PHYSIOLOGY

Structural and Topical Bases of Picrotoxin-Induced Choreomyoclonic Hyperkinesis

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Multiple bilateral microinjections of 1 μg picrotoxin (blocker of $GABA_A$-receptor chlorine channels) into the rostral neostriatum cause choreomyoclonic hyperkinesis of the paws, head, and body in rats. The most pronounced hyperkinesis was observed when the cannula was localized in the most anterior regions of the rostral neostriatum with irradiation of its action to the white matter (*corpus callosum*) and when the cannulas were located asymmetrically in the right and left striatum.

Key Words: *neostriatum; GABA-ergic system; picrotoxin; extrapyramidal hyperkinesis*

Intensive molecular biological and genetic studies revealed the loci and types of chromosome aberrations associated with Huntington's chorea [5]. This condition is characterized by imperative hyperkinesis of the limbs and body. Progressive neuronal death in the subcortical nuclei and dysfunction of neostriatum (NS; putamen and caudate nucleus) is associated with the appearance of a modified protein huntidin [7]. The main component of neuropathochemistry of Huntington's chorea is insufficiency of GABA-ergic system of NS. At the same time, many aspects of the pathogenesis of this disease remain unclear, which can be explained by neurochemical heterogeneity and functional heterogeneity of NS nuclei. The contribution of various NS structures (segments) in the genesis of motor disturbances is poorly studied.

We previously elaborated a functional model of extrapyramidal hyperkinesis similar to choreic hyperkinesis in human [1,2]. Hyperkinesis is induced by repeated injections of $GABA_A$ receptor antagonist and chlorine ionophore blocker, picrotoxin (PT) into the rostral NS. Here we used this model for evaluation of the role of various NS regions in the reproduction of hyperkinesis.

MATERIALS AND METHODS

The experiments were carried out on 34 albino male Wistar rats weighing 250-300 g. Polyethylene cannulas containing 1 μg picrotoxin (Serva) in 0.75 μl sterile apyrogenic physiological saline were bilaterally inserted into NS by stereotaxic coordinates (1-2 mm rostrally to the bregma, 2.0-2.5 mm laterally to the middle line, and 6.0-6.5 mm ventrally to the scull surface) under hexanal narcosis.

The technique of injections was described earlier [2,3]. The experiments were started 2-3 days after surgery. The preparations were injected daily for 3 weeks. Behavioral tests were conducted 3 times a week with 1-2-day intervals. Fifteen-twenty minutes after PT injection spontaneous motor activity in an open field was evaluated for 5 min. Proper localization of the canullas was verified morphologically after the experiments. In all animals, the cannula tips were located in NS area, however, their localization within this structure differed.

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The means, standard errors, and standard deviations were calculated. The significance of differences was evaluated using Student's *t* test at $p<0.05$. The parameters on the day of microinjections were compared with those before implantation of the cannula (spontaneous motor activity) or with the parameters of various groups on the corresponding experimental day (parameters of hyperkinesis).

RESULTS

Hyperkinesis was observed in 22 of 34 rats (70%). The type and temporal parameters of hyperkinesis were similar to those described elsewhere [1] and had the following manifestations. On experimental day 1 the rats demonstrated psychomotor excitation (increased motor activity), which was associated with forelimb twitching, imperative chewing movements, and vertical head movements (nodding) on min 10 postinjection. During the subsequent 20-25 min the movements became rhythmic and the amplitude and frequency of head and limb movements increased. In 16 rats, both forepaws (in some cases the ipsilateral hind paw), head, and body were involved in hyperkinesis (generalization phase). Hyperkinesis peaked on minutes 40-50 of the experiment, thereafter its intensity decreased. In further experiments, hyperkinesis usually started from the same limb. Free movements over the cage and grooming were preserved during hyperkinesis except the generalization phase. Hyperkinesis decreased and completely disappeared on experimental weeks 2 and 3, respectively.

Detailed analysis of the obtained data showed that the severity and duration of hyperkinesis, and the presence and absence of the generalization phase (reflects adequacy of the model) depended on the zone of PT diffusion in NS and surrounding white matter. Correspondingly, all rats were divided into 3 equal groups. Group 1 (n=12) included animals with symmetrical localization of cannula tips in the central and dorsal NS segments (Fig. 1, *a*). In group 2 animals (n=12), the cannula tips were located near the rostral boundary of NS and PT diffused also in the white matter surrounding NS (Fig. 1, *b*). In group 3 rats (n=10) localization of the cannulas was similar to that in group 1, but the tips were visualized at the dorsal boundary of NS and PT partly diffused in the white matter of the corpus callosum (Fig. 1, *c*).

In group 2 rats hyperkinesis was observed in all animals. In group 1 rats (nominally the best PT localization) hyperkinesis was observed in 7 of 12 rats, while in group 3 only in one third of rats. Hyperkinesis

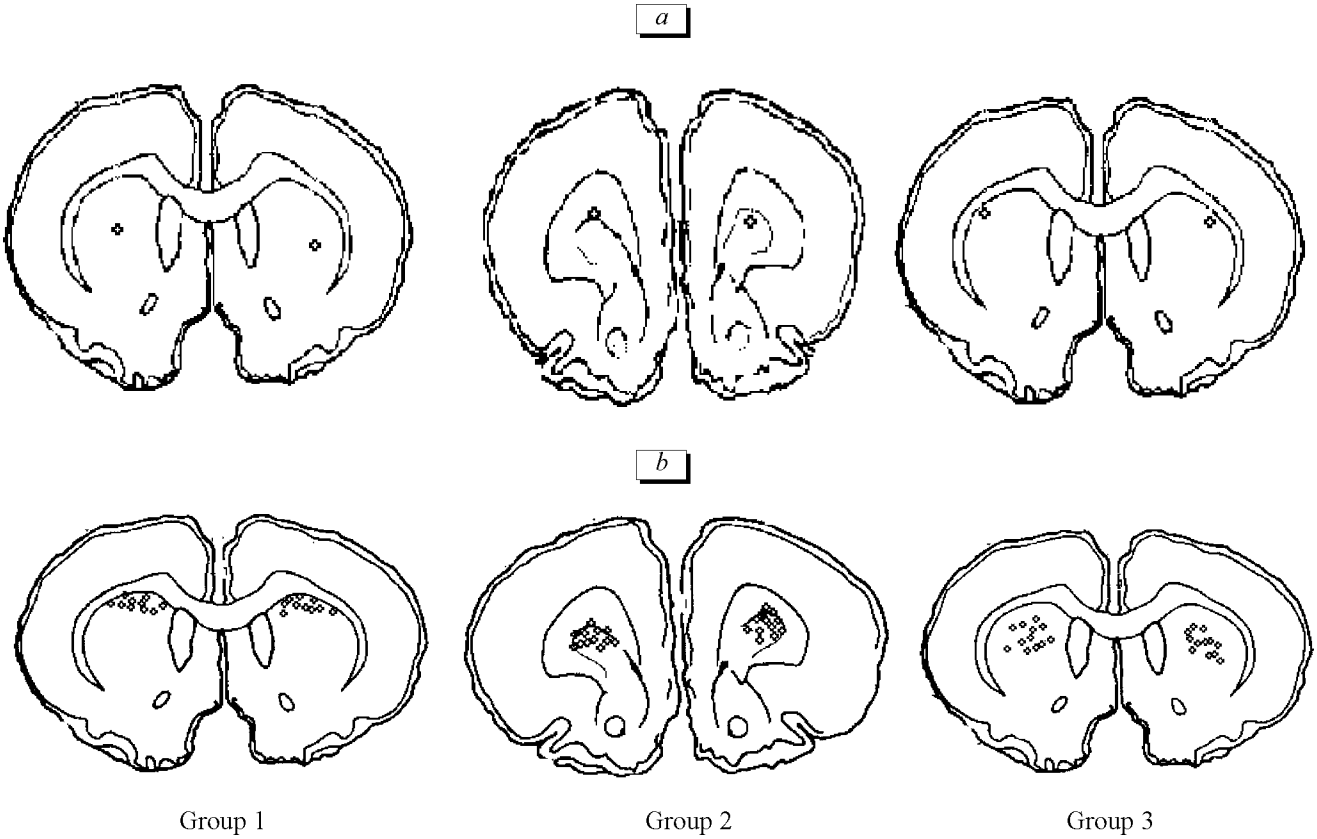

Fig. 1. Localization of cannulas (*a*) and their tips (*b*) in rostral neostriatum after bilateral injections of 1 μg picrotoxin in rats. Cannula track (*a*) and its localization in the brain (*b*).

was most pronounced during asymmetric localization of the cannula in the left and right NS. This localization was found in all groups (presented in Fig. 1, *b*).

Comparative analysis of hyperkinesis parameters revealed no differences between the groups. Thus, the latencies of hyperkinesis (from PT injection to the first paw or head jerks) in groups 1 and 2 were 9.6±4.2 and 9.5±5.2 min, respectively. The data obtained on group 3 were not processed statistically because of low reproducibility of hyperkinesis. The mean duration of hyperkinesis in groups 1 and 2 differed insignificantly (91.9±55.6 and 61.0±26.2 min). The differences between hyperkinesis parameters during generalization phase and spontaneous motor activity in the open field were also insignificant.

The reproducibility and duration of hyperkinesis were lower after PT injection into the caudal ventral NS region (analogues to the putamen in primates and subprimates) compared to injections into the rostral NS: no generalization phase was observed in this case [3]. Hyperkinesis was not reproduced after PT injection into the globus pallidus (paleostriatum). Thus, true choreomyoclonic hyperkinesis was observed after PT injections into the rostral NS, which verified the key role of this brain region, an analogue of the caudate nucleus in primates.

Thus, we showed that experimental hyperkinesis was completely reproduced when the cannulas were localized at the rostral boundary of NS and the PT diffusion zone includes the adjacent white matter. Histochemical studies [6] revealed GABA-ergic elements not only in NS, but also in the surrounding white matter. Neurophysiological mechanisms underlying the effect of PT cannot be deduced from the obtained data, but the problem is very actual. Significant asymmetry of PT action in the right and left NS probably reflects functional peculiarities of this subcortical structure.

Thus, we identified regions of striatal nuclei participating in the pathogenesis of Huntington's chorea and counteracting to this disease in healthy brain. Stereotaxic methods are now widely used in neurosurgery of extrapyramidal pathology, e.g. for the correction of hyperkinesis (similar to parkinsonism neurosurgery), and the first steps in transplantation of embryonic tissue in Huntington's chorea were done [4]. Our data can be used for determining preferable zones for such surgery.

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