SPECIFIC BINDING OF ^{3}H-NALOXONE WITH ISOLATED RAT ENTEROCYTES

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KEY WORDS: opioid receptors; naloxone; rat enterocytes.

Much evidence has now been obtained to show that opioid peptides and opioid receptors are present not only in the central nervous system, but also in other organs, including the intestine [5, 10]. It is traditionally considered that peripheral opioid receptors are associated with nerve cells, whereas binding of opioids has been found with some cells that are unrelated to the nervous system: stimulated human lymphocytes [4], rat mast cells [11], and also pituitary cells [6]. These findings suggest the existence of as yet unknown regulatory mechanisms connected with opioids.

Data on the specific binding of naloxone with isolated rat enterocytes are reported below.

EXPERIMENTAL METHOD

Isolated rat enterocytes were obtained by the method in [2] with certain modifications. Male rats deprived of food for 24 h were decapitated and the small intestine was removed and everted on a plastic pipette with a volume of 1 cm^3 (Falcon, USA). The pipette with the everted intestine was cut into segments 10 cm long and washed in medium 199. To remove mucus and microorganisms more effectively the segments were incubated for 10 min at 37°C in medium 199 containing 1 mg/ml of hyaluronidase (type I, from Sigma, USA) [12]. The segments were then washed in cold 0.14 M NaCl solution, dried, and placed in 40 ml of incubation medium. The incubation medium was a solution consisting of 65.7% of Dulbecco's phosphate buffer and 34.3% of distilled water, and contained 1% of polyvinylpyrrolidone (mol. wt. 40,000 daltons, from Sigma). The samples were incubated in this medium for 10 min at 37°C, then for 1 h at 25°C, with stirring at the rate of 200 rpm, and were finally shaken on a vortex for 1-2 min. The cells were sedmented by centrifugation for 10 min at 500g and 4°C, washed twice with medium 199 containing 1 mg/ml of bovine serum albumin (BSA), and filtered through a nylon filter. The number of cells was counted with a hemocytometer and their viability was determined by the trypan blue test: It was 80-90%. On average $5 \cdot 10^7$ cells were obtained from one animal.

Naloxone was bound with the cells in medium 199 containing 1 mg/ml of BSA. The incubation mixture (volume 1 ml) contained 5 × 100 mM ^{3}H-naloxone (42 Ci/mmole, Amersham Corporation, England) and, if indicated, other substances also. The samples were incubated at 0, 25, and 37°C and filtered through a Whatman GF/C filter; the filters were washed and dried, and radioactivity bound with them was determined by means of a scintillation counter. Nonspecific binding was determined in the presence of 10 μM of unlabeled naloxone.

EXPERIMENTAL RESULTS

Dose dependence of binding of naloxone with rat enterocytes is shown in Fig. 1. Specific binding (i.e., that inhibited in the presence of 10 μM of unlabeled naloxone) accounted for the greater part of the total binding. Saturation was reached at relatively high concentrations of the ligand. From 300 to 800 femtomoles of naloxone bound with 10^6 cells (i.e., $3 \cdot 10^5$-$8 \cdot 10^5$ molecules per cell). Addition of 5 mM HEPES to the incubation medium reduced binding of naloxone unexpectedly sharply (not indicated). The kinetics of specific binding of naloxone with enterocytes at different temperatures is shown in Fig. 2.

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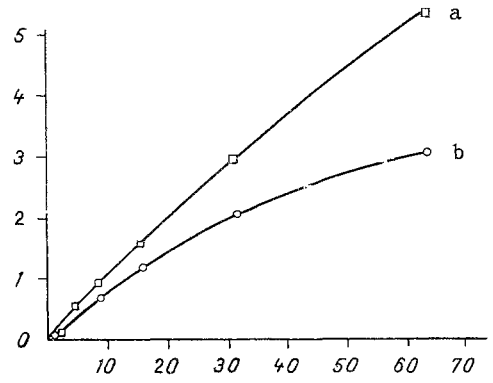

Fig. 1

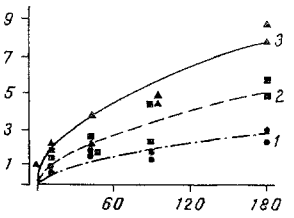

Fig. 2

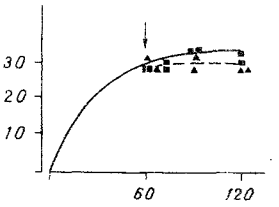

Fig. 3

Fig. 1. Binding of ^{3}H-naloxone with isolated rat enterocytes. Abscissa, concentration of ^{3}H-naloxone (in nM); ordinate, binding of naloxone (in cpm$\cdot 10^{-3}$). a) Total binding; b) specific binding. The incubation mixture contained $5\cdot 10^5$ cells and 1-64 nM ^{3}H-naloxone in 1 ml of medium 199. The samples were incubated for 60 min at 37°C.

Fig. 2. Kinetics of specific binding of ^{3}H-naloxone with isolated rat enterocytes at 0°C (1), 25°C (2), and 37°C (3). Abscissa, time (in min). Remainder of legend as to Fig. 1.

Fig. 3. Irreversibility of binding of ^{3}H-naloxone with isolated rat enterocytes. Abscissa, time (in min); ordinate, binding of naloxone (in cpm$\cdot 10^{-3}$ per $0.5\cdot 10^6$ cells). Cells ($5\cdot 10^5$ per sample) were preincubated in the presence of 17.5 nM ^{3}H-naloxone for 1 h at 37°C. Next, 10^4 nM of unlabeled naloxone was added to half of the samples and binding of ^{3}H-naloxone with enterocytes was determined at intervals of 0, 10, 30, and 60 min in samples containing (squares) and not containing (triangles) unlabeled naloxone.

TABLE 1. Inhibition of Specific Binding of ^{3}H-Naloxone with Isolated Rat Enterocytes by Opioid Ligands

Ligand	Concentration, nM	Inhibition of specific binding of ^{3}H-naloxone, %
Naloxone	10^3	42
	10^4	87
Morphine	10^3	42
	10^4	90
Bremazocine	10^3	29
	10^4	40
SKF-10047	10^3	28
	10^4	40

On addition of an excess of unlabeled ligand 1 h after the beginning of incubation no dissociation of the labeled ligand could be observed, evidence of irreversibility of binding of naloxone with enterocytes (Fig. 3).

Different ligands of opioid receptors can inhibit binding of naloxone competitively (Table 1). Morphine inhibits binding almost as actively as naloxone, whereas ligand SKF-10047 and bremazocine have weaker activity. Sodium azide inhibited naloxine binding by 85-90%.

Binding of naloxone with isolated rat enterocytes was thus demonstrated. The binding is specific, depends irreversibly on temperature, and is sensitive to the presence of sodium azide. This suggests that binding of the ligand is accompanied by internalization — a phenomenon well known for several membrane receptors, for example, receptors of insulin [1], nerve growth factor [9], epidermal growth factor [3], prolactin [7], and opioid peptides [8]. Internalization may be part of the transport pathway through enterocytes. At the same time, opioids may play an important role in the regulation of enterocyte function. It will be noted

that whereas considerable progress has been made in the study of the functional role of central opioid receptors, the physiological role of the peripheral binding sites of opioids is only little understood. However, the quite extensive distribution of these centers may be evidence of the existence of hitherto unknown opioidergic regulatory mechanisms.

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USE OF NEUROTROPIC DRUGS TO PREVENT APHTHOUS STOMATITIS

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Disturbances of neurotropic control during frequently repeated exposure to stress, of which the most important is due to impulses arising from injured organs of the digestive system, occupy an essential place among the factors involved in the mechanism of development of aphthous lesions of the oral mucosa [2, 5-13]. However, metabolic disturbances in the tissue substrate of the oral mucosa (as the direct expression of disturbed nutrition) have not yet been explained.

The aim of the present investigation was to study changes in levels of sympathetic nervous system mediators in the oral mucosa associated with injury to the abdominal organs and the possibility of using neurotropic drugs to prevent the development of aphthous lesions.

EXPERIMENTAL METHOD

Experiments were carried out on 22 dogs weighing from 7 to 15 kg and aged from 3 to 5 years. All the animals were subjected to quarantine for 3 weeks in the animal house. The mucosa of all the dogs was intact. Pentobarbital sodium in a dose of 0.03 g/kg was used as the anesthetic.

The animals as a whole were divided in three groups: 1) control, consisting of eight healthy dogs; 2) seven dogs in which aphthous lesions on the oral musoca were produced experimentally by ligation of the common bile duct [8]; 3) seven dogs receiving an intraperitoneal injection of the β-adrenoblocker propranolol (anaprilin, obsidan) in a dose of 1 mg/kg 30 min before ligation of the common bile duct. Areas of the oral mucosa were excised from all the animals 2 h after the operation from sites most frequently affected by pathological changes (the cheek, the retromolar space (RMS), and the adrenalin and noradrenalin (NA) concentrations in them were determined [1].

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