EFFECT OF SUBSTANCE P ON NEURONAL ACTIVITY IN THE ANTINOCICEPTIVE SYSTEM

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Substance P (SP) has an analgesic effect against both physiological and pathological pain [3, 7, 11, 12]. Various hypotheses have been put forward to explain the mechanism of SP-induced analgesia. It has been shown [11, 12] that the analgesic effect of SP and its fragments depends on the initial level of pain sensitivity, and analgesia arises when SP is injected into animals with increased pain sensitivity. Our previous investigation showed that the strongest and most prolonged analgesia arises when SP is given by microinjection into the dorsal nucleus raphe (DNR) – a structure of the antinociceptive system [3]. The results obtained in this study led to enunciation of the hypothesis that SP-induced analgesia may be based on a mechanism of activation of the antinociceptive system in response to the action of SP [3]. To test this hypothesis, activity of DNR neurons was investigated after injection of SP into the nucleus.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. SP was injected into DNR in a volume of 1 μl and in a dose of 1 μg through a glass microcannula by means of a hydraulic device in the course of 60 sec at the coordinates AP −6.0, α 0, H 5.8 mm [15]. SP was dissolved in 0.9% NaCl immediately before the experiment. Animals receiving SP served as the experimental group; the control group consisted of animals receiving 1 μl of 0.9% NaCl. The 3rd group consisted of intact animals. To assess the analgesic effect of SP the latent period (LP) of the pain response of the animal was recorded to nociceptive temperature stimulation (the hot plate test; 55°C). Unit activity was recorded extracellularly by means of glass microelectrodes filled with 2.5 M NaCl solution. Spontaneous spike activity (SA) of the neurons was investigated under chloral hydrate anesthesia (400 mg/kg) in tracks of DNR located in the frontal planes AP −5.8 and −6.0 mm, extending in depth from 5.5 to 6.5 mm, and operated from one another in the mediolateral direction by a distance of 0.2 mm. In each track SA was assessed at 11 points, located a distance of 0.1 mm apart in the vertical direction. If SA was present the point was assessed as active and potentials were recorded. To characterize activity of DNR the number of active points, their distribution in the nucleus, the mean discharge frequency, and the character of sequence of the discharges were determined.

EXPERIMENTAL RESULTS

LP of pain responses to nociceptive temperature stimulation in animals of the experimental group were significantly increased (Table 1). The analgesic effect of SP against this type of nociceptive stimulation could be detected immediately after the animal recovered from the anesthetic (1.5-2 h after the stereotaxic operation of insertion of the microcannula containing SP); in the 24 h following the operation the analgesic effect remained. In the control group, after injection of 0.9% NaCl LP of the nociceptive responses remained virtually unchanged (Table 1).

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TABLE 1. LP (in sec) of Nociceptive Responses to Temperature Stimulation in Animals of Different Groups

Group of animals	LP before microinjection	LP after microinjection into DNR
Intact (n = 20)	11,6±1,6	—
Control (n = 8)	11,8±1,9	12,8±1,7
Experimental (n = 12)	11,3±1,2	25,8±4,1

TABLE 2. Mean Discharge Frequency of DNR Neurons in Animals of Different Groups

Group of animals	Number of neurons	Frequency, spikes/sec
Intact (n = 17)	43	5,7±0,7
Control (n = 8)	67	4,7±0,7
Experimental (n = 12)	50	11,6±1,7

TABLE 3. Distribution of DNR Neurons by Types of Activity in Animals of Different Groups

Group of animals	Number of neurons	Percentage of neurons with single type of activity	Percentage of neurons with modulated type of activity	Percentage of neurons with burst type of activity
Intact	43	33	30	37
Control	67	39	4	57
Experimental	50	4	18	78

Investigation of SA of DNR neurons in these animals showed that during the 24 h after injection of SP there was a statistically significant ($p < 0.01$) increase in the mean running discharge frequency of the neurons compared with the discharge frequency in intact animals and in animals of the control group (Table 2). The difference between the mean discharge fequencies of DNR neurons in intact animals and after injection of 0.9% NaCl was not significant.

Analysis of the distribution of neurons by their spontaneous discharge frequency showed (Fig. 1) that in animals after injection of 0.9% NaCl into the nucleus the percentage of neurons with the lowest frequency (0-4 spikes/sec) was increased, while the overall range of frequencies did not change significantly. After injection of SP new classes of neurons appeared: besides low-frequency neurons, others with high frequency (20-48 spikes/sec) were recorded; neurons with this discharge frequency were never recorded in intact animals or in animals receiving an injection of 0.9% NaCl.

The study of the character of spike activity of the DNR neurons showed that all types of activity — single, modulated, and burst — were almost equally characteristic of the intact animals. In animals of the control group, single and burst types of activity were represented the most. After injection of SP the number of neurons with the burst type of activity rose sharply whereas the number of neurons with the single type of activity was greatly reduced (Table 3).

To study the distribution of spontaneously active neurons throughout the volume of the nucleus activity was recorded at fixed points (the step in the dorsoventral direction was 100 μ, in the mediolateral direction 200 μ). Altogether 287 points were studied under normal conditions, 231 points after injection of 0.9% NaCl, and 192 points after injection of SP.

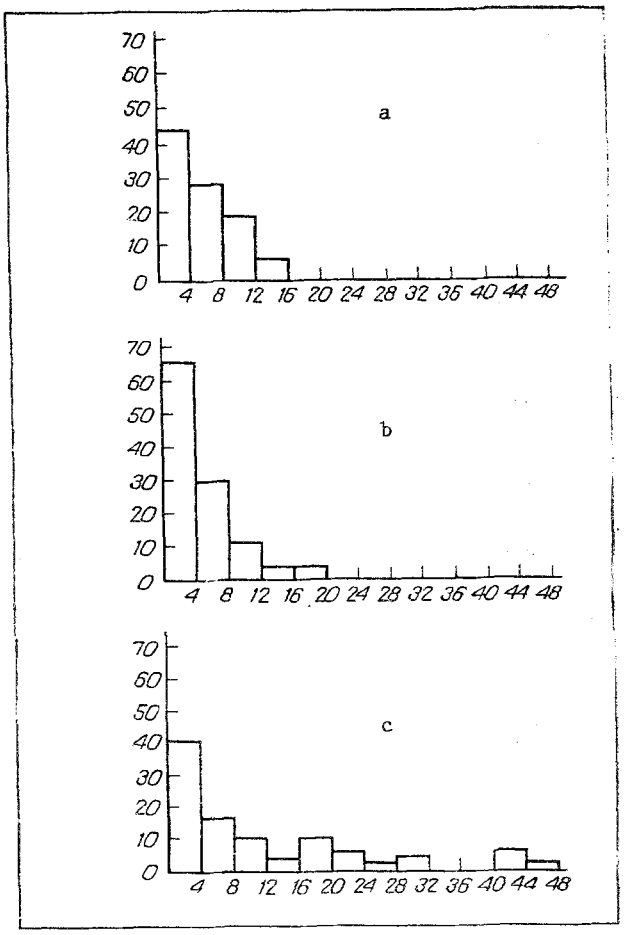

Fig. 1. Histogram of distribution of DNR neurons by discharge frequency in intact animals (a), in animals of control group after injection of 0.9% NaCl (b), and in animals of experimental group after injection of SP (c). Abscissa, discharge frequency (in spikes/sec); ordinate, number of neurons (in % of total number of neurons).

Analysis of the mosaic of active and inactive points showed that DNR was tonically active in animals of all three groups: in intact animals 28%, in the control animals 31%, and in the experimental animals 35% of the total number of recorded points were observed. The distribution of active points by depth of their location in the tracks is shown in the histogram (Fig. 2). After injection of SP a significant increase was observed in the number of active points in the lower third of the nucleus. The greatest differences were discovered at a depth of 6.3-6.5 mm.

The increase observed in the number of spontaneously active neurons in the nucleus after injection of SP, the increase in their mean spontaneous discharge frequency, and the appearance of a class of high-frequency neurons and of neurons with a burst type of activity reflect hyperactivation of DNR, and this may lie at the basis of the mechanism of SP-induced analgesia.

Analgesia induced by other methods may also be based on a mechanism of activation of neurons of the antinociceptive system. Oleson et al. [13, 14] give data showing a great increase in multineuronal activity of the periaqueductal gray matter in analgesia induced by injection of morphine or by electrical stimulation of brain structures. Results indicating an increase in electrical activity of DNR in the period of analgesia induced by creation of a generator of enhanced excitation in the nucleus with the aid of tetanus neurotoxin, which disturbs inhibitory mechanisms [1, 4, 8], have been obtained in Kryzhanovskii's laboratory [2, 8]. These findings are in agreement with those obtained by Urca et al., Fields et al. [5, 6], and Nicoll et al. [10], who showed that during analgesia induced by systemic or intracerebral injection of morphine and of opioid peptides, increased activity of neurons of the

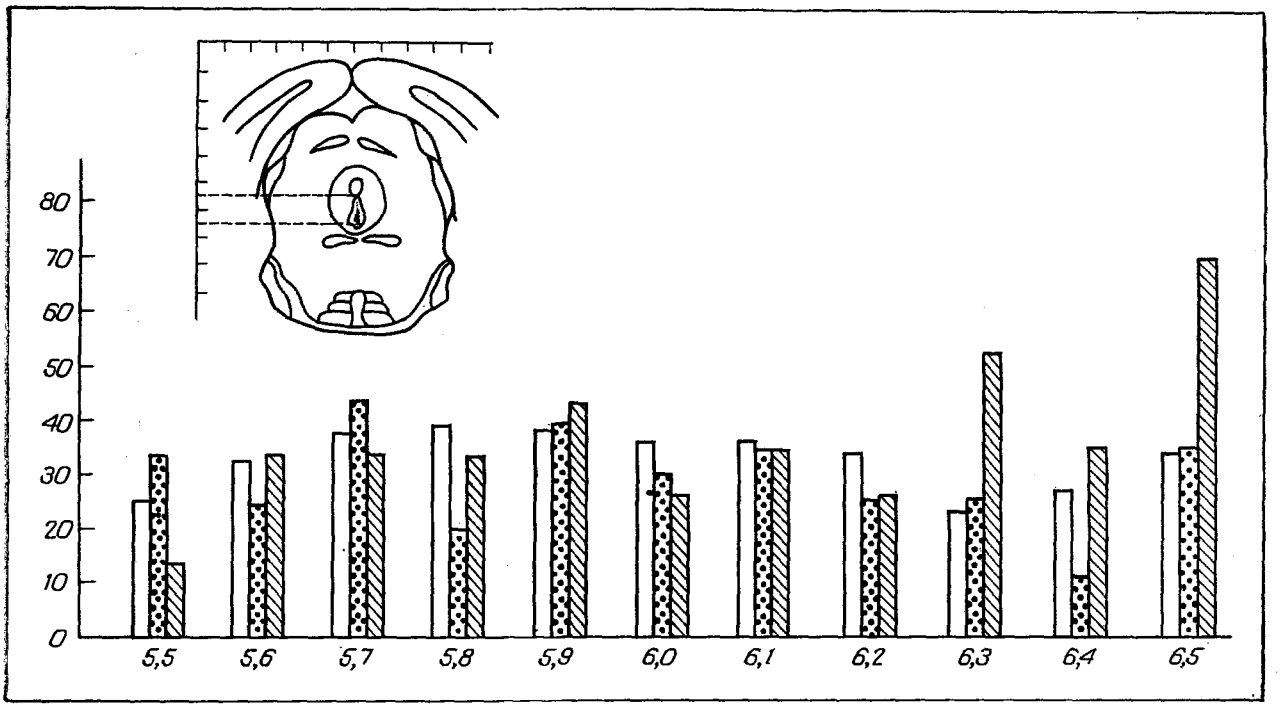

Fig. 2. Diagram of frontal section through brain region seen through DNR and histogram of distribution of number of active points in dorsoventral track. Abscissa, depth (in mm); ordinate, number of active points (in % of total number of points tested at the given depth). Obliquely shaded columns – % of active points in corresponding zone of nucleus after injection of SP; dotted columns – after injection of 0.9% NaCl; unshaded – in intact animals.

antinociceptive system is observed. Activation of the antinociceptive system is thus a common mechanism of central analgesia induced by various agents. The prolonged change (for 24 h) in activity of the antinociceptive system after a single injection of peptide is a fact which deserves special attention. It cannot be explained by the continuing action of SP, for the life of this peptide is only a few minutes; a single injection of SP evidently triggers a cascade of neurochemical changes which continue for a long time, and which probably activates different structures of the antinociceptive system.

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