OPIATE ACTIVITY OF BONE MARROW HUMORAL FACTOR STIMULATING ANTIBODY PRODUCTION

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The humoral antibody production stimulating factor of bone marrow (APSF) is one of the mediators of the immune system that controls antibody production [4, 9]. It is produced by bone marrow cells in the course of their normal metabolism and potentiates antibody formation at the time of maximal development of the immune response [2]. This mediator has been isolated from the supernatant bone marrow cell cultures from animals of different species and its properties have been described. It is a ribonucleotide with molecular weight of about 13,000 daltons [5].

It has recently been shown that APSF not only performs an immunoregulatory function, but also has an opiate-like analgesic action [3]. Administration of APSF to animals caused selective depression of nociceptive sensation, characteristic of the action of opiates. This effect is evidently realized through binding of the mediator with opiate receptors.

In the present investigation we describe the ability of APSF to bind with opiate receptors of μ and δ types in the brain and with specific binding sites of met-enkephalin on lymphocytes.

EXPERIMENTAL METHOD

APSF was obtained from the supernatant of bone marrow cell cultures from (CBA × C57BL)F_1 hybrid mice by the method described previously [4]. The dose of APSF was estimated as its protein content, determined by Lowry's method. The ability of APSF to interact with opiate receptors of the rat brain and with specific binding sites of met-enkephalin on lymphocytes was analyzed by the method of competitive replacement of [^{3}H]morphine and [^{3}H]-met-enkephalin by the APSF preparation.

Female Wistar rats weighing 200-250 g were used. The membrane fraction of brain cells was obtained by a modified Simantov's method [10]: The rat was decapitated, the brain was removed in the cold, the corpus striatum, mesencephalon, and diencephalon were isolated from it and homogenized in 50 volumes of cold buffer (50 mM Tris-HCl, pH 7.7) in a glass—Teflon homogenizer. The resulting suspension was centrifuged at 30,000g for 20 min at 4°C. The residue was suspended in the original volume of the same buffer and kept for 40 min at 37°C. The suspension was then recentrifuged under the same conditions. The residue was resuspended in 50 mM Tris-HCl, pH 7.7 (25°C) containing 0.1% of human serum albumin (HSA). HSA was added to the incubation medium to abolish any possible nonspecific effect of proteins added to the sample in the composition of the APSF on opiate binding. The reaction mixture, in a volume of 1 ml, contained 0.7 ml of membrane protein suspension and 4 nanomoles of labeled morphine or 4 nanomoles of labeled met-enkephalin, and 50 μg of bacitracin. To displace the label by the APSF preparation, it was used in concentrations of from 1 μg/ml to 1 mg/ml. During investigation of interaction of APSF with specific binding sites of [^{3}H]-met-enkephalin on lymphocytes, peripheral blood from healthy donors aged 20-40 years was used. Lymphocytes were isolated in a Ficoll—Verografin gradient and washed twice with 50 mM Tris-HCl, pH 7.4. The radioceptor investigation of interaction of [^{3}H]-met-enkephalin with lymphocytes was carried

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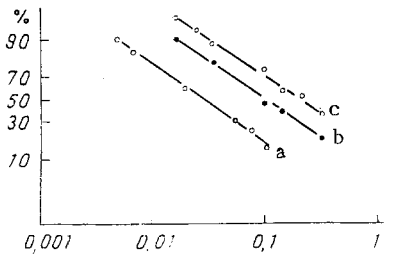

Fig. 1

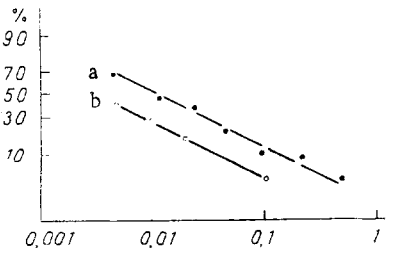

Fig. 2

Fig. 1. Comparative ability of **morphine** and APSF to displace [^{3}H]morphine from rat brain opiate receptors: a) morphine, b) APSF with bacitracin, c) APSF without bacitracin. Abscissa, concentration of morphine (in μM) and APSF (in mg/ml) in reaction mixture; ordinate, level of specific binding of [^{3}H]morphine with rat brain opiate receptors (in % of control). Each value is mean of four determinations, differing from each other by less than 10%.

Fig. 2. Displacement of [^{3}H]-met-enkephalin from rat brain opiate receptors by unlabeled met-enkephalin (a) and APSF (b). Abscissa, concentration of met-enkephalin (in μM) and APSF (in mg/ml); ordinate, level of specific binding of [^{3}H]-met-enkephalin with rat brain opiate receptors (in % of control). Reaction mixture contained bacitracin (50 μg/ml). Each value is mean of four determinations differing from each other by less than 10%.

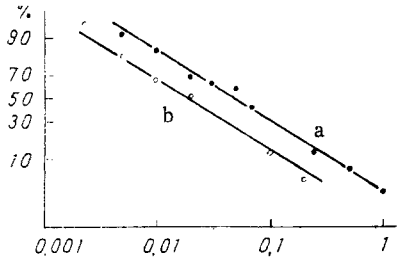

Fig. 3. Displacement of [^{3}H]-met-enkephalin from specific binding sites on lymphocytes by unlabeled met-enkephalin (a) and APSF (b). Abscissa, concentration of met-enkephalin (in μM) and APSF (in mg/ml); ordinate, level of specific binding of [^{3}H]-met-enkephalin on lymphocytes (in % of control). Reaction mixture contained bacitracin (50 mg/ml). Each value is mean of three determinations differing from each other by less than 10%.

out as described previously [1]. The level of specific binding of the label was determined as the difference between binding of [^{3}H]-met-enkephalin or [^{3}H]morphine in the presence and absence of 2 μM of met-enkephalin or morphine, **respectively, in the** reaction mixture. Bound and unbound labels were separated by rapid filtration (not more than 10 sec per sample) through glass GF/B filters (from Whatman, England). To study interaction of labeled opiates with rat brain membranes, the filters were washed with 7.5 ml cold 50 mM Tris-HCl buffer, pH 7.7, and for the work with lymphocytes, with 7 ml cold 50 mM Tris-HCl buffer, pH 7.4. The filters were transferred to flasks with 8 ml "Lipoluma" scintillator (from Lumac, Switzerland) to measure radioactivity, and kept there for 12 h; the radioactivity was measured on a "Mini Beta" counter (from LKB, Sweden).

The following reagents were used: [^{3}H]-met-enkephalin (39 Ci/mmole, from Amersham **Corporation, England**), [^{3}H]morphine (22 Ci/mmole, from the same firm), met-enkephalin (from Miles, USA), morphine -- pharmaceutical preparation, bacitracin (53,500 IU/g, from Sigma, USA), Tris (from Serva, West Germany), Ficoll (from Pharmacia, Sweden), Verografin (from Spofa, Czechoslovakia), and human serum albumin (from Reanal, Hungary).

EXPERIMENTAL RESULTS

The concentration of APSF causing 50% replacement of the label ([^{3}H]morphine) from specific binding sites with brain receptors (EC_{50} — effective concentration) in incubation medium containing the protease inhibitor bacitracin, was 96 μg/ml (Fig. 1b), whereas in medium without bacitracin it was 190 μg/ml (Fig. 1c). Hence it follows that APSF can bind specifically with rat brain opiate receptors. It can also be postulated that this property is due to ligands of peptide nature.

The comparative ability of met-enkephalin and APSF to displace [^{3}H]-met-enkephalin competitively from rat brain opiate receptors is shown in Fig. 2. As Fig. 2 shows, EC_{50} for APSF was 3.2 μg/ml. It can be concluded from a comparison of EC_{50} values for APSF obtained during displacement of morphine (Fig. 1b) and met-enkephalin (Fig. 2b) from opiate receptors that opiates in the composition of APSF are more able to interact with opiate receptors of the δ type than of the μ type.

The ability of met-enkephalin and APSF to displace [^{3}H]-met-enkephalin competitively from the specific binding sites of this ligand on human lymphocytes is demonstrated in Fig. 3. In these experiments EC_{50} for APSF was 18 μg/ml (Fig. 3b).

The results of this investigation demonstrate the ability of APSF to bind specifically with opiate receptors and they show that APSF possesses opiate activity; these observations agree with previous data on the analgesic action of this mediator [3].

Experimental results indicating interconnection between the immune and nervous sytems have recently been obtained. For instance, receptors for opiate peptides — β-endorphin [8] and met-enkephalin [1, 11] — have been found on immunocompetent cells. At the same time, it has been shown that interferon isolated from lymphocytes contains substances similar in their properties and structure to ACTH and β-endorphin [6, 7].

Interaction between the immune and nervous systems is thus effected through the participation of soluble mediators. Discovery of the opiate activity of APSF suggests its possible role in the transmission of information from the immune system to the nervous system and it opens up the prospects of a search for opiate-like substances among the various lymphokines.

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