EFFECT OF THE C-TERMINAL FRAGMENT OF SUBSTANCE (SP_{5-11}) ON NEURONAL ACTIVITY OF THE DORSAL NUCLEUS RAPHE

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Substance P has been shown to have both hyperalgesic and hypoalgesic effects [2, 6, 11, 12, 14]. The antinociceptive effect of substance P is well defined in the case of intracerebral injection [2, 6, 10], and in that case it is due to activation of the antinociceptive system [3]. The writers showed previously [2] that not only the whole molecule of substance P has an analgesic effect, but also its SP_{5-11} fragment, if injected into the dorsal nucleus raphe (DNR), which is one of the structures of the antinociceptive system [1, 5, 9]. It might be supposed that the SP_{5-11} fragment causes activation of DNR neurons, and that its analgesic effect is connected with such activation.

The aim of this investigation was to study changes in activity of DNR neurons and pain reactions in rats after injection of the SP_{5-11} fragment into DNR.

EXPERIMENTAL METHOD

Experiments were carried out on 36 male Wistar rats weighing 250-300 g. In experiments to study behavioral reactions the latent period (LP) of the pain response of rats to nociceptive thermal (55°) stimulation was determined in the hotplate test. In electrophysiological experiments, on animals anesthetized with chloral hydrate (400 mg/kg), neuronal spike activity (n = 189) was investigated by the standard method of extracellular derivation of potentials by glass microelectrodes filled with 2.5 M NaCl solution. The behavioral responses and electrophysiological parameters were studied in the same animal before injection of the substance and 24 h after injection either of 1 μl of 0.9% NaCl solution (control) or SP_{5-11} (dose 1 μg in 1 μl) The microinjection of SP_{5-11} or of 0.9% NaCl solution into the nucleus was given in the course of 60 sec, using stereotaxic coordinates [13]: AP = 6.0; L = 0; H = 5.8 mm. Unit activity was studied in tracks in DNR, located in frontal planes AP = 5.8 and AP = 6.0 mm, between depths of 5.5 and 6.5 mm, and with a distance apart of 0.2 mm in the mediolateral direction. In each track activity was measured at 11 points, 0.1 mm apart in the vertical direction. The method of step by step recording of neuronal activity enabled the density of distribution of the active neurons to be studied. The following parameters of neuronal spike activity were investigated: the average momentary discharge frequency of the neurons, the firing pattern, and the density of distribution of active neurons. The locations of the electrodes were verified histologically. The significance of differences was determined by Student's test and by the difference between fractions test.

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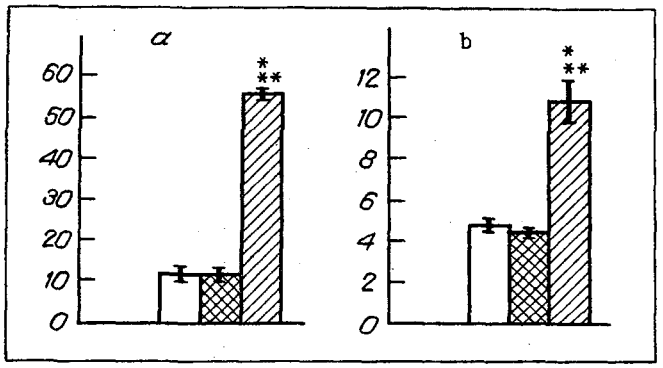

Fig. 1. Changes in latent period of pain response (a) and discharge frequency of DNR neurons (B) after injection of SP_{5-11} into nucleus. Ordinate: a) latent period (in sec); b) discharge frequency of neurons (spikes/sec). Unshaded columns – values for intact animals; cross hatched – after injection of 0.9% NaCl solution into nucleus; obliquely shaded – after injection of fragment SP_{5-11}. *) Differences significant compared with values for intact rats ($p < 0.001$); **) compared with values for control animals (injection of 0.9% NaCl solution; $p < 0.001$).

EXPERIMENTAL RESULTS

After microinjection of SP_{5-11} into the nucleus LP of pain responses to nociceptive thermal stimulation were significantly longer than LP of the response of both intact animals and animals receiving an injection of 0.9% NaCl solution (Fig. 1). This analgesic effect could be found 1.5-2 h after microinjection, i.e., after the animal had recovered from the anesthetic; in the next 24 h the analgesic effect persisted, and in some animals the increased LP lasted 5 days.

Analysis of the mean momentary discharge frequency of DNR neurons before injection of the substances, after injection of SP_{5-11}, and after injection of 0.9% NaCl solution showed that 24 h after microinjection of SP_{5-11} into DNR, i.e., at a time when LP was considerably (by up to 500%) lengthened, the mean discharge frequency of the neurons rose significantly (Fig. 1). In intact rats the mean discharge frequency was 5 ± 0.4 spikes/sec, compared with 4.8 ± 0.7 spikes/sec in the control rats after injection of 0.9% NaCl solution, and 10.9 ± 1.1 spikes/sec in rats after microinjection of SP_{5-11}. Analysis of histograms of distribution of neurons by discharge frequency showed that after injection of the peptide, classes of high-frequency neurons (from 18 to 60 spikes/sec) were recorded, which were absent in the intact and control animals (Fig. 2).

The neuronal firing pattern in animals of all three groups (intact, control, and receiving SP_{5-11}) was of three types: 1) regular spike activity characterized by regular sequences of single spikes with virtually identical interspike intervals; 2) irregular spike activity with fluctuation of interspike intervals; 3) bursting type of activity in the form of group discharges separated by intervals. In intact animals all three types of neuronal firing pattern were equally represented: the number of neurons with regular, irregular, and bursting types of activity amounted to 37, 27, and 36% respectively (Fig. 2). After microinjection of 0.9% NaCl solution some changes were observed in the ratio between the numbers of neurons with different types of activity (Fig. 2): there were fewer neurons with an irregular type of pattern, an increase in the percentage of neurons with a regular type of activity, and the number of neurons with the bursting type remained the same (Fig. 2). Frequency characteristics of neurons of the animals after injection of 0.9% NaCl solution did not differ from values of the average frequency in intact animals. Injection of SP_{5-11} into DNR caused a sharp change in the relative numbers of neurons with different patterns: the number of neurons with regular (13%) and irregular (5%) types of activity remained the same, whereas the number of neurons with the burst type of activity increased to 82% (Fig. 2).

In some cases an increase was observed in the firing rate within the burst.

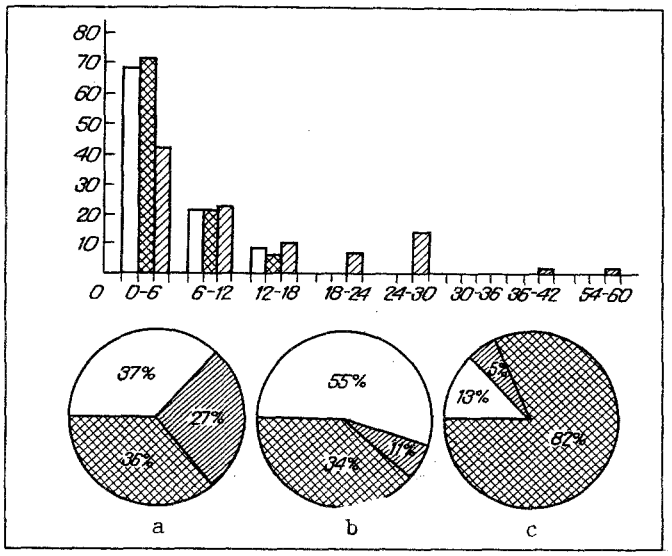

Fig. 2. Changes in number of neurons with different firing patterns after injection of SP_{5-11} into nucleus. On histogram – spectrum of distribution of neurons depending on discharge frequency. Abscissa, discharge frequency, in pulses/sec; ordinate, percentage of neurons with corresponding discharge frequency. Unshaded columns – values in intact animals; cross-hatched – after injection of 0.9% NaCl into nucleus; obliquely shaded columns – after injection of fragment SP_{5-11} On pie charts: relative percentages of number of neurons with regular (unshaded sector); irregular (cross-hatched sector), and burst (obliquely shaded sector) type of activity: a) intact animals; b) after injection of 0.9% NaCl solution; c) after injection of SP_{5-11}.

Step by step recording of activity in the nucleus enabled the density of distribution of the active neurons to be tested. The analysis showed that the percentage of active neurons recorded in intact rats was equal to the percentage of active neurons in the control animals, into whose nucleus 0.9% NaCl solution was injected. After injection of SP_{5-11} into the nucleus the density of the active neurons was doubled (Table 1).

The investigation thus showed that after microinjection of the C-terminal fragment SP_{5-11} into DNR, LP of the pain response to nociceptive thermal stimulation increased, and during the same period changes took place in electrical activity of the neurons of DNR: the number of active neurons increased, their mean discharge frequency increased, high-frequency neurons were recorded, and the number of neurons with a burst type of activity increased. These observations indicate hyperactivation of DNR.

If the results are compared with those of a previous study of the effect of SP_{1-11}, when injected into DNR [3], it will be clear that the effects of fragment SP_{5-11} and of the whole peptide have common features: both SP_{1-11} and SP_{5-11} suppress pain responses and increase neuronal activity in the nucleus. Meanwhile, the intensity of the effects of SP_{5-11} and SP_{1-11} is different: the effect of SP_{5-11} on behavioral reactions and on some parameters of spike activity is stronger. SP_{5-11} inhibits behavioral pain responses more effectively than SP_{1-11}, and the duration of the effect of SP_{5-11} is much longer than that of SP_{1-11}. The density of active neurons in the nucleus after injection of the fragment is greater than that of active neurons after injection of SP_{1-11}; the percentage of high-frequency burst neurons also is higher after injection of SP_{5-11}. The stronger effect of SP_{5-11} on the antinociceptive system compared with the whole SP_{1-11} molecule may be attributed to several factors. SP_{1-11} is known to be predominantly an agonist of NK_1-receptors, whereas SP_{5-11} binds to a greater degree with NK_2-receptors [13]. Probably during the action of SP_{1-11} and SP_{5-11} different types of neuroendocrine receptors are involved differently in activation of the nociceptive and antinociceptive systems. The analgesic effect of the whole peptide molecule (SP_{1-11}) may be weakened due to the action of the N-terminal fragment formed from SP_{1-11}, for as was shown in [7], it gives an antagonistic modulating effect toward the whole SP_{1-11} molecule.

TABLE 1. Changes in Number of Active Neurons in DNR after Injection of SP_{5-11}

Procedure	Number of zones tested	Number of active neurons, %
Intact animals	287	29
0.9% NaCl solution	231	28
SP_{5-11}	253	57**

Legend. *) Differences significant compared with intact animals ($p < 0.001$), **) compared with values in control animals ($p < 0.001$).

The results are evidence that analgesia induced by the C-terminal fragment of substance P (SP_{5-11}), when injected into the dorsal nucleus raphe, is due to activation of the neurons of this structure of the antinociceptive system. A similar mechanism lies at the basis of the analgesic effects of some other procedures, as has been shown by our previous studies [1, 9], and also those of other workers [4, 5, 8]. It is an interesting fact that the analgesic effect connected with activation of DNR neurons can be achieved by the action of nonspecific convulsants on this nucleus also [1, 9]. Substance P and, in particular, its SP_{5-11} fragment can be regarded as specific activators of the antinociceptive system.

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