SPECIFIC BINDING OF μ- AND δ-LIGANDS BY RAT BRAIN OPIATE RECEPTORS IN THE PRESENCE OF REAFERON

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Recent publications have demonstrated the existence of a link between opiate systems and α-interferon (α-IF), an endogenous regulator of many cellular processes and belonging to the class of immune polypeptides [3, 4, 7]. It has been shown that if α-IF is injected into the cerebral ventricles of mice, it induces β-endorphin-like effects, namely analgesia and catatonia [5], and also exhibited the property of modifying the course of the withdrawal syndrome in animals dependent on morphine [6]. Direct experiments using the radioreceptor method demonstrated the possibility of competitive displacement of opioid ligands from receptors by α-IF, obtained from human leukocytes [3, 5]. It is considered that activity of α-IF relative to opiate receptors is probably due to fragments of its amino-acid sequence that are similar to the sequence of β-endorphin [9]. However, the functional analogy based on the hypothetical structural similarity is not observed in every case. After microiontophoretic application of α-IF to rat brain cells, inhibition of spontaneous neuronal discharges, characteristic of the action of morphine, was not found [7]. Interaction of α-IF with opiate receptors, at least those of the μ-type, in vitro also is effected, according to the definition of the "sodium shift" by an antagonistic mechanism [3]. These contradictions may perhaps be explained by the fact that the methods of the effect of α-IF on opiate receptors may differ from the classical competitive interaction in the active center of the receptor [1]. The role of peptide and carbohydrate components of the α-IF molecule in reception likewise remains unexplained.

It was accordingly decided to use biochemical approaches to assess the opiate activity of reaferon, which is a recombinant α_2-interferon obtained by genetic engineering methods, and is the polypeptide part of the interferon molecule without its sugar residues.

EXPERIMENTAL METHOD

Male albino rats weighing 180-220 g were used. Isolation of the membrane fraction of brain cells (without the cerebellum), containing opiate receptors, and radioreceptor analysis were carried out as described previously [2]. To separate the bound and freely dissolved label, a filtration method was used [8]. Specific interaction of labeled ligands with opiate receptors was determined as the fraction of their binding by membranes which depended on the presence of 2.5 μM D-ala^2-met-enkephalinamide (DALA) — a nonselective opioid ligand. The results were subjected to statistical analysis by Student's method. Protein in the samples was determined by Lowry's method.

The following compounds were used: ^{3}H-D-ala^2, D-leu^5-enkephalin (^{3}H-DADL) — 45 Ci/mmole; ^{3}H-RX 783006 (^{3}H-DAGO) — 60 Ci/mmole; ^{3}H-morphine — 28 Ci/mmole; ^{3}H-dihydromorphine (^{3}H-DHM) — 67 Ci/mmole, ^{3}H-naloxone-60 Ci/mmole (from Amersham International, England); DALA (from Serva, West Germany); bacitracin (from Sigma, USA). A commercial preparation of reaferon was used in a dose of 10^6 U in an ampul ("Biopreparat" All-Union Production Combine).

EXPERIMENTAL RESULTS

The reaferon preparation, on addition to the reaction medium, induced concentration-dependent inhibition of specific interaction of ^{3}H-DADL with opiate receptors. The inhi-

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bition constant (K_i) of reception of the δ-agonist by reaferon, calculated from the results of three independent experiments, was 8.50 ± 2.60 $U \cdot 10^{-3}$/ml. If the assumption was valid that all of the determined activity in relation to opiate receptors is due to the interferon molecule itself, the reduced value of K_i is 1.7 nM reaferon. Consequently, the effectiveness of reaferon relative to displacement of ^{3}H-DADL from binding sites is equivalent to the analogous action of morphine and enkephalins.

Reaferon had a similar effect on receptor binding of μ-agonists — ^{3}H-morphine, ^{3}H-DHM, and ^{3}H-DAGO: in the presence of the immune peptide in concentrations of above 500 U/ml, a fall of the level of binding of these labeled compounds was observed. The relative ability of reaferon to inhibit reception of ^{3}H-DAGO — a μ-selective compound of peptide nature — was found to be rather less than its action on alkaloids. The values of K_i for ^{3}H-morphine, ^{3}H-DHM, and ^{3}H-DAGO were 3.25 ± 0.35, 4.28 ± 0.81, and 6.51 ± 1.27 $U \cdot 10^{-4}$/ml respectively.

It is easy to see that the ability of reaferon to reduce specific interaction of the δ-agonist ^{3}H-DADL with opiate receptors by an order of magnitude exceeds its effect on μ-agonist binding. This indicates a preferential reaction of reaferon with δ-receptors. According to data published previously, the same property is possessed also by α-IF obtained from human leukocytes [3]. Considering the differences in the structure of leukocytic and recombinant α-IF, it can be accepted that selectivity in interaction with a particular type of opiate receptors is determined, not by the carbohydrate, but probably by the peptide component of the native glycopeptide molecule.

The character of the effect of reaferon on reception of ^{3}H-naloxone was found to be different from its action on agonist binding.

In concentrations of up to 10^5 U/ml reaferon did not lower the level of receptor interaction of the antagonist. Conversely, in the presence of more than $5 \cdot 10^3$ U/ml of reaferon activation of specific binding of ^{3}H-naloxone was observed. The isotherm shown is "saturating" in character, evidence of restriction of the number of sites reacting with reaferon. It will be noted that further addition of 100 mM NaCl to the reaction mixture led to reversal of the activating effect of reaferon throughout the range of concentrations tested.

The observed increase in the level of binding of ^{3}H-naloxone may have been the result of interaction of reaferon both with the macromolecular receptor complex and also with the label. The latter suggestion appears unlikely, for evidence against it is given by the fact that we could find no mention in the literature of the possibility of binding of reaferon with morphine and DHM, which closely resemble naloxone in their structure, although they differ from it in their pharmacological properties. Nevertheless, we tried to obtain experimental data refuting or confirming this view. Analysis carried out in the absence of membranes in the reaction mixture, just as also in the presence of previously heated (10 min at 98°C) membranes, revealed no specific binding of ^{3}H-naloxone by reaferon in concentrations of up to 8 nM and $5 \cdot 10^4$ U/ml respectively. Thus the only possible explanation of the phenomenon remains the suggestion that reaferon interacts with a naloxone-binding receptor. Since binding of a substance in an active center implies competitive displacement of the label from it, there is good reason to postulate the existence of a special site, reacting with reaferon. Consequently, the effect of the immune polypeptide on reception of ^{3}H-naloxone may be realized by a mechanism of allosteric regulation type.

Previously the writers showed that allosteric regulation of reception of ^{3}H-naloxone by a preparation of leukocytic α-IF is possible [1]. In accordance with the data described above, reaferon may also probably possess this same property. Meanwhile the effect of these compounds on specific binding of the antagonist is not identical. For example reaferon, unlike α-IF, cannot displace ^{3}H-naloxone from the active center and it exhibits activating properties only in a preparation of freshly prepared membranes, which have not been frozen. It can accordingly be concluded that although the presence of the amino-acid sequence of α-IF or of its fragment is essential for activation of ^{3}H-naloxone binding, the intensity of the regulatory effect depends on the presence of the carbohydrate component of the immune glycopeptide molecule.

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EFFECT OF A HELIUM-NEON LASER ON PIAL MICROCIRCULATION IN RATS

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Lasers, which have a photodynamic and biostimulating action on tissues, are being increasingly used to study the morphological and functional changes in organs and tissues [2, 5, 8]. The use of lasers in microcirculatory research has made it possible to apply precise conditions of stimulation to different parts of the microcirculatory bed [6] and also a means of assessing the microcirculation of the blood in vivo [7]. The use of low-energy lasers, inducing a biostimulating effect, is promising as a means of studying the effect of outside influences on vessels of the microcirculatory bed [1, 3]. Among these lasers, the helium-neon laser (HNL) has achieved the most widespread popularity.

The aim of this investigation was to study the effect of HNL on the microcirculatory bed of the pia mater and the microcirculation in rats in order to discover the sensitivity of arteriolar and venular microvessels to this agent at different ages of postnatal development.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats aged 7, 30, 45, 60 and 90 days. Preparation of the animal and biomicroscopy of the pial microvessels were carried out by the method described previously [4]. The MBB-1 contact microscope, through the optical system of which the radiation of an HNL (the LGN-104) with a wavelength of 0.63 μ and a power of 40 mW was passed, was used. The duration of irradiation of the microvessels was 1, 3, 6, 12, 18, and 30 min. The diameter of the laser beam in the focal plane of the microscope was 10-20 μ, so that it was possible to subject the wall of arterioles, precortical arterioles, capillaries, postcortical venules, larger venules, and arteriolo-arteriolar anastomoses to the action of the laser beam.

EXPERIMENTAL RESULTS

High-precision application of HNL to the pial microvessels induces local changes in the microcirculation. In the zone of action of the laser on the wall of an arteriole dilatation develops, with the result that a zone of local dilatation is formed. Above and below the zone of dilatation, constriction of the microvessel is observed (Fig. 1). The amount of dilatation is directly proportional to the duration of exposure, and inversely proportional

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