10^{-13}-10^{-8} g/mouse causes a naloxone-dependent modulatory effect on mouse algesthesia threshold with a predominant analgetic effect.

Key words: *antibody production; algesthesia threshold; naloxone; hexapeptides*

It is known that bone marrow cells of various animal species and of human origin produce humoral factors (myelopeptides - MP) which influence immune response development and algesthesia [3,10]. There are reports that MP can produce dose-dependent opposite effects on algesthesia correlating with the immune response [1,13]. The presence of opioid peptides in MP and the naloxone-dependence of its antibody-stimulating and analgetic effects point to the participation of bone marrow opioids along with other MP in the realization of these effects [2,10]. However, the identification of individual MP molecules responsible for immuno- and neurotropic properties requires their isolation from the mixture.

At present two such compounds, MP1 and MP2, are structurally characterized. They are hexapeptides, identical to the N-terminal fragments of the α- and β-chains of swine hemoglobin [5]. Endogenous peptides isolated from swine bone marrow cell culture supernatant possess nocicepcio-modulating activity. Synthetic analogs of these peptides have been obtained and their properties investigated on various experimental models.

The objective of the present investigation was to study the influence of hexapeptides on antibody production and algesthesia in mice and the dependence of these effects on naloxone.

MATERIALS AND METHODS

Experiments were carried out on (CBA×C57Bl/6) F_1 hybrid mice and on CBA strain mice of 18-20 g weight. Mice were immunized with a single intraperitoneal injection of 0.5 ml of 5% or 0.05% sheep red blood cell suspension 20, 60, or 180 min after algesthesia threshold (AT) determination.

The enhancing effect of MP1 and MP2 on antibody production was assessed by estimation of the number of antibody-producing cells (APC) in

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mouse spleens using a modified method of Cunningham [4]. Peptides were injected intraperitoneally in concentrations of 10^{-6}-10^{-13} g/mouse one day before immunization with antigen simultaneously, or on day 1, 2, 3, or 4 after immunization in 0.2 ml volume. Animals were killed on the 5th day after primary immunization with antigen. The coefficient of antibody production stimulation (C) was calculated as the ratio between APC number in experimental to control groups.

The effect of MP1 and MP2 on algesthesia was assessed with the hot plate method at a temperature of 50 or 55°C [7]. Each group consisted of 6-8 mice. The experimental groups received an intraperitoneal injection of 0.2 ml peptide solution (10^{-5}-10^{-13} g/mouse). The control group received 0.2 ml of solvent. The effect was estimated 15, 30, 45, and 60 min after injection at the same time of day (between 13:00 and 15:00 h). Each animal was used for one measurement only. In some experiments hexapeptides were injected simultaneously with naloxone (10^{-5}-10^{-8} g/mouse). Statistical evaluation of the data obtained was carried out using Student's t test.

RESULTS

The effect of various MP1 and MP2 doses on the inductive and productive phases of the primary immune response in mice was investigated. The experimental results are presented in Figs. 1 and 2. Peptide injection simultaneously with 5% sheep red cells and on the 4th day after immunization caused a reliable increase of the spleen APC count. At the inductive stage of the immune response MP1 expresses its maximal stimulative effect in doses of 10^{-10} and 10^{-8} g/mouse (C=1.5 and 1.7, respectively), while MP2 expresses its maximal effect in doses of 10^{-13} and 10^{-8} g/mouse (C=1.5 and 1.8 respectively, Fig. 1). At the productive stage of the immune response both MP1 and MP2 administration in doses of 10^{-8} g/mouse enhance antibody production 1.5-1.4-fold (Fig. 2). Combined injection of MP and naloxone, which in itself does not stimulate antibody production, does not abolish the the MP-mediated stimulative effect (Table 1). It should be noted that in the absence of an MP2 effect its combined injection with naloxone leads to a 4-5-fold rise of antibody production (Table 2), evidently due to naloxone-mediated blocking of opioid receptors (primarily of the μ- and β-types), causing suppression of antibody production [6].

The effects of naloxone and MPs depend on the initial level of immune response, on the functional status of experimental animals, and, specifically, on the AT level. Earlier we showed that most experimental groups of mice exhibited a negative correlation between antibody production and AT determined in the hot plate test. Nociceptive thermal stimulation caused a reliable decrease of APC number in animals immunized with a suboptimal dose of sheep red cells (0.05%) 20 min, 1 h, and 3 h after AT estimation. The maximal decrease of APC, approximately 4-fold, was observed after 1 h. MP1 (10^{-8} g/mouse) injected simultaneously with antigen against the background of immune response suppression, i.e., 1 h after AT measurement, caused a 3.3-fold increase of antibody production to the primary level. The antibody production-stimulating effect of the peptide was

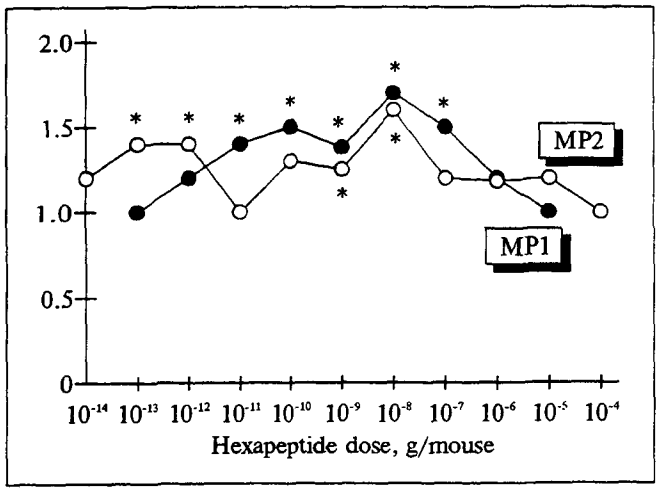

Fig. 1. Dose–dependent antibody–stimulating effect of bone marrow hexapeptides MP1 and MP2 in inductive phase of immune response. Mice were immunized with 5% sheep red cell suspension. Peptides were injected simultaneously with antigen (day 0). Here and in Figs. 2 and 3: *$p<0.05$

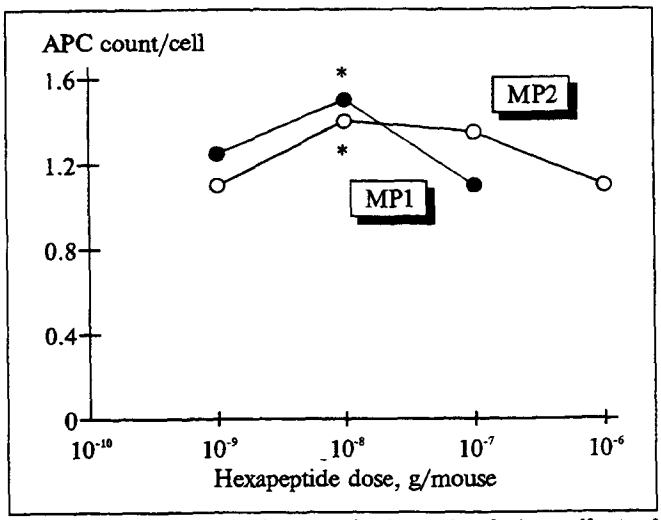

Fig. 2. Dose–dependent antibody–stimulating effect of bone marrow hexapeptides MP1 and MP2 in productive phase of immune response. Peptides were injected on the 4th day after immunization with 5% sheep red cell suspension.

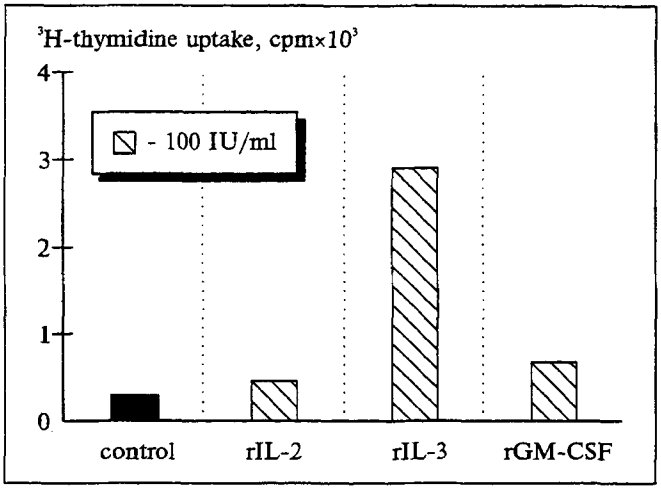

Fig. 3. Abolishment of antibody–stimulating effect of hexapeptides MP1 and MP2 by naloxone against the background of immune response suppression caused by hot plate. Preparations were administered at same time as immunization with 0.05% sheep red cell suspension. *2–8*) groups with administration at 1 h after hot plate test: *2*) 0.9% NaCl (control group); *3*) MP1 – 10^{-13} g/mouse; *4*) MP1+naloxone; *5*) MP2 – 10^{-13} g/mouse; *6*) MP2+naloxone; *8*) naloxone – 10^{-5} g/mouse; *1* and *7*) 0.9% NaCl and naloxone without hot plate test, respectively. Ordinate: ratio of APC count per spleen in experimental groups to control.

naloxone-dependent, i.e., it was totally abolished when peptide and naloxone were injected simultaneously (Fig. 3). The administration of MP2, in contrast to MP1, had a more marked antibody production-stimulating action (C=5). The effect of MP2 was also naloxone-dependent (Fig. 3).

Based on the presented data, it can be assumed that opioid receptors are involved in the realization of the antibody-stimulating reactions of bone marrow hexapeptides against the background of stress-mediated immunodeficiency. In the absence of stress the stimulating effect of MP is realized through nonopioid receptors.

Since immunostimulating effects of synthetic MP may be mediated by opioid receptors, we investigated their influence on algesthesia. The experiments carried out showed that each peptide expresses a different action (Table 3). In almost all experiments MP1 in concentrations of 10^{-13}-10^{-8} g/mouse caused a 30-40% prolongation of the latency of the pain reaction. The hypoalgesic effect of MP1 developed 30 or 45 min after peptide administration. This effect was totally abolished by naloxone (10^{-5} g/mouse). In some experiments MP1 in the same concentrations caused a 20-30% shortening of the latency (Table 3).

MP2 produced a reliable hypoalgesic effect in a concentration of 10^{-13} g/mouse and a 20-50% prolongation of the latency of the pain reaction (Table 3). In some experiments along with an expressed hypoalgesic effect, this peptide had no effect on AT. Here the effect of naloxone was ambiguous. If the peptide had a hypoalgesic effect, this was abolished by naloxone. In the absence of an MP2 effect, combined peptide-naloxone administration caused a 60% shortening of the latency of the pain reaction (Table 3).

These equivocal results may be interpreted in two ways. First, the peptide fragments may be considered as enkephalin-endorphin-releasing factors or as regulators of proteolytic enzymes of endog-

Table 1. Effect of Naloxone on Antibody–Stimulating Activity of Bone Marrow Hexapeptides MP1 and MP2 ($M\pm m$, $n=7$)

Preparation	Dose, g/mouse	APC count/spleen	C
0.9% NaCl		290 000±32 240	–
Naloxone	10^{-5}	267 600±36 600	0.92
MP1	10^{-8}	395 700±24 600*	1.36
MP1+naloxone	10^{-8}	378 200±15 700*	1.30
MP2	10^{-8}	406 700±30 440*	1.40
MP2+naloxone	10^{-8}	401 700±50 340*	1.39

Note. Preparations were administered on the 4th day after immunization with 5% sheep red cell suspension. Here and in Table 2: *$p<0.05$ in comparison to NaCl injection, n=number of animals.

Table 2. Effect of Naloxone on Initially Undetected Antibody–Stimulating Activity of Hexapeptide MP2 ($M\pm m$, $n=10$)

Preparation	Dose, g/mouse	APC count/spleen	C
0.9% NaCl		4 036±1 537	
Naloxone	10^{-8}	5 227±2 427	1.30
MP2	10^{-13}	3 128±680	0.81
MP2+naloxone	10^{-13}	17 128±4 191*	4.20

Note. Preparations were injected at same time as immunization with 0.05% sheep red cell suspension (day 0).

Table 3. Modulating Influence of Bone Marrow Hexapeptides MP1 and MP2 on Algesthesia ($M \pm m$)

Hexapeptide	Peptide dose, g/mouse	Number of animals	Temperature of plate, °C	Latency of pain reaction (sec) to:			% of control
				0.9% NaCl	Peptide	Peptide+ naloxone	
MP1	10^{-13}	30	50	25.7±3.1	35.4±2.0*	–	138.0
	10^{-8}	40	50	23.3±1.0	28.7±1.7*	–	123.2
						23.7±1.6*	100.2
	10^{-13}	10	50	26.0±2.3	22.0±1.6*	–	85.0
	10^{-13}	10	55	13.2±2.0	9.0±1.8*	–	68.2
MP2	10^{-13}	20	50	19.8±2.3	31.0±4.1*	–	156.6
	10^{-13}	20	55	8.3±1.1	12.5±2.8*	150.6	
					8.2±1.7*	98.5	
	10^{-13}	30	55	13.4±2.0	14.2±2.3	–	106.0
					9.6±1.2**	71.6	

Note. Naloxone (10^{-5} g/mouse) had no influence on algesthesia. The joint action of Naloxone and MP1 was estimated using the inversion test. *$p<0.05$, **$p<0.01$ in comparison to NaCl injection.

enous opioids (aminopeptidases, enkephalinases). The basis for such a view may be the results obtained for kyotorphin, the C-terminal fragment of the α-chain of hemoglobin. Administration of this peptide in a dose of 10^{-5} M cause a 2-3-fold increase of met-enkephalin release into the striatum. Simultaneously suppression of proteolytic enzyme degradation was observed [11].

The second interpretation proceeds from the assumption that the hexapeptides can have a direct influence on algesthesia regulation. The possibility of obtaining both hypo- and hyperalgesic effects for hexapeptide administration may be explained on the basis of the initial AT. It is known that a high AT level leads to the development of analgetic tolerance [8]. Here, an analysis based on absolute individual differences of AT is not sufficient because of individual basal level variability. The same numerical value of hot plate test parameters may correspond in one animal to a state of stress-induced analgesia (as a result of social tension, for example [9,12]), and in another to a spontaneous, stress-free state. Such circumstances evidently account for the differences in the modes of action of bone marrow hexapeptides on algesthesia.

The results of this study indicate that the two isolated and characterized hexapeptides exert antibody-stimulative and nocicepciomodulatory effects. However, their mechanisms of action only partially coincide with the mechanism of action of the integral MP composition. The more marked antibody-stimulative and analgesic effects of MP are probably linked with other components of the mixture, or manifest themselves in combined use.

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