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EFFECT OF β-ENDORPHIN, ENKEPHALINS, AND THEIR SYNTHETIC ANALOGS ON UNIT ACTIVITY IN THE BULBAR RESPIRATORY CENTER

V. V. Yasnetsov, V. A. Pravdivtsev, and V. G. Motin

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KEY WORDS: endogenous opioid peptides; respiratory center neurons; microiontophoresis.

Endogenous peptides with morphine-like activity inhibit respiration [2, 7, 9, 10] as a result of the direct action of opioid peptides on the bulbar respiratory center and on suprabulbar structures concerned in the regulation of respiration [8]. Data in the literature on the direct effect of endogenous opioid peptides and their synthetic analogs on neurons of the bulbar respiratory center are scarce [6, 11].

It was accordingly decided to study the effect of microiontophoretic application of β-endorphin, enkephalins, and their synthetic analogs on single unit activity in the bulbar respiratory center. The observed effects of the opioid peptides were compared with the action of morphine.

EXPERIMENTAL METHOD

Unit activity in the bulbar respiratory center was recorded extracellularly in rabbits and cats anetsthetized with pentobarbital sodium (50 mg/kg, intraperitoneally) [1, 4, 5], and the test substances were applied by microiontophoresis through multibarreled glass microelectrodes [3]. In the course of the experiment an "Élektronika DZ-28" mimicomputer, coupled to the apparatus for recording unit activity, processed information on unit activity and plotted it graphically. Parallel with unit activity, the pneumogram was recorded (to identify respiratory neurons). The following freshly prepared solutions were used for microiontophoresis: β-endorphin 0.001 M; Met- and Leu-enkephalin, Tyr-D-Ala-Gly-Phe-NH_2, Tyr-D-Ala-Gly-Phe-D-Leu, Tyr-D-Ala-Gly-(Me)Phe-Gly-ol 0.02 M (all opioid peptides were synthesized at the All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, under the direction of Doctor of Chemical Science M. I. Titov); naloxone hydrochloride (from "Endo Laboratories," USA) 0.1 M; morphine hydrochloride 0.05 M. The solvent was 0.03 M NaCl, which also was used (3 M solution) to fill the recording and compensating barrels of the microelectrode. The substances were applied by currents of 10-50 nA with positive polarity.

To study the effect of the above-mentioned opioid peptides on respiration a series of experiments was carried out on waking rabbits. The frequency and volume of respiration were

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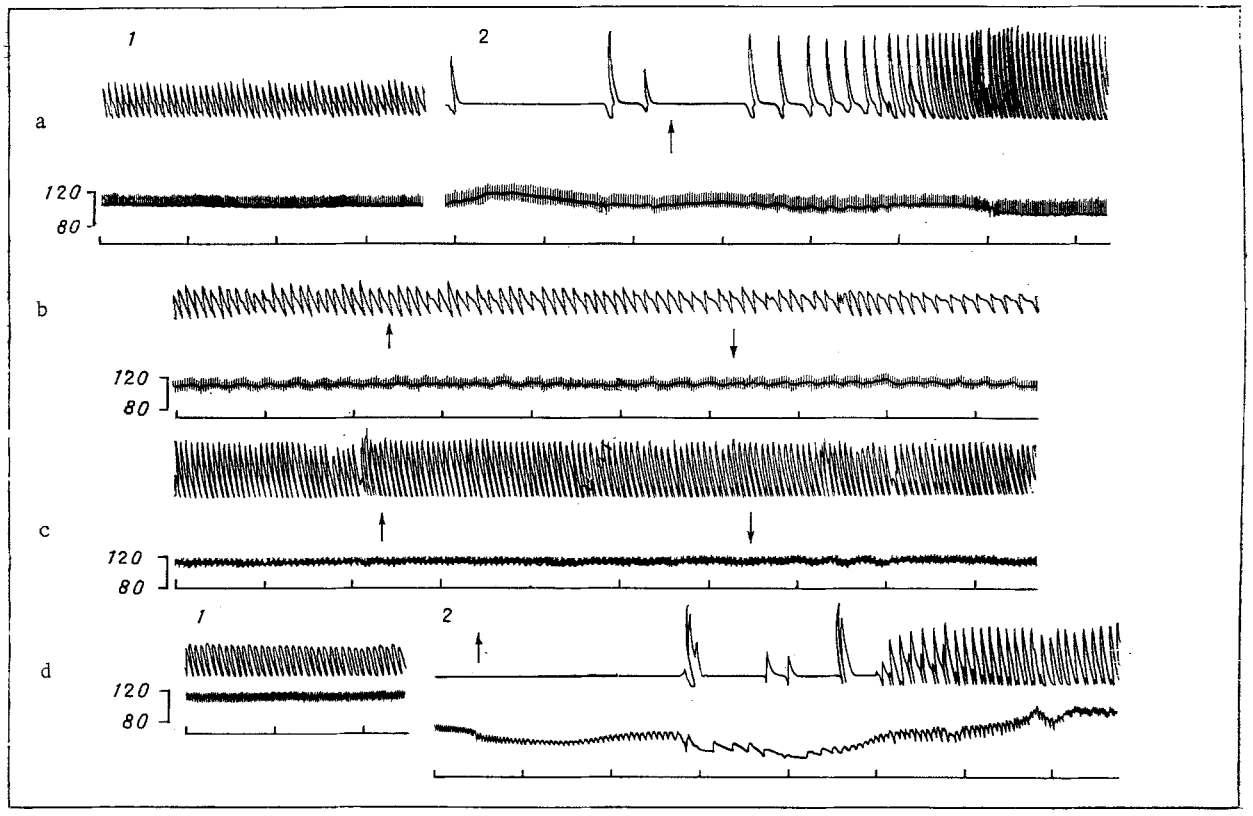

Fig. 1. Inhibitory effect of opioid peptides on respiration in waking rabbits. Abolition of their effect by naloxone. a-d) Different opiates. a: 1) Background, 2) 34 min after injection of Tyr-D-Ala-Gly-Phe-D-Leu in a dose of 200 µg (peptide injected into third ventricle in 30 µg of physiological saline). Arrow indicates beginning of injection of nalozone in a dose of 0.4 mg/kg, intravenously; b) arrows mark beginning and end of injection of Leu-enkephalin in a dose of 1000 µg (peptide injected into third ventricle in 30 µg of physiological saline); c) the same as b, but against the background of naloxone, 0.4 mg/kg, intravenously; d: 1) background, 2) 30 min after injection of Tyr-D-Ala-Gly-(Me)Phe-Gly-ol in a dose of 500 µg (injected into third ventricle in 30 µl of physiological saline). Arrow indicates beginning of injection of naloxone in a dose of 0.5 mg/kg, intravenously. In all experiments from top to bottom; respiration, arterial pressure (in mm Hg), time marker (10 sec).

recorded in 26 tracheotomized animals with the aid of the PDM-3 instrument, blood pressure was measured in the left common carotid artery by the direct method, and the ECG was recorded in standard lead II. The preparations were injected, within a wide range of doses, both intravenously (0.5-5.0 mg/kg) and into the third ventricle of the brain (10-1000 µg).

EXPERIMENTAL RESULTS

In waking rabbits β-endorphin, enkephalins, their synthetic analogs, and also morphine, depending on dose, induced inhibition of respiration whether administered systemically or into the third ventricle. Naloxone, a specific antagonist of narcotic analgesics, completely prevented or abolished this effect of the preparations (Fig. 1). Consequently, inhibition of respiration by opioid peptides is evidently due to their interaction with opiate receptors of various brain structures concerned in the regulation of respiration.

Opioid peptides and morphine, when applied microiontophoretically to inspiratory neurons of the respiratory center had a predominantly inhibitory effect on unit activity. Of 56 inspiratory neurons studied 36 (64.3%) responded by inhibition to microapplication of the substances (Fig. 2) and 20 (35.7%) neurons were areactive. No activation responses of neurons to the substances were observed. There were no qualitative differences in the action of β-endorphin, enkephalins, their analogs, or morphine on spike discharges of inspiratory neurons. If cell responded (did not respond) to one of the preparations, it responded (did not respond) similarly to the others. Naloxone prevented inhibitory effects of opioid pep-

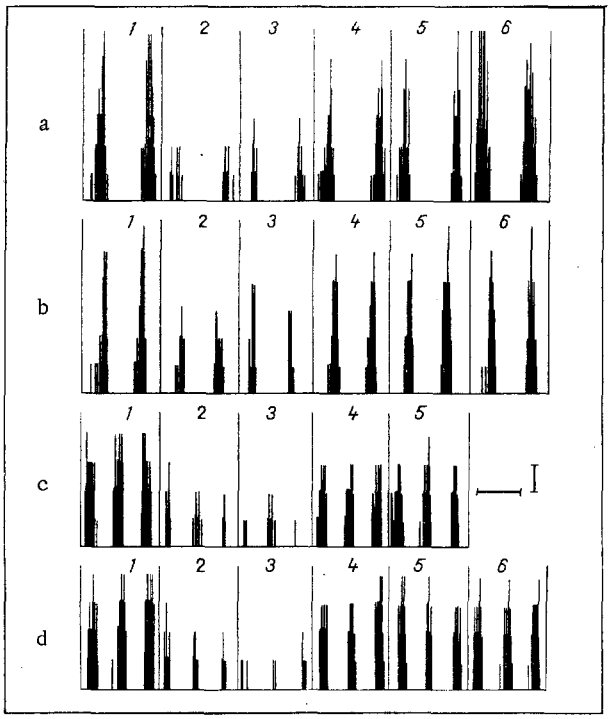

Fig. 2. Effect of opioid peptides on unit activity of inspiratory neurons of bulbar respiratory center. a: 1) Background, 2) morphine (20 nA, 4.5 min), 3) Leu-enkephalin (30 nA, 2.5 min), 4) morphine (30 nA, 5 min) against the background of naloxone (30 nA, 6 min), 5) Leu-enkephalin (30 nA, 3 min) against the background of naloxone (30 nA, 6 min), 6) Na^+ (30 nA, 6 min); b: 1) background, 2) β-endorphin (20 nA, 2 min), 3) Met-enkephalin (20 nA, 1 min), 4) Met-enkephalin (20 nA, 1 min) against the background of naloxone (30 nA, 3 min), 5) β-endorphin (20 nA, 2 min) against the background of naloxone (30 nA, 4 min), 6) Na^+ (30 nA, 4 min); c) 1) background, 2) Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (30 nA, 30 sec), 3) Tyr-D-Ala-Gly-Phe-D-leu (30 nA, 50 sec), 4) Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (30 nA, 30 sec) against the background of naloxone (30 nA, 1 min), 5) Tyr-D-Ala-Gly-Phe-D-leu (30 nA, 1 min) against the background of naloxone (30 nA, 2 min); d: 1) background, 2) Leu-enkephalin (30 nA, 20 sec), 3) Tyr-D-Ala-Gly-Phe-NH_2 (20 nA, 30 sec), 4) Leu-enkephalin (30 nA, 30 sec) against the background fo naloxone (30 nA, 1 min), 5) Tyr-D-Ala-Gly-Phe-NH_2 (20 nA, 30 sec) against the background of naloxone (30 nA, 1 min), 6) Na^+ (30 nA, 1 min). Here and in Fig. 3, a-d represents different neurons. Calibration: ordinate, 1 spike every 100 msec; abscissa, 5 sec.

tides and morphine (Fig. 2). This suggests that the inhibitory effect of opioid peptides and morphine on electrical activity of inspiratory neurons is connected with interaction of the preparations with opiate receptors located on these cells.

Opioid peptides and morphine had a similar action on unit activity when applied microiontophoretically to expiratory neurons of the respiratory center. For instance, of 42 cells recorded 26 (61.9%) of neurons were inhibited and 16 (38.1%) were areactive. No differences likewise were found in the action of the preparations and there were no activation responses to morphine-like substances. Naloxone prevented the inhibitory effect of opioid peptides and morphine (Fig. 3). Consequently, the inhibitory effect of the preparations on expiratory unit activity was due to interaction of β-endorphin, enkephalins, and their analogs with opiate receptors.

Considering that reticular neurons in the region of the respiratory center (they have no obvious connection with the rhythm of respiration) participate in the regulation of respiration [4, 5], the effect of opiod peptides and morphine on their discharge patterns was

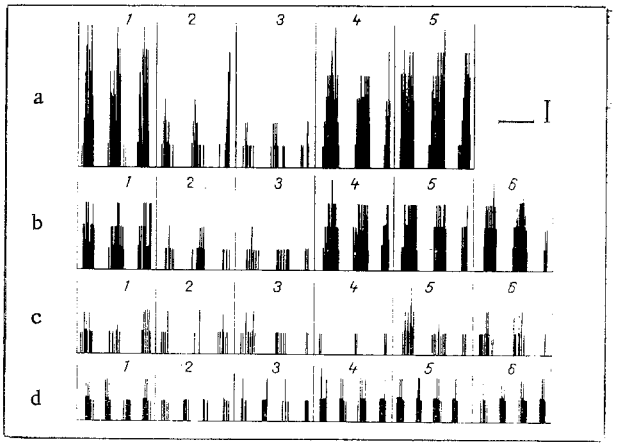

Fig. 3. Effect of opioid peptides on spike discharges of expiratory neurons of bulbar respiratory receptors. a: 1) Background, 2) morphine (30 nA, 2 min), 3) Leu-enkephalin (30 nA, 2 min), 4) morphine (30 nA, 3 min) against the background of naloxone (30 nA, 6 min), 5) Leu-enkephalin (30 nA, 2 min) against the background of naloxone (30 nA, 6 min); b: 1) background, 2) β-endorphin (30 nA, 1 min) against the background of naloxone (30 nA, 3 min), 5) Met-enkephalin (30 nA, 1 min) against the background of naloxone (30 nA, 3 min), 6) Na^+ (30 nA, 3 min); c: 1) background, 2) Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (20 nA, 2 min), 3) Tyr-D-Ala-Gly-Phe-D-leu (20 nA, 2 min) against the background of naloxone (30 nA, 4 min), 4) Tyr-D-Ala-Gly-Phe-D-leu (20 nA, 2 min), 5) Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (20 nA, 2 min) against the background of naloxone (30 nA, 4 min), 6) Na^+ (30 nA, 4 min); d: 1) background, 2) Leu-enkephalin (20 nA, 30 sec), 3) Tyr-D-Ala-Gly-Phe-NH_2 (20 nA, 30 sec), 4) Leu-enkephalin (20 nA, 30 sec) against the background of naloxone (30 nA, 2 min), 5) Tyr-D-Ala-Gly-Phe-NH_2 (20 nA, 30 sec) against the background of naloxone (30 nA, 2 min), 6) Na^+ (30 nA, 2 min).

studied. Reticular neurons in the region of the respiratory center were found to respond to microapplication of the preparations in the same way as respiratory neurons: of 37 cells 21 (56.8% were inhibited and 16 (43.2%) did not respond. No qualitative differences were observed in the action of β-endorphin, enkephalins, their analogs, and morphine, and there were no activation responses to the substances. Naloxone blocked the inhibitory effect of the preparations and, on that basis, it can be concluded that opiate receptors participate in these effects.

The results thus demonstrate the high sensitivity of neurons (probably on account of opiate receptors located on them) of the bulbar respiratory center to β-endorphin, enkephalins, their analogs, and morphine (about 60% of both respiratory and reticular neurons were inhibited by microiontophoretic application of the substances). Consequently, it can be postulated that the inhibitory action of endogenous opioid peptides and of their synthetic analogs on respiration is largely due to the direct effect of morphine-like substances on neurons of the bulbar respiratory center.

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PHARMACOETHOLOGIC STUDY OF ANALGESIA INDUCED BY INTRASPECIFIC CONFRONTATION

Yu. D. Ignatov, Yu. V. Petrova, and V. P. Poshivalov

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KEY WORDS: pain, aggression; analgesia; pharmacoethology.

Psychological aspects of pain and analgesia, especially under conditions of intraspecific conflict, have so far received little study. One line of research in this field is to study dependence of pain perception and response to pain on experience of victory or defeat in intraspecific conflicts. For a long time the view has been held that in a situation of conflict, confrontation, fighting, or strong emotions, pain sensation is depressed. This phenomenon, in its general form, has been named "stress-induced analgesia," and its experimental principles have been established [15]. However, pharmacoethologic aspects of this phenomonon have not been analyzed in detail.

The aim of this investigation was to study the dynamics of pain thresholds in animals under intraspecific confrontation conditions (fighting), when one animal is the attacker, another is simply the defender, which loses the fight. The aims of the investigation also included a pharmacoethologic analysis of the phenomenon of analgesia induced by intraspecific confrontation.

EXPERIMENTAL METHOD

Experiments were carried out on 49 male CC57BL/6 mice, 35 of which were kept in groups (10 at a time), and 14 mice were kept in isolation in single cases. Only highly aggressive dominant mice were isolated, and only those animals whose latent period of first attack did not exceed 5 sec were used in the experiments. In special tests nonaggressive grouped mice were subjected to "measured" (controlled) attacks from dominant mice on their territory in single cages. Each cycle of attack was limited artificially to 20 bits, which induced submissive and defensive behavior in nonaggressive mice in the form of specific acts and postures [2, 4].

Pain thresholds in dominant and subordinate mice were measured in seconds by the tail flick test, with heating from focused light, by means of a special instrument with automatic recorder. Measurements were made before attacks and 2, 5, 15, 30, 45, and 60 min after 20 attacks. Success of the attacks was monitored by analysis of the behavior of the mice which were defeated — they lay on their side, then stood vertically in a defensive posture, and very rarely, lay supine, with the ventral surface toward the oponent [1-3]. Analgesia was considered to be complete if the mice did not flick their tail in the course of over 14 sec.

For pharmacologic analysis of the phenomenon, naloxone, an antogonist of opiate receptors, in doses of 0.1-1 mg/kg, and the GABA antagonist bicuculline, in doses of 1-2 mg/kg, were used. All drugs were injected intraperitoneally.

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