CHANGES IN NEURONAL ELECTRICAL ACTIVITY OF THE DORSAL NUCLEUS RAPHE DURING DEVELOPMENT OF A GENERATOR OF PATHOLOGICALLY ENHANCED EXCITATION IN THE NOCICEPTIVE SYSTEM

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Analgesia induced by various exogenous influences on the body is based on activation of the antinociceptive system (ANS) [1, 3, 5-7, 9, 12, 13]. Data on the development of analgesia after exposure to pain [4, 8, 10, 11, 14] suggest that the main mechanism of onset of this type of analgesia is also a process of ANS activation: nociceptive impulsation activates structures of the ANS. Research into the study of the effect of activation of the nociceptive system (NS) on activity of the ANS has been conducted mainly on models of physiological pain and during exposure to short-term nociceptive stimulation. The aim of the present investigation was to study neuronal activity of the dorsal nucleus raphe (DNR), a structure forming part of ANS, in the period of formation of a generator of pathologically enhanced excitation (GPEE) [1, 12] in NS, activity of which lies at the basis of the pain syndrome of spinal origin [1, 2, 12].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-280 g. The animals were divided into three groups: 1) intact rats (20), 2) control rats undergoing a mock operation (18), and 3) experimental animals undergoing an operation to form a GPEE in the nociceptive structures of the dorsal horns of the spinal cord, and thus creating a model of a pain syndrome of spinal origin (23 rats). The GPEE was created in the dorsal horns of the lumbar division of the spinal cord of the rats with the aid of penicillin, contained in an agar wafer (25 U penicillin in 1 mm^3), which was applied to the right half of the dorsal surface of the lumbar division of the spinal cord. Characteristic spontaneous and evoked attacks of pain appeared and increased in severity in the unrestrained rats 10-30 min after application of penicillin, in the form of motor excitation, vocalization, flexion of the right hind limb, and licking and biting of an area of skin on the right hind limb (Stage I); after 30-90 min maximal development of the pain syndrome was observed (Stage II), after which there was a gradual decrease in the intensity and frequency of the painful episodes (Stage III) in the course of 90-180 min. The particular features of the onset of the pain syndrome of spinal origin were described in detail previously [2]. Action potentials were recorded extracellularly from neurons of DNR of rats anesthetized with chloral hydrate (400 mg/kg) by means of glass microelectrodes filled with 2.5 M NaCl solution, by the standard electrophysiological method. Electrical activity was studied in part of the nucleus bounded by the following coordinates [15]: AP 5.8-6.2, L 0-0.2, H 5.5-6.5 mm. For the purpose of analysis the nucleus was divided conventionally into two parts: dorsal and ventral (Fig. 1). To record the spike activity (SA) of the neurons in a series of steps, the following parameters were chosen: the step in the dorsoventral direction was 100 μ, and in the mediolateral and rostrocaudal directions 200 μ. For the method of step by step extracellular recording of spike activity (SA) of the neurons the following parameters were chosen: the step in the dorsoventral direction was 100 μ and in the mediolateral and rostrocaudal directions 200 μ. The method of step by step extracellular recording of SA of DNR neurons makes it possible to study the density of distribution of spontaneously active neurons. To assess SA data on the mean momentary firing rate of the neurons, histograms of distribution of the neurons by frequency of SA

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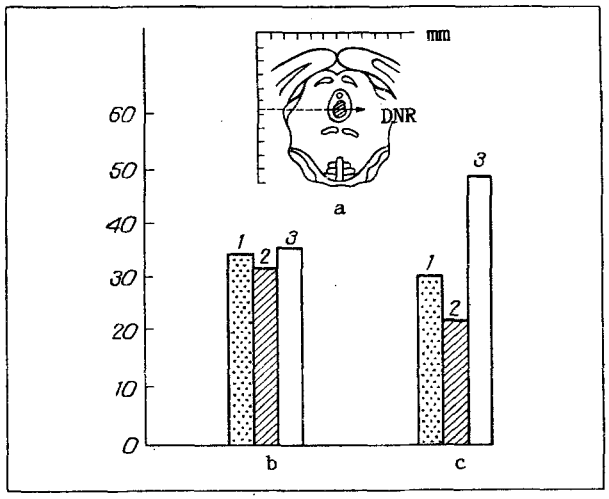

Fig. 1. Frontal section through DNR (broken line indicates conventional division of nucleus into dorsal and ventral parts) and diagram of distribution of active zones in dorsal (b) and ventral (c) parts of DNR in animals of different groups. Ordinate, number of active zones (in per cent of total number of zones recorded). 1) Parameters for Group 1, 2) Group 2, 3) Group 3.

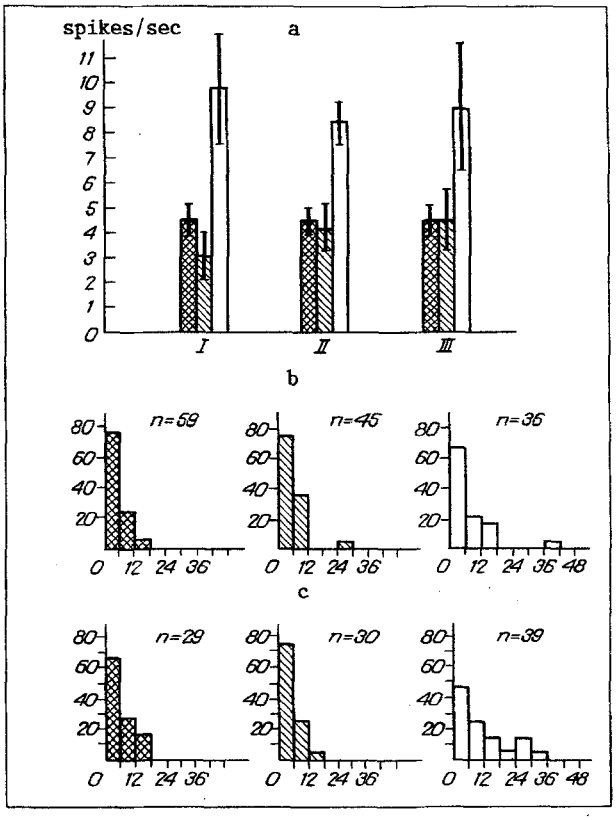

Fig. 2. Changes in mean momentary firing rate of DNR neurons during development of GPEE in dorsal horns of spinal cord: a) mean momentary firing rate of neurons, I) 10-30 min after penicillin application, II) during next 60 min (30-90 min after application), III) 90-180 min after application, b, c) histograms of distribution of neurons in dorsal (b) and ventral (c) parts of DNR by firing rate of neurons. Abscissa, discharge frequency (spikes/sec); ordinate, number of neurons (as per cent of total number of neurons in corresponding part). Significance of columns for animals of the three groups the same as in Fig. 1.

TABLE 1. Neuronal Activity in Dorsal and Ventral Parts of DNR, as Reflected in Mean Momentary Frequency and Density of Active Zones

Group of animals	Dorsal part of DNR		Ventral part of DNR	
	frequency, spikes/sec	per cent of active zones	frequency, spikes/sec	per cent of active zones
1 (n=20)	4,1±0,5	33	5,3±0,8	30
2 (n=18)	4,4±0,5	31	5,3±0,9	21
3 (n=23)	6,8±1,2	34	9,9±1,4	50

Legend. $p < 0.05$ compared with Group 1; $p < 0.05$ compared with Group 2.

in different parts of the nucleus, the density of distribution of active neurons in the nucleus, and the dynamics of the changes in SA depending on the phase of development of GPEE, were analyzed. The "ATAC" (Japan) analyzer and the "Nord-100" (Sweden) and "Olivetti" (Italy) computers were used to process the data.

EXPERIMENTAL RESULTS

The investigation showed that characteristically for the intact animals (Group 1) there was a uniform distribution of tonically active neurons in both dorsal and ventral parts of the nucleus; the number of active zones was 30% of the total number of zones recorded (n = 287; Fig. 1). In animals undergoing the mock operation (Group 2) no distinguishing features could be found in the distribution of active zones in the part of the nucleus studied (n = 287), by contrast with what was observed in the intact animals (Fig. 1b, c). Significant changes in the distribution of active neurons were found in the experimental animals (Group 3). In these animals, 30-90 min after creation of the GFEE in the spinal cord, i.e., at the peak of development of the syndrome, the density of active zones was increased; the maximum of enhanced bioelectrical activity in this case was observed in the ventrocaudal part of the nucleus, where the number of active zones reached 50% (Fig. 1). The number of active zones in the ventral part of DNR in the experimental animals differed significantly from that in rats undergoing the mock operation and in intact rats ($p < 0.05$).

The momentary discharge frequency of neurons of the animals of Groups 1 (number of neurons n = 88) and 2 (n = 76) did not differ throughout the period of observation but remained at 4.5-5.5 spikes/sec. By comparison with these two groups, in the animals of Group 3 the discharge frequency of the neurons (n = 75) was significantly higher ($p < 0.05$). This increase began as early as in Stage I of development of GPEE (Fig. 2a, I), although in this period a considerable increase in firing rate was observed in not all the rats. The effect of an increase in the mean firing rate (up to 8.5 ± 0.9 spikes/sec) was seen most clearly 30-90 min after application of the penicillin wafer to the spinal cord, i.e., at the time of maximal development of the pain syndrome (Fig. 2a, II). Later this increase still continued in the period of Stage III, when the syndrome was subsiding (Fig. 2a, III). Final restoration of the mean momentary discharge frequency (5.1 ± 0.7 spikes/sec) of the neurons (n = 54) of DNR did not take place until the 5th-7th day.

Comparison of the histogram of distribution of neurons by average discharge frequency showed that in the animals of Group 3, as early as Stage I of development of the syndrome, it began to be possible to identify a class of high-frequency neurons (Fig. 2b, c), which were absent in the intact rats and those undergoing the mock operation; more of these high-frequency neurons were found in the ventral part, moreover, than in the dorsal part of the nucleus.

Characteristics of neuronal activity in the dorsal and ventral parts of DNR are illustrated in Table 1.

The study of the firing pattern of the DNR neurons (Fig. 3) showed that under normal conditions (animals of Group 1) there were three types of neuronal activity: 1) irregular SA, when fluctuations were observed in the duration of interspike intervals, but the spikes were not grouped into distinct bursts, separated by long intervals (Fig. 3, 1); 2) rhythmic SA, characterized by a regular train of single spikes with virtually identical interspike intervals (Fig. 3, 2); 3) Bursting activity, in the form of bursts separated by intervals (Fig. 3, 3).

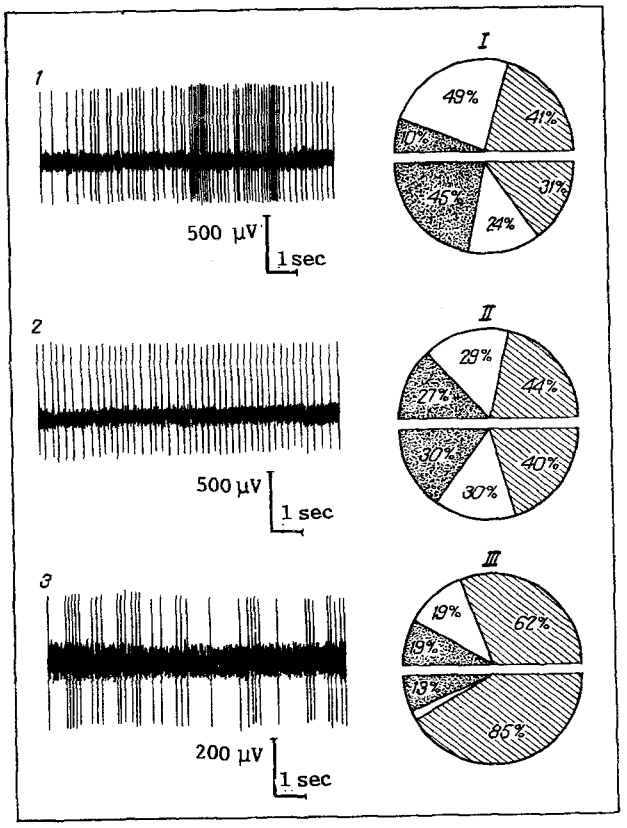

Fig. 3. Types of patterns of SA of DNR neurons in rats of three groups. I) Trace recorded from neuron with irregular type of SA; II) the same, with regular type of SA; III) the same with bursting type of SA. Pie charts indicate each type of neurons in dorsal (top semicircle) and ventral (bottom semicircle) parts of DNR in animals of Groups 1, 2, and 3 (I, II, and III respectively). Dotted sector of circle denotes percentage of neurons with irregular type of SA, unshaded sector — with regular type of SA, obliquely shaded sector — with bursting type of SA.

Neurons with rhythmic activity were observed most frequently in the dorsal part of the nucleus, those with irregular activity — in the ventral part (in animals of Group 1); these were mainly low-frequency neurons with a mean firing rate of not more than 6 spikes/sec (Fig. 3, I).

In animals undergoing the mock operation changes were observed in the relative numbers of neurons with different firing patterns: in the dorsal part the number of neurons with regular rhythmic activity was reduced but the number with irregular SA was increased, but in the ventral part the number of neurons with an irregular type of SA was reduced and the number with a bursting type was increased (Fig. 3, II); the frequency characteristics of this group of neurons were virtually identical with those of normal animals.

Considerable changes in the character of neuronal activity were observed after creation of the GPEE in the nociceptive apparatus of the spinal cord. The most significant change was expressed as a sharp increase in the fraction of neurons with a bursting type of activity (Fig. 3, III); this increase in the number of neurons of bursting type, moreover, was observed in both dorsal (62%) and ventral (85%) parts, whereas the number of low-frequency cells with regular activity was considerably reduced (the number of these cells in the dorsal part was 19% but in the ventral part only 2%).

A distinguishing feature of neurons with the bursting type of activity in neurons of Group 3 was the higher average discharge frequency. The increase in the relative proportion of high-frequency neurons in animals with GPEE took place as a rule on account of neurons of this class. Among neurons with a bursting type of activity a group could be distinguished with a clear rhythm, during which bursts followed one another after virtually equal and long interburst intervals, and there was an increase in the discharge frequency within the bursts themselves. In cells with initially irregular activity, during the development

of GPEE there was a gradual division of the firing pattern into bursts. In some cases activity of mixed type was observed in the animals of Group 3: bursts with an increasing frequency alternated with periods of irregular or rhythmic activity.

The investigation thus showed that during the development of a GPEE in the nociceptive apparatus of the dorsal horns of the spinal cord, inducing a pain syndrome of spinal origin, changes took place in neuronal activity in an antinociceptive structure (DNR). An increase in the number of active zones of the nucleus, an increase in the mean discharge frequency of the neurons and in the number of neurons with a bursting type of activity, as well as other parameters are evidence of activation of DNR. It can be tentatively suggested that activation of this structure of ANS makes its own contribution to limitation of the intensity of the pain syndrome and its abolition. The fact will be noted that for several days after abolition of the pain syndrome, activity of single neurons of DNR remained enhanced. This tonic activation of DNR may play the role of a reliability factor, contributing to resistance to increased algogenic influences, as was shown previously [4].

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