PHYSIOLOGY

Modulation of Peripheral Opioid Receptors Affects the Concentration of μ-Opioid Receptors in Rat Brain

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Radioligand binding assay was used to evaluate characteristics of central μ-opioid receptors after peripheral administration of μ-opioid receptor agonist loperamide and antagonist methylnaloxone. These substances do not cross the blood-brain barrier. Loperamide and methylnaloxone produced opposite effects on the density of μ-opioid receptors in the frontal cortex of rat brain. These data confirm our hypothesis on reciprocal interactions between central and peripheral compartments of the endogenous opioid system.

Key Words: *central and peripheral μ-opioid receptors; methylnaloxone; loperamide; radioligand binding*

The endogenous opioid system is present not only in CNS, but also in various peripheral organs and tissues. The structure of opioid receptors and opioid peptides is similar in CNS and other organs and tissues. The endogenous opioid system differs in central and peripheral functions, which is related to an impermeability of the blood-brain barrier (BBB) for most opioid peptides [4,5]. We hypothesized the existence of reciprocal interactions between the central and peripheral compartments of the endogenous opioid system [1]. According to this hypothesis, functional suppression of the peripheral compartment should be followed by activation of the central compartment. Activation of peripheral opioid receptors is probably accompanied by inhibition of central receptors. The proposed hypothesis was confirmed by experimental data. We showed that peripheral administration of opioid receptor antagonist methylnaloxone, which does not cross BBB, is followed by significant reduction of withdrawal syndrome in morphine-dependent rats [2] and has a strong analgesic effect. μ-Opioid receptor agonist loperamide, which does not cross BBB, insignificantly decreases the tail-flick latency [1].

We assume that methylnaloxone has an antagonistic effect on peripheral μ-opioid receptors, which results in an increase in the concentration or affinity of central μ-opioid receptors, development of the analgesic effect, and prevention of withdrawal syndrome. By contrast, loperamide activates peripheral receptors. The resulting inhibition of central μ-opioid receptors causes hyperalgesia. To test this hypothesis, we studied the characteristics of central μ-opioid receptors after peripheral administration of opioid receptor ligands, methylnaloxone and loperamide.

MATERIALS AND METHODS

Experiments were performed on 24 male Wistar rats weighing 140-180 g. The animals were housed in ca-

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ges (8 specimens per cage) and had free access to water and mixed food (standard diet). After adaptation to vivarium conditions, the animals were divided into 3 groups (8 rats per group). They received an intraperitoneal injection of loperamide (5 mg/kg, Sigma; group 1), naloxone methiodide (5 mg/kg, Sigma; group 2), or equivalent volume of 0.9% NaCl (group 3). The animals were decapitated 30 min after injection. The brain was removed and placed in a Petri dish (dorsal surface downward) on ice. The hypothalamus, midbrain, and frontal cortex were isolated.

μ-Receptors were identified by radioligand binding assay. The brain structure was homogenized in a Downs homogenizer with 25 ml 50 mM Tris-HCl buffer (pH 7.7) at 4°C. The suspension was centrifuged at 30,000*g* and 4°C for 15 min. The supernatant was removed. The pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.7) at 25°C. The suspension was incubated in a water bath at 37°C for 40 min and repeatedly centrifuged. The pellet was resuspended in 25 ml 50 mM Tris-HCl buffer (pH 7.7) at 25°C. Protein content in the supernatant was measured by the method of Lowry. The binding capacity of μ-opioid receptors was estimated by radioreceptor assay. [^{3}H]-DAGO (Amersham) served as a μ-opioid receptor ligand.

The reaction mixture for evaluation of the binding capacity of μ-opioid receptors (0.5 ml) consisted of 50 μl bacitracin (final concentration 50 μg/ml), 50 μl labeled ligand, 150 μl membrane protein suspension (final concentration 0.6-0.8 mg/ml), and 300 μl 50 mM Tris-HCl buffer (pH 7.4). When the reaction mixture contained 50 μl labeled ligand and 50 μl unlabeled ligand, the volume of 50 mM Tris-HCl buffer (pH 7.4) was 250 μl. The binding reaction was performed at 25°C for 1 h under thorough agitation.

The specific interaction of tritium-labeled DAGO with μ-opioid receptors was estimated as the difference between membrane-bound radioactivity under conditions of total binding (without unlabeled DAGO) and nonspecific binding (100-fold excess of unlabeled DAGO).

The reaction was stopped by rapid filtration of samples through GF/B fiberglass filters (Whatman) on a Millipore device. The filters were washed with 6 ml Tris-HCl buffer (pH 7.4), dried in air, and placed in flasks for scintillation counting. The standard dioxane scintillator (7 ml) was added. Radioactivity was measured on a RackBeta 1219 scintillation spectrometer (LKB). The efficiency of counting was not less than 30%.

The dissociation constant (Kd; inversely proportional to the receptor affinity for ligand) and Bmax (number of ligand-binding sites) were used as the criteria for receptor binding capacity.

Protein concentration was measured by the method of Lowry. The membrane suspension was pretreated with sodium deoxycholate.

The data of a radioreceptor assay were processed with LIGAND software. The results were analyzed by Student's *t* test.

RESULTS

Single injection of μ-opioid receptor agonist loperamide was followed by a significant decrease in the number of these receptors in the midbrain and frontal cortex (Table 1). However, the number of μ-opioid receptors in the frontal cortex increased significantly after single treatment with μ-opioid receptor antagonist naloxone methiodide (Table 2). It should be emphasized that the receptor affinity for the ligand did not change after injection of loperamide or naloxone methiodide.

These data confirm our hypothesis on reciprocal interactions between the central and peripheral com-

TABLE 1. Binding Capacity of μ-Opioid Receptors after Single Injection of Loperamide

Groups of animals/brain structures	Kd, nM	Bmax, fmol/mg protein
Midbrain		
Control	5.2±1.0	157±60
Loperamide	4.2±0.7	51±18*
Hypothalamus		
Control	2.9±0.9	77±38
Loperamide	3.7±1.5	90±45
Frontal cortex		
Control	4.4±0.6	281±75
Loperamide	3.7±0.4	107±39*

Note. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Binding Capacity of μ-Opioid Receptors after Single Injection of Naloxone Methiodide

Groups of animals/brain structures	Kd, nM	Bmax, fmol/mg protein
Midbrain		
Control	5.2±1.0	157±90
Naloxone methiodide	3.9±0.8	180±63
Hypothalamus		
Control	2.9±0.9	77±38
Naloxone methiodide	4.9±1.3	58±34
Frontal cortex		
Control	4.4±0.6	281±75
Naloxone methiodide	6.1±1.1	421±90*

partments of the endogenous opioid system. Inactivation of the peripheral compartment with an antagonist methylnaloxone was followed by an increase in the density of μ-opioid receptors in the cerebral cortex. These changes probably contribute to the analgesic effect. Published data show that opiate withdrawal syndrome is accompanied by low activity of the central compartment of the endogenous opioid system [3,6]. The increase in the number of μ-opioid receptors in various structures of the brain will be probably followed by the reduction of withdrawal symptoms. The decrease in the density of opioid receptors in rat brain after peripheral administration of loperamide was followed by mild hyperalgesia. Our results illustrate the opposite effects of peripheral treatment with loperamide and methylnaloxone on the subsystem of opioid receptors in the frontal cortex of rat brain. It is manifested in changes in the density of receptors (Bmax) in this structure.

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