α-INTERFERON–BRAIN OPIATE RECEPTOR INTERACTION

L. F. Panchenko, T. N. Alyab'eva,
V. V. Malinovskaya, and A. M. Balashov

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Interferons (IF) are a class of endogenous compounds which participate in the regulation of various cellular processes [4]. According to the accepted classification [2] three types of human IF are distinguished: leukocytic (α-IF), fibroblastic (β-IF), and immune (γ-IF), with different spectra of physiological activity. It is considered [3, 9] that the biological importance of antiviral, immunomodulating, anticellular, radioprotective, and other effects of IF is the maintenance of cellular homeostasis by these related compounds.

Recent investigations [5, 6] have demonstrated that IF possess structural and functional similarity with other physiologically active substances, including the endorphins. For instance, IF have been shown to induce analgesia and catatonia, and to partially block the inhibitory effect of morphine on neuronal electrical activity in the rat brain [8]; common amino-acid sequences have been found in molecules of IF, ACTH, and β-endorphin [5]. These data are evidence that the opiate like effects of IF may be realized through interaction with specific opiate receptors of the CNS.

A biochemical study of the effect of IF on opiate reception in the brain showed [6] that only α-IF preparations actively bind opiate sites on biological membranes, and this is not characteristic of β- and γ-IF. It has been shown that the ability of α-IF to displace ^{3}H-dihydromorphine from opiate receptors is inversely proportional to its specific antiviral activity. Meanwhile the problem of selectivity of the opioid effects of α-IF on different types of opiate receptors, and its agonist-antagonist properties, remains unsolved.

EXPERIMENTAL METHOD

Male noninbred laboratory albino rats weighing 200-250 g were used. The animals were decapitated in the morning, the brain was quickly removed in the cold, the cerebellum was detached, and the remaining tissues were frozen and kept for 2-3 months at −80°C until use. The membrane fraction of the brain cells, containing opiate receptors, was obtained immediately before the radioreceptor investigations, as described previously [1]. The reaction of binding of labeled opiates and α-IF with opiate receptors was carried out in the presence of bacitracin, a nonspecific protease inhibitor, at 25°C for 40 min. To separate the membrane-bound and freely dissolved radioactive label, a filtration method [11] was used. Specific interaction of the label with the receptors was determined from the difference between membrane-bound radioactivity determined in the presence and absence of 2.5 μM D-ala^2-metenkephalinamide (DALA). The experimental results were subjected to statistical analysis by Student's t test.

The following compounds were used: ^{3}H-D-ala^2, D-leu^5-enkephalin (^{3}H-DADL), 32.1 Ci/mmole (Amersham International, England); ^{3}H-naloxone, 48 Ci/mmole (New England Nuclear, USA); DALA (Serva, West Germany); bacitracin, Tris (Sigma, USA). The α-IF preparation, with specific antiviral activity of $2.5 \cdot 10^3$ U/mg protein, was generously provided by the Department of Interferon Biosynthesis, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. The remaining reagents were of Soviet origin and of the chemically pure and highly pure grades.

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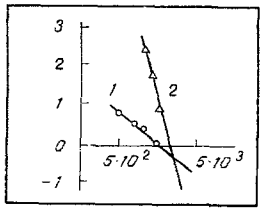

Fig. 1. Comparative ability of α-IF to displace ^{3}H-DADL (1) and ^{3}H-naloxone (2) from opiate receptors. Abscissa, α-IF concentration (in U/ml); ordinate, logit B/B_0, where B_0 is specific binding of the label in the absence of α-IF, and B specific binding of the label in the presence of the given concentration of α-IF. Results of one of three independent experiments are shown.

EXPERIMENTAL RESULTS

The results of comparative determination of the ability of α-IF to displace selective ligands (^{3}H-naloxine and ^{3}H-DADL) from the binding sites of opiate receptors are given in Fig. 1. The α-IF preparation was more effective at inhibiting specific binding of ^{3}H-DADL than of ^{3}H-naloxone; the corresponding values of EC_{50} (the concentration of α-IF replacing 50% of the label in the receptors), determined by log-logit transformation, were 1250 ± 250 and 2190 ± 150 U/ml ($n = 3$, $p \leqslant 0.05$). Preliminary experiments showed that in a concentration of 1.5 nm ^{3}H-DADL and ^{3}H-naloxone interacts selectively, mainly with delta- and mu-receptors respectively; moreover, the equilibrium dissociation constant (K_d) was 0.22 nm for ^{3}H-DADL and 1.67 nM for ^{3}H-naloxone. From the experimentally determined values of K_d and EC_{50}, inhibition constants of receptor binding of the ligands used by α-IF were calculated, and were found to be 1150 ± 80 U/ml for ^{3}H-naloxone and 160 ± 30 U/ml for ^{3}H-DADL. Consequently, the α-IF preparation interacts 7.2 times more preferentially with opiate receptors of the delta-type than with mu-receptors.

Interaction of morphine agonists and antagonists with opiate receptors is known to differ in sensitivity to Na^+ cations [2]. Accordingly the ability of α-IF to displace ^{3}H-naloxone from the binding sites was determined in the absence and presence of 100 mM NaCl. The addition of Na^+ cations to the reaction mixture did not change the effectiveness of interaction of α-IF with ^{3}H-naloxone-binding receptors. The "coefficient of the sodium shift," determined by the ratio $EC_{50}{}^{+NaCl}/EC_{50}{}^{-NaCl}$, was 1. According to data in [12], independence of receptor interaction of a compound tested by this method of the presence of Na^+ cations in the medium predetermined its antagonistic properties. It can thus be concluded that α-IF interacts antagonistically with opiate receptors.

The antagonistic character of interaction of the α-IF preparation with opiate receptors, incidentally, does not mean that it exhibits only the properties of a morphine antagonist in other test systems. There is evidence [6] of parallel changes in motor activity and sensitivity to pain following central administration of opioid, morphine, and a highly purified α-IF preparation, i.e., in vivo α-IF can exhibit pharmacological activity as a morphinomimetic. On the other hand, the results of electrophysiological experiments are evidence of the development of the opposite effects following electrophoretic application of morphine and α-IF to cells of the cerebral cortex [10]. The results of the present experiment are in agreement with this last observation, for it can be concluded from them that the effect of α-IF on opiate systems is not necessarily mediated by activation of postreceptor biochemical and physiological processes. This state of affairs probably explains the data on modification by α-IF of the naloxone-induced withdrawal syndrome in morphine-dependent rats [7]. α-IF itself evoked a weak withdrawal-like response in morphine-dependent animals, but when combined with naloxone, it reversed its behavioral effects. The final solution to the problem of whether agonistic or antagonistic properties of α-IF predominate in vivo is difficult because of the multiplicity of points of application of its activity; in vitro, however, when interaction of neuronal mechanisms is excluded, α-IF behaves as a "pure" morphine antagonist.

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OPIOID ACTIVITY OF PEPTIDES AND HEALING OF SKIN WOUNDS

V. A. Vinogradov, S. E. Spevak, K. N. Yarygin, N. V. Korobov, A. I. Solov'eva, A. B. Shekhter, and M. I. Titov

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It has been shown experimentally and clinically in recent years [2-5] that certain peptides, which are enkephalin analogs, can stimulate repair of injuries to many tissues. At the same time, enkephalins are known to play an important role in the activity of peptidergic antinociceptive centers [8, 14]. The change in their functional activity arises under the influence of nociceptor stimulation [9], indicating a disturbance of the structural integrity of the tissues [6]. Hence it may be postulated that endogenous opioids not only regulate sensitivity to pain in vivo, but also are evidently involved in the system regulating structural homeostasis. Exogenous ligands, structural analogs of the enkephalins, may act in accordance with the same principle. Pharmacological investigations [1, 7] have shown that most of them have an analgesic action when injected intracisternally and systemically. However, to elucidate the mechanism of the effect of endogenous peptides and their structural analogs on the healing process, the first essential is to establish the link between opioid activity and regulation of repair processes.

The aim of this investigation was to study peripheral and central opioid activity of dalargin, its four analogs, and also of FK-33824, DADLE, met-enkephalin, morphine, and naloxone and to compare it with their effects on the healing of dorsal skin wounds in rats.

EXPERIMENTAL METHOD

Peripheral opioid activity of the peptides was assessed by their ability to inhibit contractions of an isolated segment of the ileum of noninbred male guinea pigs [11] and of the isolated vas deferens of noninbred mice [10], evoked by electrical stimulation. The preparations were placed in a constant-temperature cell 10 ml in volume, with modified Krebs' solution and stimulated by an electric current: the ileum — by pulses 1 sec in duration with a frequency of 0.1 Hz, the vas deferens by series of 4-6 pulses with a duration of 0.5 msec, an interval of 2 msec, and a frequency of 0.15 Hz. Contractions of the organs were recorded under isometric conditions by means of a K 30 transducer (Hugo Sachs Elektronik) on an MS 6601 automatic writer (Watanabe). The peptides were dissolved in distilled water and added to the surrounding solution in a volume of 5-50 μl. Activity of the peptides was expressed as the index EC_{50} — the concentration causing a reduction of 50% in the amplitude

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