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SUBARACHNOID ANALGESIA INDUCED BY SEROTONIN AND GAMMA-AMINOBUTYRIC ACID

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An essential component in the analgesic action of narcotic analgesics is narrowing of the afferent input for nociceptive impulses at the spinal level. This action is based on the ability of opiates to enhance depolarization of high-threshold spinal afferents and to inhibit interneuronal activity in laminae V-VI of the gray matter of the spinal cord [3].

Changes in the functional state of neurons concerned with transmission of nociceptive information at the spinal level can be induced not only by opiates and endogenous opioids [6, 10, 11], but also by certain neurotransmitters: serotonin [7, 9], noradrenalin [9], and gamma-aminobutyric acid (GABA) [1, 12].

In this paper the effects of morphine, GABA, and serotonin on function of primary afferents and the analgesic activity of these substances when injected by the subarachnoid route are compared.

EXPERIMENTAL METHOD

The effect of morphine, serotonin, and GABA was studied on neurons and synaptic transmission in preparations of isolated spinal cord from rats aged 9-14 days. Electrotonic dorsal root potentials arising during superfusion of the isolated spinal cord for 30 sec with solutions of morphine, serotonin, or GABA were recorded. To discover whether the drugs tested act directly on primary afferents or through spinal interneurons, in the experiments of series I synaptic transmission in the spinal cord was blocked by superfusion with a solution containing an excess (10 mM) of Mg^{++} ions and a deficiency (0.2-0.4 mM) of Ca^{++} ions.

The effect of morphine, GABA, and serotonin on synaptic transmission in the spinal cord was studied by recording changes in dorsal root potentials evoked by electrical stimulation of the dorsal root of the neighboring segment (the DR_2-DR_1 potential, see diagram in Fig. 1), or in the orthodromic polysynaptic ventral root potential (DR_2-VR_1). Both potentials were evoked by stimulation of dorsal root L3 by square pulses of current with a duration of 0.3 msec, frequency 0.1 Hz, and intensity 8-10 thresholds. Potentials were derived respectively from dorsal root L4 and ventral root L4. Details of the technique were described previously [1].

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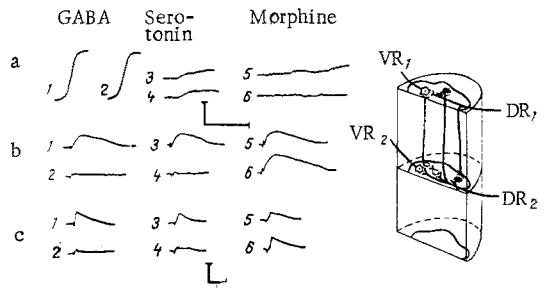

Fig. 1. Effect of GABA, serotonin, and morphine on polarization of dorsal roots (a) evoked by dorsal root potentials (b) and polysynaptic ventral root potentials (c). a) Electrotonic potentials recorded during superfusion of spinal cord by solutions containing GABA (10^{-3} M), serotonin (5×10^{-5} M), and morphine (10^{-5} M), in the absence (1, 3, 5) and in the presence (2, 4, 6) of an excess of Mg^{++} ions; b) dorsal root potentials evoked by electrical stimulation of dorsal roots of neighboring segment (DR_2-DR_1) before (1, 3, 5) and during superfusion of spinal cord with solutions of GABA (2), serotonin (4), and morphine (6) in concentrations indicated above; c) ventral root potentials evoked by electrical stimulation of dorsal root of neighboring segments (DR_2-VR_1) before (1, 3, 5) and during superfusion of spinal cord with solutions of GABA (2), serotonin (4), and morphine (6). Vertical calibration – 1 mV (a: 1-4; b, c) and 600 μV (a: 5-6). Horizontal calibration 1 min (a) and 100 msec (b, c). Upward deviation of curves denotes negativity of proximal electrode. Diagram on right explains relations of neurons in neighboring spinal cord segments: DR) dorsal root, VR) ventral root; cells shaded black are neurons of substantia gelatinosa, which inhibits the afferent input.

In the experiments of series II the analgesic activity of morphine, GABA, and serotonin was compared after their subarachnoid injection into adult rats of both sexes weighing 150-200 g. The method of gradually increasing nociceptive stimulation was used [2]. The painful stimulus was electrical stimulation of the base of the rats' tail by square pulses of current with a duration of 10 msec and a frequency of 50 Hz. Thresholds of sensitivity of four components of the behavioral reaction to a nociceptive stimulus were measured: restlessness (twitching, changing from one limb to another), turning, squeaking, running. The rat was then anesthetized with ether for 2 min, during which time subarachnoid puncture was carried out at coordinates described in [5] and the test drug was injected. Different doses of drugs were injected in a constant volume of 0.1 ml. After 15-20 min the rate recovered completely from the anesthetic, and 1, 2, and 3 h after the injection the thresholds of nociceptive sensation were again measured in relation to the same components of the behavioral response. The action of morphine hydrochloride was studied in doses of 0.02, 0.1, 0.3, 0.6, 1, and 2 mg, of serotonin creatinine-sulfate in doses of 0.1 and 0.3 mg, of GABA in doses of 0.1, 0.3, and 0.6 mg, and of sodium hydroxybutyrate in a dose of 0.6 mg. The action of each dose was studied on 9-12 rats (the action of morphine in doses of 1 and 2 mg was studied on four animals). The experimental results were subjected to statistical analysis by the usual methods.

EXPERIMENTAL RESULTS

The action of morphine and serotonin in concentrations of 10^{-5} M and also of GABA (10^{-3} M) caused primary afferent depolarization (Fig. 1a). Depolarization induced by morphine and serotonin developed slowly, depolarization induced by GABA was stronger and developed quickly. The depolarizing effect of GABA and serotonin was completely preserved after synaptic transmission in the spinal cord had been blocked by an excess of

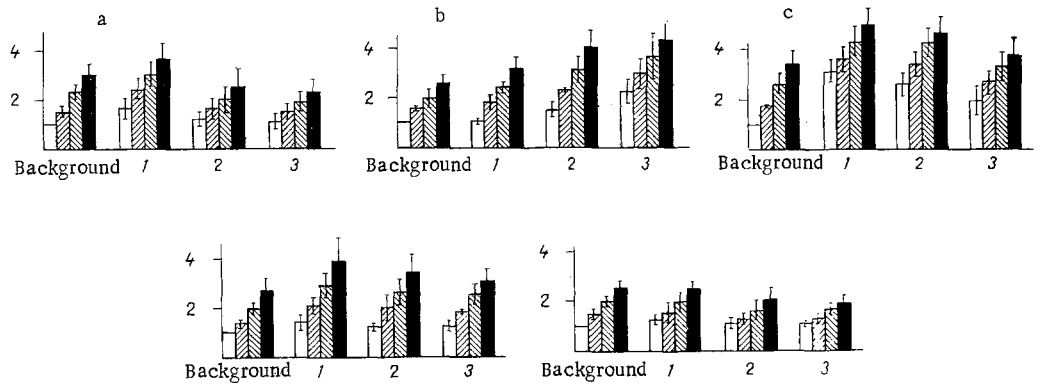

Fig. 2. Effect of subarachnoid injection of sodium hydroxybutyrate in a dose of 600 μg (a), GABA in a dose of 600 μg (b), serotonin in a dose of 300 μg (c), and morphine in doses of 100 (d) and 20 μg (e) on thresholds of four components of behavioral response arising in rats to nociceptive stimulus. Mean levels of thresholds and their confidence intervals (P = 0.05); threshold of first component (restlessness) taken as 1. Abscissa, time after injection of drug (in h). First columns – restlessness, 2nd – turning, 3rd – squeaking, 4th – running.

Mg^{++} ions, but the depolarizing effect of morphine under the same conditions disappeared (Fig. 1a). Consequently, unlike GABA and serotonin, morphine induces primary afferent depolarization indirectly, through spinal interneurons. Since the level of primary afferent polarization is determined by activity of neurons of the substantia gelatinosa [12], this suggests that morphine depolarizes primary afferents (Fig. 1a) by exciting neurons of the substantia gelatinosa [4].

Differences in the mechanisms of the depolarizing effect of morphine on primary afferents, on the one hand, and of GABA and serotonin on the other hand, were clearly revealed by a study of the action of these drugs on evoked dorsal root potentials (DR_2-DR_1), which, in the existing view, are due to excitation of cells of substantia gelatinosa [12]. Under the influence of morphine the dorsal root potential is increased whereas GABA and serotonin suppress it completely (Fig. 1d). Morphine thus merely strengthens depolarization inhibition of primary afferents due to activation (during electrical stimulation of DR_2) of substantia gelatinosa neurons, whereas GABA and serotonin evoke depolarization of central terminals of primary afferents.

By disturbing the functions of primary afferent terminals GABA and serotonin significantly inhibit orthorhombic polysynaptic ventral root potentials (DR_2-VR_1), evoked by stimulation of dorsal roots of the neighboring segment (Fig. 1c). Morphine, on the other hand, enhances polysynaptic ventral root potentials (Fig. 1c). Inhibition of polysynaptic reflexes by morphine described by other workers in cats in situ is probably due to simultaneously evoked circulatory disturbances [8] or to species differences in the action of morphine. Potentiation of polysynaptic ventral root potentials of the isolated rat spinal cord (Fig. 1c) evidently lies at the basis of the ability of morphine to induce tremor and seizures when given by subarachnoid injection to rats in large doses (1-2 mg).

The ability of morphine, serotonin, and GABA to induce primary afferent depolarization (Fig. 1a) provided the basis for a comparative study of their analgesic properties when given by subarachnoid injection, for the two latter substances do not pass through the blood-brain barrier.

Subarachnoid injection of 0.9% NaCl, like that of morphine in a dose of 20 μg, into rats (Fig. 2) had no analgesic action. Moreover, the application of nociceptive stimuli to the animals and the repeated experimental situation were accompanied by a gradual (after 2 and 3 h) lowering of the levels of pain sensation. Conversely, subarachnoid injection of morphine in a dose of 100 μg (0.5 mg/kg) significantly raised the threshold of three (restlessness, turning, squeaking) of the four components of the behavioral response evoked by nociceptive stimulation (Fig. 2). The maximal analgesic effect took place 1 h, but the effect still continued 3 h after injection. Morphine, injected subarachnoidally in a dose of 300 μg, induced rather stronger and more prolonged analgesia, but in a dose of 600 μg the analgesic effect of morphine was less marked and less constant than in doses of 300 and 100 μg. Morphine, in doses of 1 and 2 mg, had a toxic action, manifested as scratching movements, flexion of the hind limb, catalepsy, convulsions, and death of some animals.

Serotonin caused marked analgesia when given by subarchnoid injection in a dose of 300 μg, but in a dose of 100 μg analgesia was observed only 1 h after injection. The analgesic effect of GABA was observed after sub-

arachnoid injections in a dose of 300 μg, but in a dose of 600 μg GABA had a powerful and prolonged analgesic action (Fig. 2). Sodium hydroxybutyrate, a structural analog of GABA in the same dose caused weaker analgesia, comparable with the effect of morphine in a dose of 100 μg (Fig. 2).

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EFFECT OF RAUSEDIL AND PYRROXAN ON AUTOMATIC CONTROL OF THE CEREBRAL BLOOD FLOW

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In essential hypertension the limits of automatic control reactions of the cerebral vessels are known to be shifted toward higher arterial pressure (AP) levels [12, 13]. Under these conditions a rapid fall of AP to the normal level may lead to disturbances of the cerebral circulation.

It was accordingly decided to study the effect of reserpine and pyrroxan on automatic control of the cerebral blood flow. No corresponding information about these hypotensive agents could be found in the literature.

EXPERIMENTAL METHOD

Acute experiments were carried out on 20 dogs of both sexes weighing 8-12 kg. The use of general anesthetics was avoided because they may change the character of automatic control reactions (ACR) of the cerebral vessels [1, 8], and for that reason morphine (1 mg/kg subcutaneously) was used for general analgesia and a 0.25% solution of procaine was used for local anesthesia, and listhenon for immobilization; artificial ventilation of the lungs was carried out with monitoring of pH and blood gas composition on the AZIV-2 instrument. The oxygen saturation of the arterial blood was maintained at 96-98% and pH between 7.36 and 7.42.

The cerebral blood flow (CBF) was recorded continuously with an RKÉ-01 electromagnetic flowmeter, the transducer of which was located on the vertebral artery. The general AP was recorded in the common carotid artery by a mercury manometer, the venous pressure (VP) by a water manometer in the cranial segment of the jugular vein. The perfusion pressure (PP) in the main arteries of the brain was determined as the difference between AP and VP.

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