Impulse Activity of Neurons of the Sensorimotor Cortex of Rabbits with Experimental Diabetes Mellitus

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At least three types of spontaneous impulse activity of neurons are identified: single spikes, short bursts of impulses, and alternating periods of single impulses and bursts. In rabbits with experimental diabetes mellitus a marked shift of the middle-frequency distribution of spontaneously-active neurons to higher frequencies is observed.

Key Words: *rabbits; diabetes mellitus; sensorimotor cortex; neuronal activity; histology*

Since the discovery of insulin and insulin receptors in brain tissue [9], the state of the central nervous system (CNS) during diabetes mellitus has become an object of increasing interest [4-6]. Along with biochemical and molecular-biological studies, electrophysiological investigations (using electroencephalography [12] and the method of evoked potentials [8,11] are being carried out; however, studies directly devoted to specificities of neuronal activity are lacking.

The aim of the present study was to investigate the impulse activity of neurons of the sensorimotor cortex (SC) of rabbits with experimental diabetes mellitus (EDM).

MATERIALS AND METHODS

The experiments were carried out on adult freely moving alert Chinchilla rabbits with EDM (the experimental group, 3 rabbits) and on healthy animals (the control group, 3 rabbits).

Insulin-dependent diabetes mellitus was created by injecting 16 mg/kg body weight dithizone intravenously [2]. On day 30 this resulted in stable hyperglycemia (12-29 mmol/liter), glucosuria, weight loss, and an increased water intake. The developed diabetes had a long-term course: studies were performed during the 3rd-6th month of disease. Insulin therapy was not performed, except for the rare cases when the state of the animal markedly deteriorated beyond the experimental period. The animals were placed in a shielded box (65×85×85 cm), and the impulse activity of SC neurons was extracellularly led off using glass mi-

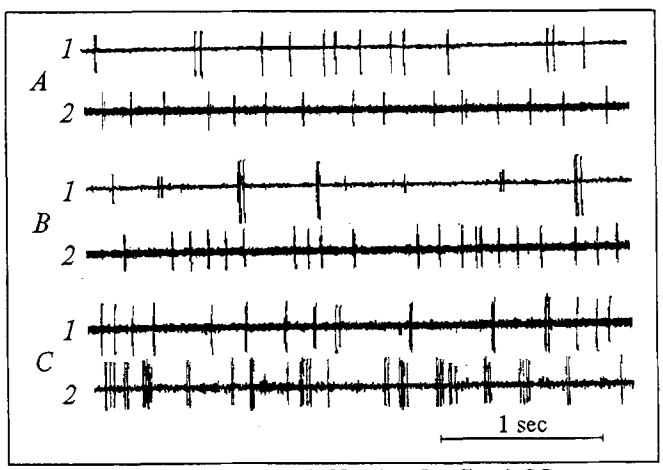

Fig. 1. Typical patterns of SIA (*A*, *B*, *C*) of SC neurons. Fragments of neuronal activity of distribution neurons for the absence (*1*) and presence (*2*) of regular impulse activity in the impulse flow.

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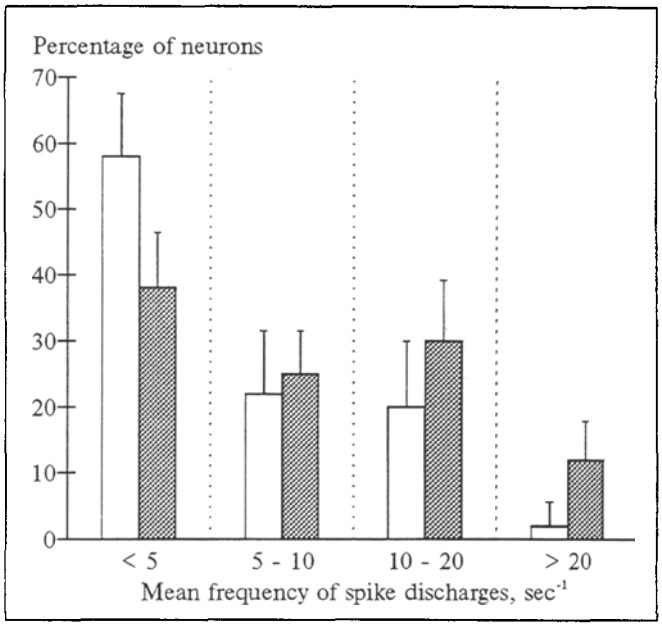

Fig. 2. Distribution of SC neurons over middle-frequency impulse activity in healthy rabbits (open bars) and in rabbits with EDM (dark bars). Confidence intervals correspond to $p<0.05$.

croelectrodes, which were filled with KCl (2.5 mmol/liter) and inserted in the brain with a micromanipulator attached to the skull [1] and a set of amplifying and recording equipment. The rabbit's behavior in the box was examined in parallel with the investigation of the impulse activity. The techniques were described in more detail previously [3]. The spontaneous impulse activity (SIA) of neurons during calm periods of alertness was analyzed not earlier than 1 min and not later than 2 min after the gradual advance of the microelectrode along the track, provided that the record of cell discharges was stable for 10 min or longer. The study of neuronal activity involved: 1) a qualitative description of the pattern of SIA and its classification; 2) determination of the middle frequency of cell firings and/or bursts; 3) detection of regular impulse activity in the impulse flow and determination of its parameters. In all, SIA of 306 neurons was analyzed in rabbits with EDM and SIA of 81 neurons in the control animals. Morphological changes of the brain tissue of layers III and V of the sensorimotor cortex were assessed by Golgi staining in animals with EDM (7 rabbits), in the rabbits in which EDM did not develop after dithizone injection (4 rabbits) and in healthy animals (3 rabbits).

RESULTS

Analysis of SIA of SC neurons in rabbits of both groups showed the presence of at least three types of cell elements with different patterns of spontaneous impulse activity (Fig. 1). SIA of the overwhelming majority of neurons (Table 1, A) comprised single irregular (Fig. 1, *A*, *1*) or, more seldom, regular (rhythmic), pacemaker-type (Fig. 1, *A*, *2*) impulses. In a small fraction of examined neurons (Table 1, B) SIA consisted of short bursts of impulses formed by 3-8 spikes with an interspike interval of 5-20 msec (Fig. 1, *B*). A rhythmic pattern of bursts in the range of 3-8 Hz was typical of the majority of these neurons (Fig. 1, *B*, *2*). An intermediate type of SIA in the form of alternating periods of single irregular discharges and bursts was characteristic of the third group pf neurons, of which there were also few (Fig. 1, *C*, Table 1, C). Some neurons of the three abovementioned types exhibited a virtual absence of spontaneous impulse activity (1-3 spikes and/or a burst of impulses during 10 sec and longer), and, depending on the pattern of discharges, were identified as "silent" neurons of one or another type (Table 1). Comparison of the results obtained on the animals of the experimental group with the control data demonstrated that the number of neurons with the burst component in SIA was relatively higher (approximately 2-fold) in rabbits with EDM (Table 1, B and C). The fraction of "silent" neurons in animals of the experimental group was also more than 1.5 times larger (Table 1). On the other hand, the relative number of pacemaker-

TABLE 1. Distribution of Neurons with SIA of Different Types (A, B, and C) in SC of Healthy Rabbits (1) and Rabbits with EDM (2)

Pattern of SIA	Number of neurons, %							
	total		irregular		rhythmic		"silent"	
	1	2	1	2	1	2	1	2
A	92.6	86.6	70.4	62.7	6.2	1.3	16.0	22.6
B	3.7	5.6	—	1.0	3.7	3.9	—	0.7
C	3.7	7.8	1.2	3.2	2.5	3.6	—	1.0
In all	100	100	71.6	66.9	12.4	8.8	16.0	24.3

Note. A dash shows that neurons of this type were not detected.

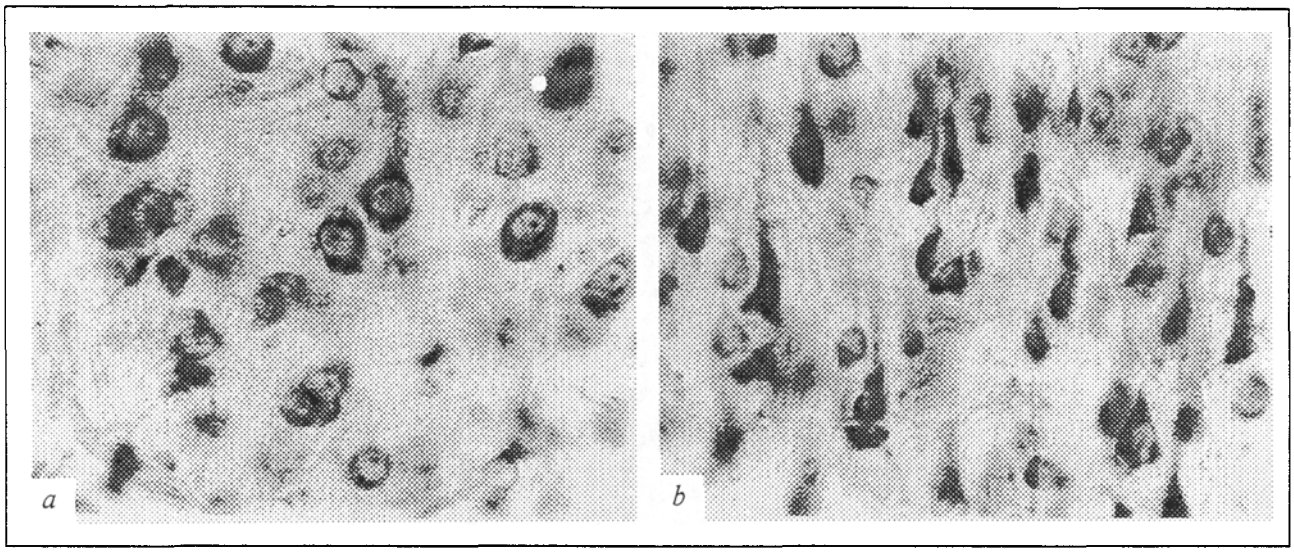

Fig. 3. Neurons of layer V of SC of healthy rabbit (*a*) and of rabbits with EDM (*b*). Golgi staining, ×400.

type neurons in the animals with EDM was almost 5 times lower than in the control.

Analysis of the distribution of spontaneously active neurons over the middle-intensity impulsation in animals with EDM (Fig. 2) demonstrated a marked shift of the distribution of neuronal activity toward an increased frequency of spontaneous discharges, i.e., an increase in the number of neurons with a high-frequency SIA coupled with a simultaneous decrease of the percentage of cells with a low-frequency SIA.

Assessment of morphological changes showed, along with the traditionally described gross vascular and tissue disorders [7,10], pronounced changes in the form of wrinkling, hyperchromatism, and/or hypertrophy of individual neurons in nerve cells of the brain of rabbits with EDM (Fig. 3). These changes may be directly associated with the pathology (EDM) (since in the rabbits which were given dithizone but in which EDM did not develop these changes were not observed) and, evidently, with the described changes in the neuronal activity, which reflect functional disorders in EDM.

These findings provide evidence that the activity of SC neurons is transformed in rabbits with EDM and indirectly corroborate the earlier reported data on the effect of long-tern hypoinsulinemia on the parameters of neuronal maintenance of the food-procuring behavior [3].

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