COMPARISON OF EFFECTS OF MET-ENKEPHALIN ON SECRETION OF ANTIBODIES TO VARIOUS ANTIGENS BY MOUSE LYMPHOCYTES

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A central theme in neuroimmunology is the study of the immunomodulating properties of neuropeptides and, in particular, their effect on the development of humoral immunity. Involvement of the opioidergic system in the regulation of psycoemotional behavior and of the adaptive reactions of the individual organism, and also the problem of the relationship between stress and immunity have led to a cycle of research into the immunomodulating properties of opioid peptides [3, 11]. The early work into the effect of enkephalins on active E-rosette formation by lymphocytes showed that different opioid peptides may differ significantly in the types of effects manifested by them [8]. Experiments in vivo have shown that injection of enkephalins normalizes the immune response in immunodepressions. The most interesting of them is Met-enkephalin (ME), which is active in low doses [6]. The study of the effect of ME in experiments in vitro has shown that the peptide suppresses the number of IgM-antibody-forming cells (AFC) to sheep's red blood cells (SRBC) [7] and to trinitrophenol-lipopolysaccharide [4] in the primary immune response, but without affecting the number of IgG-AFC to SRBC during the secondary immune response [1], and that it modulates secondary response in a culture of human lymphocytes to tetanus toxoid antigen dose-dependently [9].

The aim of this investigation was to compare the effect of ME on secretion of specific antibodies by mouse lymphocytes in vitro on different models of the immune response.

EXPERIMENTAL METHOD

Experiments were carried out on female (CBA × C57BL/6)F_1 mice weighing 18-22 g. Bovine γ-globulin and ovalbumin (both from "Serva" Germany) and trinitrobenzenesulfosepharose 4B (TNBS-SP) were used for immunization. The TNBS-SP was prepared by incubating a 100-fold molar excess of TNBS (from "Merck," USA) with AN-sepharose 4B ("Pharmacia," Sweden) in 0.1 M Na-borate buffer (pH 9.2), followed by washing out the conjugate by triple centrifugation in 0.01 M Na-phosphate buffer (0.15 M NaCl, pH 7.4) (PBS) at 500*g* for 8 min. The mice were immunized subcutaneously in the footpads of the fore and hind limbs in a dose of 100 μg protein per mouse, using Freund's complete adjuvant ("Boehringer," Germany) for primary immunization and a solution of the antigen in PBS for secondary immunization. At the peak of the primary (6th-7th day) or secondary response (4th-5th day) the mice

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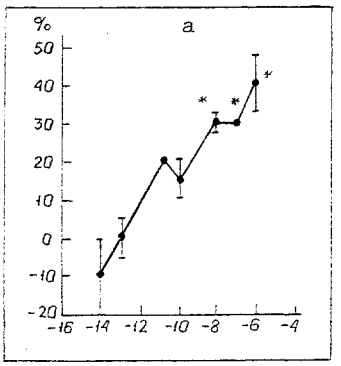

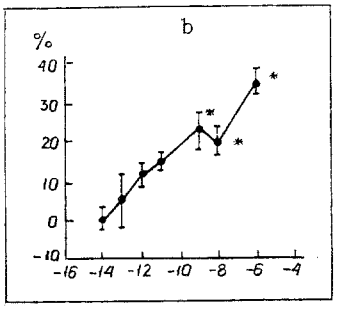

Fig. 1. Dependence of IgG-antibodies against bovine γ-globulin by mouse lymphocytes on dose of Met-enkephalin in productive phase of primary (a) and secondary immune response (b). Number of experiments – 3; *p < 0.05 indicates values differing significantly from control.

were killed and lymph nodes removed and homogenized, after which the lymphocytes were washed off by centrifugation in medium 199 (1000*g*, 8 min). The cells were incubated in a concentration of 10^6/well in 96-well immunologic planchets ("Costar," USA) in medium RPMI 1640 ("Serva," Germany), containing 2 mM glutamate ("Serva," Germany), 10 mM HEPES buffer, pH 7.2 ("Serva," Germany), 100 μg/ml penicillin and streptomycin, and 5% fetal serum for 24 h at 37°C in 5% CO_2 in an incubator with the addition of 10^{-15}-10^{-6} M ME ("Boehringer," Germany) or D-Ala^2-Met^5-enkephalinamide (DAMEA) ("Serva," Germany) or D-Ala^2-D-Leu^5-enkephalin (DADLE) "Serva," Germany). The opioid antagonist naloxone ("Sigma," USA) was used in a concentration of 10^{-6} M.

The cell supernatant was subjected to enzyme immunoassay on specific antibodies by the method in [12]. Conjugates of rabbit antibodies against mouse IgG and IgM with horseradish peroxidase ("Sigma," USA) and orthophenylenediamine ("Merck," USA) were used as the enzyme substrate. The spectrophotometric measurements were made at 492 nm on a "Multiscan" apparatus ("Flow Laboratories," USA).

Statistical analysis was carried out by Student's test for 3 or 4 repetitions ($p < 0.05$).

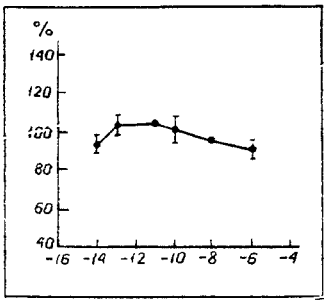

Fig. 2. Effect of Met-enkephalin on secretion of IgM-antibodies against TNBS by mouse lymphocytes during primary immune response. Number of experiments – 3.

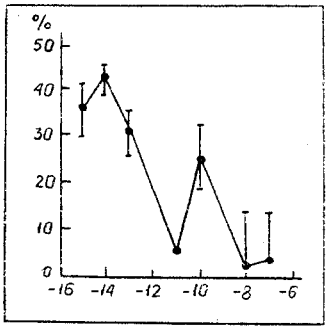

Fig. 3. Dependence of suppression of secretion of IgG-antibodies against ovalbumin by mouse lymphocytes in productive phase of secondary response. Number of experiments – 3.

EXPERIMENTAL RESULTS

Figure 1 shows that in the case of the strong T-dependent antigen bovine rglobulin, ME in concentrations of 10^{-8}-10^{-6} M, leads to dose-dependent inhibition of 24-hourly secretion of specific IgG-antibodies at the peak of both the primary and the secondary immune response. Introduction of the opioid antagonist naloxone abolished the suppressive effect of ME.

By contrast with ovalbumin and bovine γ-globulin, it was impossible with the T-independent antigen TNBS-SP to demonstrate an effect of ME on IgM-antibody formation over a wide range of concentrations in mice during the primary immune response (Fig. 2).

The results of the investigation of ME on secretion of IgG-antibodies at the peak of the secondary immune response to ovalbumin in lymphocyte cultures obtained from individual animals are given in Table 1. Clearly the main type of effect is suppressive (70%). However, no effect also was Possible (10%), and even stimulation with certain doses (20%). All effects of 10^{-8}-10^{-6} M and the majority of effects of 10^{-15}-10^{-14} M ME were abolished by

TABLE 1. Effect of Met-Enkephalin on Secretion of IgG-Antibodies to Ovalbumin during Secondary Immune Response in Mice

Type of effect	Number of mice	Maximal effect, per cent	ME concentration corresponding to maximal effect, M	Remarks
Suppression	7	28	10^{-7}, 10^{-15}	Abolition by naloxone, DADLE has a similar action
		46	10^{-9}, 10^{-15}	
		38	10^{-15}	
		39	10^{-11}—10^{-9}	Abolition by naloxone, DAMEA has a similar action
		41	10^{-15}—10^{-14}	
		29	10^{-15}—10^{-12}	Naloxone abolishes action of (10^{-7}-10^{-9} M) ME
		68	10^{-9}	
Stimulation	2	22	10^{-8}	Abolition by naloxone, similar action of ADMEA, supresses effect at 10^{-15} M
		39	10^{-9}—10^{-7}	
No effect	1			

naloxone. The synthetic enkephalin analogs DADLE and DAMEA, more resistant to proteolytic degradation, demonstrated similar effects. A considerable scatter was observed in the types of individual dose-dependence of the action of ME. In most cases, however, a suppressive effect of low doses of ME (10^{-15}-10^{-13} M) was found. The typical dose-dependence of the effect of low doses of ME is shown in Fig. 3.

The following conclusions can be drawn from the results. ME suppresses secretion of specific mouse IgG-antibodies against the strong T-dependent antigen bovine γ-globulin during both primary and secondary immune responses. The maximal effect of the peptide is exhibited in concentration of 10^{-8}-10^{-6} M. Abolition of the effect of ME by naloxone indicates involvement of opioid receptors in the realization of the effect. The similarity of concentration dependencies of suppression for the primary and secondary immune response may be connected with a common mechanism of its realization, for example, with the effect on secretion of an antigen-specific T-helper factor, as has been suggested for the suppressive effect of α-endorphin [5]. By contrast with T-dependent antigens, ME does not affect the IgM-response to the typical T-independent antigen TNBS within a wide range of concentrations.

The mechanisms of modulation by ME of antibody secretion in response to a relatively weak antigen, namely ovalbumin, are probably different from those for bovine γ-globulin. Evidence of this is given by the more distinctly individual character of the trend of the effects and the dose dependencies, and also the frequent manifestation of the suppressive effect of ME in low doses (10^{-15}-10^{-13} M). Abolition of the effect of ME by naloxone and the reproduction of these effects by the use of DADLE and DAMEA instead of ME is evidence of the receptor character of the action of ME and of the negligible contribution of processes of proteolytic degradation of the peptide, which it is important to take into account when antigen-induced lymphocyte proliferation is regulated by opioids [10].

Manifestation of the action of ME in very low doses is evidence that during activation of lymphocyte by ovalbumin either a high-affinity type of receptor to ME with $K_d << 10^{-10}$ M is exhibited, or a powerful system of signal transduction and amplification is activated. Previously the effects of low doses of ME were demonstrated by the writers in the case of the proliferative response of mouse lymphocytes to mitogens [2]. The research was funded by the GKNT.*

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