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ACTION OF MORPHINE ON NEURONAL INPUT SYSTEMS OF THE SPINAL CORD INVOLVED IN NOCICEPTIVE PRESSOR REFLEX FORMATION

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The complex defensive reaction evoked by nociceptive afferent stimuli of somatic nerves includes, besides various motor reflexes, autonomic reflexes, of which one is a rapid rise of arterial blood pressure (BP) [12]. Morphine, which acts in the region of entry of afferent stimuli into the brain, namely through opiate receptors of posterior horn neurons of the spinal cord (intrathecal injection), can suppress both the sensation of pain and the motor components of this reaction [13]. Meanwhile it has been reported that the circulatory component of the defensive reaction, consisting of powerful pressor reflexes developing in response to electrical stimulation of somatic nerves, is not reduced after intrathecal injection of morphine [1, 7]. This dissociation in the action of morphine on transmission of nociceptive stimuli in the spinal cord appears enigmatic, more especially if it is recalled that: 1) after intravenous injection morphine reduces pressor reflexes developing in response to electrical stimulation of (A + C)-afferents of somatic nerves [2]; 2) if injected either intravenously [2, 8] or intrathecally, namely into the region of entry of the stimulated nerves into the brain [10], morphine suppresses reflex responses of sympathetic neurons evoked by volleys of somatic C-afferents; 3) a reflex rise of BP under general anesthesia is induced by stimuli from somatic afferents of this same type [4, 5, 9, 11]. It can accordingly be postulated that opiate receptors are present in the membrane of some of the input neurons of the spinal cord involved in the formation of nociceptive pressor reflexes, and their activation should lead to weakening of these reflexes.

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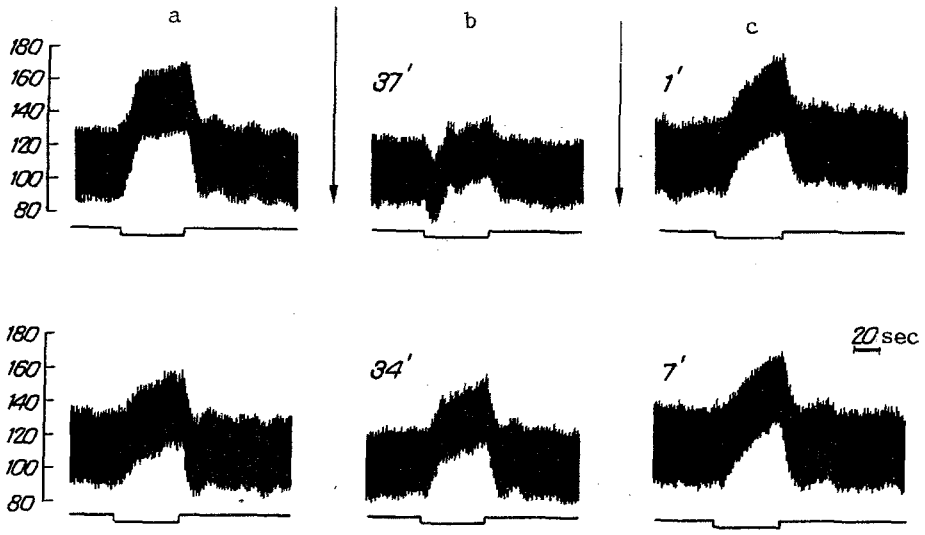

Fig. 1. Action of morphine, applied to segments L4-S2, on reflex reactions of BP evoked by volleys of (A + C)-afferents of TN. Ordinate, scale for BP (in mm Hg); marker of stimulation indicated below traces, parameters of stimulation: 15 V, 1 msec, 1 Hz.

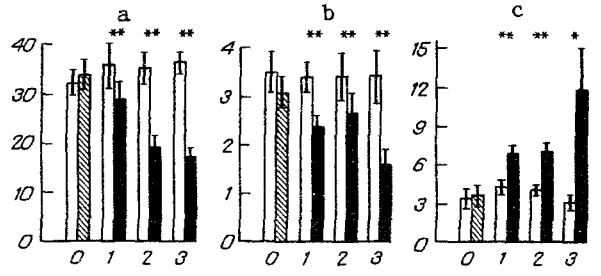

Fig. 2

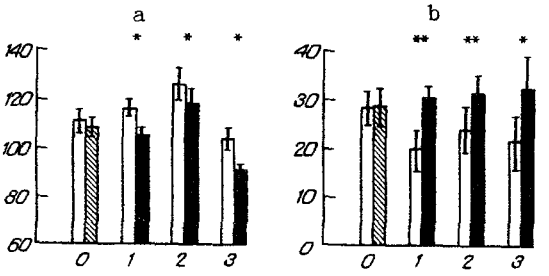

Fig. 3

Fig. 2. Changes in amplitude (a), initial steepness (b), and latent period (c) of pressor reflexes from TN on application of morphine to region of entry of its afferents into spinal cord. Ordinate, mean values of corresponding parameters (for a – in mm Hg, for b – in mm Hg/sec, for c – in sec); abscissa, procedure: 0) control application, results of 8 experiments; 1, 2, 3) application of morphine to brain in concentration of 0.02% respectively (results of 8 experiments), 0.1% (results of 7 experiments), and 0.5% (results of 7 experiments). Unshaded columns – mean values of corresponding parameters in control period of experiments, obliquely shaded columns – after control applications of Ringer's solution, black columns – after application of morphine. Significance of difference between mean values of parameters before and after application: *$p < 0.05$, **$p < 0.01$.

Fig. 3. Changes in BP in period between stimulations (a, in mm Hg) and amplitudes of pressor reflexes evoked by volleys of afferents from RN (b, in mm Hg) on application of morphine to segments L4-S2. Legend as to Fig. 2.

The aim of this investigation was to determine whether morphine, acting on neuronal input systems of the spinal cord, can weaken pressor reflexes due to the entry of volleys of nociceptive signals, initiated by electrical excitation of (A + C)-afferents of somatic nerves into the spinal cord. To limit the direct action of morphine to these particular systems only, a solution of morphine was applied to the dorsal surface of those segments of the spinal cord which receive afferent fibers

from one of the nerves chosen for stimulation, namely the radial (RN) or tibial (TN) nerve. The possible distant effects of morphine, i.e., those whose manifestations cannot be explained by a change in stimulus transmission only in those segments of the spinal cord to which morphine was directly applied, were judged from the BP level in the period between stimulations and changes in reflexes from a nerve entering segments of the spinal cord remote from the region of application.

EXPERIMENTAL METHOD

Cats weighing not less than 2 kg were anesthetized with chloralose and urethane (20-30 and 330-500 mg/kg respectively, intravenously). The methods and apparatus used to record BP, of maintaining the animal at a constant temperature, immobilizing it, and maintaining it on artificial ventilation of the lungs (with monitoring of the CO_2 concentration in the expired air, measured with an IL-200 capnograph (USA), and also methods of isolation and electrical stimulation of RN and TN, were all described previously [3]. The method of applying solutions of the test substances to several adjacent segments of the spinal cord by placing a strip of cotton wool, soaked with this solution, to their exposed surface, also was described in equal detail. Laminectomy was performed in the region of segments L4-S2 (in 27 animals) or C6-T1 (in 4 animals). The duration of stimulation (30-55 sec) and the interval between stimulations (3-4 min) were assigned by a WPI generator (USA), and remained unchanged during the experiment.

Having verified the stability of amplitude of the pressor reflexes evoked by volleys of (A + C)-afferents (parameters of stimulation: 15 V, 1 msec, 1-2 Hz) of each of the nerves, a strip of cotton wool with Ringer's solution placed on the brain was replaced by a similar strip, soaked in a warm solution of morphine hydrochloride or, in the case of control applications, again with Ringer's solution, and after 1-2 min recording of the reflex responses was resumed. In 11 experiments, after morphine had acted for 40-80 min, it was not removed from the surface of the spinal cord, but naloxone was injected (0.2 mg/kg, intravenously).

The mean BP in the period between stimulations, the amplitude of its reflex changes, and also the rate of its rise during pressor reflexes and the latent period of those reflexes were measured. From the results of each experiment the average (for 4-8 realizations) values of these parameters were determined in the control period (before application of morphine) and in the period of stable inhibition of pressor reflexes (see below). In the experiments in which the inhibitory action of morphine was not exhibited, and in the experiments with control applications, averaging was carried out for the period corresponding to stable inhibition of reflexes in the remaining experiments. The effectiveness of the action of morphine depending on its concentration was judged from the results of averaging of these mean values for each experiment relative to the corresponding experiments.

EXPERIMENTAL RESULTS

In most animals (22 of 27) morphine applied in a concentration of 0.02-0.5% to segments L4-S2 inhibited pressor reflexes evoked by volleys of (A + C)-afferents of TN (Fig. 1). The higher the morphine concentration, the faster the course of the initial period of its action, characterized by a gradual diminution of these reflexes: with a concentration of 0.02% it lasted 10-35 min, but with concentrations of 0.1 and 0.5% it lasted 5-20 min and 5-15 min respectively. At the end of this transition period and until application (or injection of naloxone) ceased inhibition of the reflexes was stable: their amplitude remained relatively constant, so that for quantitative analysis of the effects observed it was possible to use mean values of parameters of reflexes for each experiment recorded during their period of stable inhibition.

It will be clear from Fig. 2 that with an increase in the concentration of morphine, it inhibits pressor reflexes more strongly. The amplitude of the reflexes decreases significantly only with an increase in concentration of morphine to 0.1% (Fig. 2a). The same conclusion can be drawn from comparison of the relative changes in amplitude of the reflexes: for concentrations of 0.02, 0.1, and 0.5% the corresponding mean values were -19.1 ± 2.8, -45.4 ± 5.0, and -51.9 ± 5.8%. Nevertheless, with a further increase in the concentration of morphine (to 0.5%) its inhibitory action continued to increase, but this was manifested mainly as a decrease in the initial steepness of the reflexes (Fig. 2b) and a sharp increase in their latent period (see Fig. 2c).

The important fact is that in all these experiments the action of morphine was manifested as inhibition only of pressor reflexes. For instance, in 9 animals volleys of afferents of TN initially evoked biphasic reflexes: the pressor component was preceded by a small depressor component. In none of these animals was inhibition of the pressor component of the reflex by morphine (0.02% or 0.1%) accompanied by weakening of the depressor component (Fig. 1). Moreover, in 5

animals this component appeared after application of morphine to the spinal cord. The onset of depressor reflexes in response to stimulation of spinal afferents can be linked with the inhibitory action of their stimuli on tonic discharges of vasoconstrictor neurons [4, 11]. Preservation and even the appearance of initial pressor components of the reflexes with weakening of their pressor components can therefore be regarded as the result of selective inhibition by morphine of transmission of the excitatory action of stimuli from spinal afferents to vasoconstrictor neurons.

Besides the effect already examined, application of morphine to segments L4-S2 led to lowering of BP (Fig. 3a) and to a small but statistically significant increase in the pressor reflexes evoked by volleys from afferents of RN (Fig. 3b). It is evident that both effects are manifestations of the distant (in the same sense as was explained in the introduction) effect of morphine. They can be due both to the direct action of morphine, penetrating from its region of application into the blood or spreading along the cerebrospinal fluid, on neuronal systems remote from this region, and also to the indirect action, linked with the fact that a change in the state of the neuronal systems in the region of application may itself also alter the conditions of transmission of stimuli in other neuronal systems. Whatever the true mechanism of these distant effects may be, it is natural that pressor reflexes evoked by stimuli from an input remote from the region of application – from afferents of RN, were not reduced under these circumstances. The initial steepness likewise was not reduced, nor was their latent period increased.

This result is important in two respects. First, it follows from it that inhibition of pressor reflexes evoked by volleys of afferents from TN, by morphine applied to segments L4-S2, is due to its action mainly in the region of application. A similar localized action of morphine also was found when applied to segments C6-T1: in these experiments pressor reflexes evoked by stimulation of RN were significantly reduced, whereas reflexes from TN either were unchanged or were increased somewhat. Considering, therefore, that segments of the spinal cord to which morphine was applied (L4-S2 and C6-T1), with the exception of segments L4 and T1, virtually do not contain any preganglionic sympathetic neurons, it can be tentatively suggested that local inhibition of pressor reflexes by morphine which we discovered is due to the action of morphine on the input systems of the spinal cord.

Moreover, the opposite character of changes in the amplitude of the pressor reflexes, due to the local and distant action of morphine, suggests that the reason for the absence of any appreciable local inhibition of reflexes from TN in 5 (of 27) animals might be mutual balancing of these effects.

All changes in reflexes and in the background BP observed in response to application of morphine to the above-mentioned segments were quickly abolished by naloxone (Fig. 1). Consequently, all these changes were due to the action of morphine through opiate receptors.

Thus morphine, by its action through opiate receptors of neuronal input systems of the spinal cord, inhibits (at least in anesthetized animals) not only the motor components of the defensive reaction, but also autonomic and, in particular, circulatory components. Meanwhile changes in the trend of development of pressor reflexes in response to local inhibition of these reflexes by morphine (Fig. 2b and c), revealed by these experiments, are evidence that morphine, acting in the region of input of the afferent stimuli into the brain, weakens temporal summation of processes which, by triggering these signals, determine the intensity of excitation of the vasoconstrictor neurons by them. In turn, it follows from these observations that one possible cause of the negative results obtained by other workers [1, 7] is that they used only high-frequency stimulation of afferent fibers (10-20 Hz). In fact, as recent investigations have shown [6], not only the character of the processes triggered in neuron chains by repetitive stimuli, but also the spectrum of neurotransmitters released as a result of stimulation, depends essentially on the duration of the intervals between stimulations.

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ROLE OF CALCIUM IONS IN CAROTID CHEMORECEPTION

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Data relating to the direct involvement of the metabolic systems of the amphibian carotid labyrinth in the reception of certain chemical substances, which are among the most important arguments supporting the hypothesis of heterogeneity of chemoreception [5], have been confirmed also by experiments on mammals [6, 7]. In addition to this hypothesis there is also a "calcium theory" of carotid chemoreception. Its authors link the perception of chemical stimuli with changes in the "calcium homeostasis" of the glomus cells [14]. The important role of Ca^{2+} in carotid chemoreception has been demonstrated by experiments in which liposomes containing Ca^{2+} were injected into the glomus cells [9]. On the basis of experimental data showing differences in the time course of spike activity in fibers innervating the glomus cells under the influence of various modulators of calcium metabolism it has been suggested that the Ca^{2+} content in the glomus cells is dependent on the partial pressure of oxygen in the glomus tissue, and a determinant role of this cation in the mechanisms of excitation of chemoreceptors has been postulated [9, 14].

We have analyzed the role of Ca^{2+} in carotid chemoreception in mammals and have compared our results with the factual evidence in support of the hypothesis of heterogeneity of carotid chemoreception [5]. We set out from the modern view of the determinant role of Ca^{2+}, bound with membranes of organoids, in the regulation of cellular metabolism [1, 2].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 200-300 g, anesthetized with hexobarbital. Chemical stimulation of the carotid chemoreceptors was carried out with solutions of caffeine sodium benzoate (20 mM) and phosphate buffer, pH 6.2, which induced marked reflex responses of external respiration, and also changes in metabolic activity of the carotid body [6]. Effector influences on glomus cells were simulated by electrical stimulation of the peripheral end of the divided carotid sinus nerve by square pulses with a frequency of 20 Hz, for it was during similar stimulation at this same frequency that the most significant changes in cellular respiration of the glomus were recorded previously in rats [7].

Changes in the concentration of membrane-bound Ca^{2+} in cells of the carotid glomus were recorded by the aid of the fluorescent probe chlortetracycline (CTC), in a concentration of 25 μM. The time course of the intensity of fluorescence of the Ca^{2+} CTC – biomembrane (BM) complex, reflecting the kinetics of Ca^{2+} release from cell membranes [1, 11], was studied in the LYUMAM-IUF-1 luminescence microscope. The intensity of luminescence was recorded at a wavelength of 530 nm and excitation wavelength of 390 nm by means of an ÉM-1 electrometer and KSP-4 self-recording potentiometer.

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