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NALOXONE-DEPENDENT WEAKENING OF EXCITATORY RESPONSES OF SNAIL NEURONS TO DOPAMINE BY MORPHINE

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UDC 612.816.014.46:[615.357.452+615.212.7:547.943

KEY WORDS: morphine; naloxone; responses to dopamine; snail neurons.

The possible neurotransmitter and neuromodulator role of endogenous opiates is being widely discussed in the literature in connection with the use of various model systems [6, 13]. A promising way of studying this problem has been found to be by using molluscan neurons, on which opiate receptors have recently been identified by biochemical and electrophysiological methods [10-12]. The present writers have used neurons of the snail *Helix pomatia* to study the modulator functions of opiates in relation to spontaneous activity of the neurons [5] and their responses to artificially applied serotonin [1].

The aim of the present investigation, a continuation of previous studies, was to examine the effect of morphine on responses of snail neurons to dopamine.

EXPERIMENTAL METHOD

Experiments were carried out on neurons of *H. pomatia* from May through November. Neurons of all ganglia on the dorsal surface with stable discharges of high amplitude were used. The neurons were identified in accordance with Sakharov's classification [4]. For the microelectrode studies the apparatus from Nihon Kohden (Japan) was used. Potentials were recorded on a Recticorder RIG-4024 automatic ink writer. The recording microelectrode was filled with 2M potassium citrate. The cell membrane was polarized through the recording microelectrode by means of a bridge circuit. Dopamine (from Koch-Light, England), in a dose of $1 \cdot 10^{-7}$-$1 \cdot 10^{5}$ M, morphine hydrochloride in a dose of $1 \cdot 10^{-5}$ M, and naloxone (from Endo), in a dose of $1 \cdot 10^{-5}$ M, were injected into the perfusion fluid by means of a microsyringe. Otherwise the technique was the same as that described previously [1].

EXPERIMENTAL RESULTS

Of the 250 neurons studied (in 50 preparations) 120 cells responded to application of $1 \cdot 10^{-7}$-$1 \cdot 10^{-5}$ M dopamine by a marked reversible dose-dependent change in membrane potential. However, only those neurons (n = 35) whose responses to dopamine remained unchanged during frequent repeated applications were used in the experiments with morphine.

Of the 35 neurons (22 preparations), 19 cells responded to dopamine by depolarization of the membrane and quickening of the discharge, 14 responded by hyperpolarization and inhibition of the discharge, and in two cells the response was

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(Presented by Academician of the Academy of Medical Sciences of the USSR, A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 8, pp. 51-53, August, 1981. Original article submitted February 13, 1981.

0007-4888/81/9208-1061 $07.50

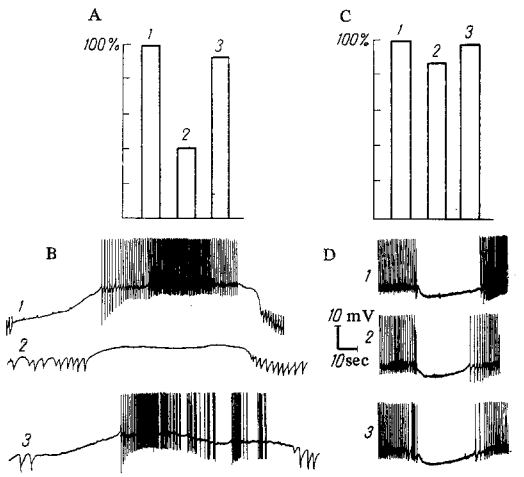

Fig. 1. Naloxone-dependent weakening of depolarizing, but not of hyperpolarizing responses of *Helix pomatia* neurons to dopamine by morphine. A: Mean values of depolarizing responses to $1 \cdot 10^{-5}$ M dopamine (n = 14): 1) before injection of morphine; 2) during administration of $1 \cdot 10^{-5}$ M morphine – 40% ($P < 0.05$); 3) after administration of $1 \cdot 10^{-5}$ M naloxone + $1 \cdot 10^{5}$ M morphine – 85% ($P < 0.05$); B: 1) depolarization of 15 mV to $1 \cdot 10^{-5}$ M dopamine; 2) decrease in depolarization to $1 \cdot 10^{-5}$ M dopamine to 6 mV under influence of $1 \cdot 10^{-5}$ M morphine; 3) depolarization by 12 mV to $1 \cdot 10^{-5}$ M dopamine, when besides $1 \cdot 10^{-5}$ M morphine perfusion fluid also contains $1 \cdot 10^{-5}$ M naloxone. Spontaneous IPSPs can be seen on trace. Results for neuron RPa4; C: mean values of hyperpolarizing responses to $1 \cdot 10^{-5}$ M dopamine (n = 19): 1) before injection of morphine – 100%; 2) after administration of $1 \cdot 10^{-5}$ M morphine – 85%; 3) after $1 \cdot 10^{-5}$ M naloxone + $1 \cdot 10^{-5}$ M morphine – 95%; D: 1) hyperpolarization by 5.3 mV to $1 \cdot 10^{-5}$ M dopamine; 2) hyperpolarization by 4.8 mV to $1 \cdot 10^{-5}$ M dopamine in presence of $1 \cdot 10^{-5}$ morphine; 3) hyperpolarization by 6 mV to $1 \cdot 10^{-5}$ M dopamine, when perfusion fluid contains $1 \cdot 10^{-5}$ M naloxone in addition to $1 \cdot 10^{-5}$ M morphine. Results obtained on neuron in F region. Calibration for C and D: 10 mV, 10 sec.

biphasic. The amplitude of the response to dopamine was 3-15 mV, the latent period 3-10 sec, and the duration 30-100 sec.

Preliminary injection of $1 \cdot 10^{-5}$ morphine into the perfusion fluid caused weakening of the excitatory responses to dopamine in 13 of 21 neurons. Weakening of the excitatory responses to dopamine varied from 20 to 70%; the mean response to dopamine was 40% of its initial level and was statistically significant ($P < 0.05$). Inhibitory dopamine responses were weakened by morphine in three of 16 cases, so that the mean response to dopamine in the presence of morphine was 88% of its initial value. The effect of morphine under these conditions was not statistically significant (Fig. 1C, D).

The opiate antagonist naloxone, if injected into the perfusion fluid immediately before morphine in a concentration of $1 \cdot 10^{-5}$ M, abolished the effect of morphine in all cases studied (n = 16). Responses to dopamine under these conditions amounted to 80-90% of the response in the control (Fig. 1). It is important to note that naloxone by itself modified neither the inhibitory nor the excitatory responses of the neurons to dopamine.

When the magnitude of the response to dopamine was assessed after injection of morphine and naloxone, the possibility of nonspecific changes in the response, which could be associated with a shift of membrane potential such as is observed in some cases after application of morphine and naloxone, was taken into account. In fact, in four of the 35 cases morphine induced hyperpolarization of the membrane by 2-6 mV, and in 10 cases it induced depolarization by 2-5 mV;

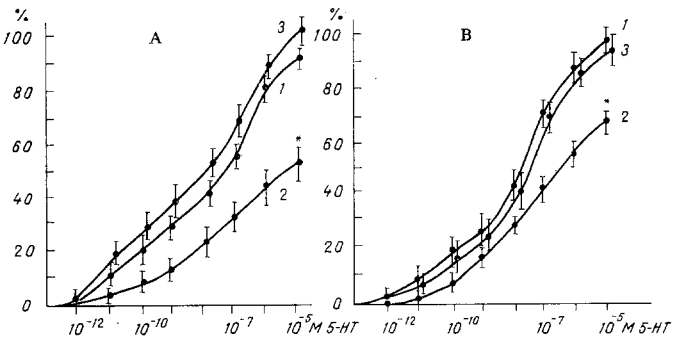

Fig. 2. Averaged dose-effect curves for depolarizing (n = 14) responses of *Helix pomatia* neurons to dopamine. Abscissa, dopamine concentration; ordinate, responses to dopamine (in % of maximal response); 1) before morphine; 2) after $1 \cdot 10^{-5}$ M morphine; 3) after combined application of $1 \cdot 10^{-5}$ M naloxone and $1 \cdot 10^{-5}$ M morphine.

naloxone, however, in four cases, depolarized the membrane by 2-5 mV. In these experiments dopamine was applied only when the membrane potential was restored to its initial value by artificial polarization or spontaneously in cases of transient changes. Meanwhile, in most experiments (in 22 of 35) no marked effects of morphine or naloxone on membrane potential and on discharge frequency could be found. This suggests that changes in magnitude of the response of the neuron to dopamine under the influence of morphine and naloxone are independent of any other factors influencing the electrical parameters of the neuron membrane.

Pharmacological analysis of interaction between morphine and dopamine was carried out by plotting dose–effect curves (n = 7) for excitatory responses to dopamine before injection of morphine (Fig. 2, curve 1), after its injection (Fig. 2, curve 2), and after combined application of naloxone and morphine (Fig. 2, curve 3). As will be clear from Fig. 2, morphine considerably weakened responses evoked by higher concentrations of dopamine. Naloxone restored responses to all concentrations of dopamine equally successfully.

Morphine rapidly and reversibly weakened excitatory responses of the neurons to dopamine. The naloxone-dependent character of the effects of morphine points to involvement of opiate receptors in the process. The character of the dose–effect on dopamine curves before and after administration of morphine indicates a noncompetitive type of interaction between morphine and dopamine and, consequently, participation of two types of receptors in this process, namely opiate and dopamine receptors. It can accordingly be postulated that morphine has no direct action on dopamine receptors, but weakens responses of neurons to dopamine indirectly.

The fact that not all excitatory dopamine responses were weakened by morphine can evidently be taken to mean that two types of receptors (dopamine and opiate) do not necessarily coexist on the membrane, or that interaction between them is not always exhibited.

The investigations showed that, unlike excitatory responses, inhibitory responses of neurons to dopamine are not weakened by morphine. This does not conflict with the modern view on the identity of the biological mechanisms of excitatory and inhibitory responses of snail neurons to dopamine.

In fact, excitatory and inhibitory responses of molluscan neurons to dopamine are known to be linked with different types of receptors, each of which has its own special agonists and antagonists [8]. There are also grounds for considering that excitatory responses to dopamine are mediated, to a greater degree than inhibitory responses, by adenylate cyclase and cyclic AMP [2, 3, 7].

These observations, and also the well known fact that morphine and endogenous opiates inhibit adenylate cyclase activity and lower the cyclic AMP level [9, 10] shed some light on the mechanism of selective weakening of excitatory responses of molluscan neurons to dopamine by morphine.

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EFFECT OF MET- AND LEU-ENKEPHALINS AND THEIR SYNTHETIC ANALOG ON ANALGESIA INDUCED BY STIMULATION AND ACUPUNCTURE

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UDC 617-089.851.41-092:616.831-008.939.43

KEY WORDS: enkephalins; analgesia; acupuncture; rat brain.

Naloxone, an antagonist of opiate receptors, is known to reduce or even completely suppress analgesia arising in response to stimulation of deep brain structures and activation of acupuncture points [2, 6, 11]. These and other indirect data [12, 14] suggested that endogenous polypeptides (enkephalins and endorphins) play an essential role in the development of acupuncture- and stimulation-induced analgesia. However, the concrete mechanism whereby this effect of the enkephalins in the formation of the pain-relieving action in these types of analgesia is realized has not yet been finally elucidated. Moreover, so far the direct effect of enkephalins on the intensity of stimulation- and acupuncture-induced analgesia has virtually not been studied.

Accordingly the aim of this investigation was to compare the action of enkephalins and of their synthetic analog on analgesia induced by stimulation and acupuncture.

EXPERIMENTAL METHOD

Male albino rats weighing 250-300 g were used. In a preliminary operation monopolar electrodes were implanted into the animals in the region of the central gray matter (CGM) and cannulas for microinjections were inserted into the lateral ventricles. Nociceptive stimulation of the base of the tail was carried out by means of removable bipolar electrodes (100 Hz, 1 msec, 1 sec, 30-150 V) and the animals were able to move freely. The response to nociceptive stimulation was assessed by a scale developed by the writers [2]. The CGM was stimulated for 30 sec by square pulses (100 Hz, 1 msec, 30-350 μA). Electroacupuncture was carried out by the method described in [2]. Polypeptides were injected, in a volume of 5 μl, over a wide range of doses (from 5 to 200 μg). The position of the electrodes was verified in serial brain sections. ED_{50} for the various substances was determined by Prozorovskii's method [5]. Nonparametric methods were used for the statistical analysis of the data.

EXPERIMENTAL RESULTS

The analgesic effect of Met-enkephalin appeared after microinjection of the peptide in a dose of 50 μg. The thresholds of onset of emotional-behavioral components of the pain response such as vocalization and running was raised in this case by 18 and 20% above the initial level, respectively. Initial changes in the structure of the response to nociceptive stimulation were observed 5-10 min after microinjection of the peptide, they reached a maximum after 15 min, and continued for a further 10-15 min. Under the influence of Met-enkephalin in a dose of 100 μg the threshold of development of the vocalization response was increased by 35-40% and of running by 38% (Fig. 1A). The value of ED_{50} for Met-enkephalin was 49.7 μg.

Department of Pharmacology, Academician I. P. Pavlov First Leningrad Medical Institute. Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 8, pp. 53-56, August, 1981. Original article submitted March 12, 1981.

 0007-4888/81/9208-1064$07.50