PHYSIOLOGY

Neostriatal Glutamatergic System Is Involved in the Pathogenesis of Picrotoxin-Induced Choreomyoclonic Hyperkinesis

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Administration of dizocilpine (MK-801, noncompetitive antagonist of NMDA glutamate receptors) into the neostriatum decreased the reproducibility and duration of hyperkinesis in rats induced by repeated microinjections of $GABA_A$ receptor antagonist picrotoxin. By contrast, glutamate potentiated the hyperkinetic and convulsive effect of picrotoxin and promoted the inhibition of conditioned avoidance response. Our results indicate that the striatal glutamatergic system is involved in the development of locomotor and cognitive disorders associated with deficiency of the neostriatal GABAergic system and playing a role in the pathogenesis of Huntington's chorea.

Key Words: *neostriatum; GABA; glutamate; MK-801; picrotoxin; hyperkinesias*

Huntington's chorea (HC), or Huntington's disease, is a progressive hereditary human disorder manifesting in deficiency of locomotor and mental functions and associated with degenerative processes in the cortex of cerebral hemispheres and subcortical nuclei [2,10]. HC is accompanied by dysfunction of GABAergic interneurons and neurons in the neostriatum (NS, caudate nucleus, and putamen) that are responsible for the realization of efferent internuclear relationships [10, 13]. The excitotoxic effect of glutamate [8] and imbalance in the brain amino acid neurotransmitter system play a key role in cascade neurochemical disturbances during HC. The interaction between neostriatal GABAergic and glutamatergic systems during hyperkinetic dysfunction of basal ganglia remains unclear. Experimental hyperkinesis of the limbs, head, and body in rats modeled by repeated administration of a $GABA_A$ receptor antagonist picrotoxin (PT) into rostral NS serves as the model of hyperkinesis in chorea patients [3]. Topical characteristics and GABAergic mechanisms of the disorder were previously studied [1]. A possible role of the neostriatal glutamatergic system in dysfunction of basal ganglia was studied on this model of hyperkinesia.

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MATERIALS AND METHODS

Experiments were performed on 43 male Wistar rats (250-300 g) with conditioned active avoidance response in a shuttle box. The animals were trained to leave a dark compartment no later than 10 sec after conditioned acoustic stimulation (1.7-20.0 kHz). Electric current (1 mA) was delivered through a metal floor and served as negative reinforcement. Each experiment included 10 presentations of conditioned and unconditioned stimuli at 20-30-sec intervals. Trained rats were narcotized with hexenal. Polyethylene cannulas were bilaterally implanted into the rostral NS (1-2 mm rostral to bregma, 2.0-2.5 mm lateral to the

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midline, 6.0-6.5 mm ventral to the skull surface). The cannulas were filled with sterile apyrogenic physiological saline (control group, *n*=9) or pharmacological preparations (experimental groups). The solution (1 μl) contained 2 μg PT (Serva; group 1, *n*=12), 2 μg PT and 5 μg glutamate (Lachema; group 2, *n*=11), or 2 μg PT and 1 μg dizocilpine (MK-801, RBI; group 3, *n*=11). The procedure of microinjections was described elsewhere [1,3]. The experiment started 2-3 days after surgery. The preparations were administered daily for 14-16 days. The behavior was studied 3 times a week at 1-2-day intervals. Spontaneous locomotor activity of rats in the open field (3 min) was recorded 15-20 min after administration of preparations. Then the animals were tested in a shuttle box. Localization of the cannula tips in the brain tissue was verified morphologically after the end of the experiments. In all rats they were localized in the rostral NS.

Statistical treatment included calculation of the arithmetic mean, standard error, and standard deviation. The significance of differences was evaluated by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

Repeated microinjections of physiological saline into NS had little effect on conditioned active avoidance response in rats. Exploratory activity of control animals in the open field decreased over the 1st week. The number of vertical rearing postures recorded for 3 min decreased from 12.3±8.2 (before treatment with physiological saline) to 2.1±3.5 (day 1) and 0.8±0.7 (day 5, $p=0.05$). After termination of microinjections exploratory activity returned to the initial level. Motor dysfunction was not revealed in control rats.

Blockade of neostriatal $GABA_A$ receptors inhibited conditioned response in group 1 rats (30-40% of response accuracy). This parameter returned to normal after termination of PT microinjections. Hyperkinesis developed in all rats of this group starting from the 1st days of PT treatment. The main parameters of dysfunction were similar to those described previously [1,3]. The duration of hyperkinesis on day 1 of PT treatment was 88.4±27.7 min. The first symptoms of hyperkinesis developed 6-10 min after microinjection (9.4±4.2 min) and included imperative movements of the forelimb, chewing movements, and vertical head movements (nodding). Rhythmic twitching was observed after 15-20 min. The amplitude and frequency of head and limb movements increased. Hyperkinesis of the forelimbs, head, and body was revealed in 50% animals. The disorder involved practically all body parts (generalization, Table 1). Locomotor activity in the open field increased by 3-4 times over the 1st week of PT microinjections. This increase might be more pronounced, but hyperkinesis seems to restrict locomotor activity of animals. The degree of hyperkinesis was maximum 40-50 min after treatment, but progressively decreased in the follow-up period. The duration of hyperkinesis decreased over the 2nd week of treatment. Hyperkinesis completely disappeared on the 3rd week. However, avoidance performance did not return to normal (Fig. 1, *1*). No direct correlation was revealed between the degree of changes in conditioned response activity and severity of hyperkinesis. Spontaneous locomotor activity returned to normal after termination of treatment.

Combined administration of PT and glutamate was followed by rapid development of hyperkinesis in group 2 rats (4-5 min after microinjection, Table 1). Hyperkinesis of masticatory and mimic muscles was manifested in rhythmic movements and resembled phenamine-induced stereotyped behavior. Treatment with glutamate produced a dose-independent toxic effect of PT. Tonic convulsions of skeletal muscles in the limbs and body developed in 4 rats at the peak of generalized hyperkinesis (1st days of microinjections). Two animals died. Progression of hyperkinesis, conditioned behavior (Fig. 1, *2*), and spontaneous locomotor activity in the remaining rats of group 2 did not differ from those in group 1 animals.

In group 3 rats noncompetitive NMDA glutamate receptor antagonist MK-801 prevented the develop-

TABLE 1. Hyperkinesis of Limbs, Head, and Body in Rats Receiving Repeated Bilateral Microinjections of Neurotransmitters in the Rostral Area of NS (*M*±*m*)

Group	Hyperkinesia			Generalized hyperkinesia		
	latency	duration	reproducibility, %	latency	duration	reproducibility, %
1	9.4±4.2	88.4±27.7	100	24.5±12.0	60.0±36.3	54
2	5.4±1.1*	97.6±49.2	100	12.2±6.0	33.8±17.8	45
3	11.2±5.1	55±22.7*	54	—	—	27

Note. *$p=0.05$ compared to group 1. Dash: generalized hyperkinesis was observed in only 2 rats, statistical treatment is impossible.

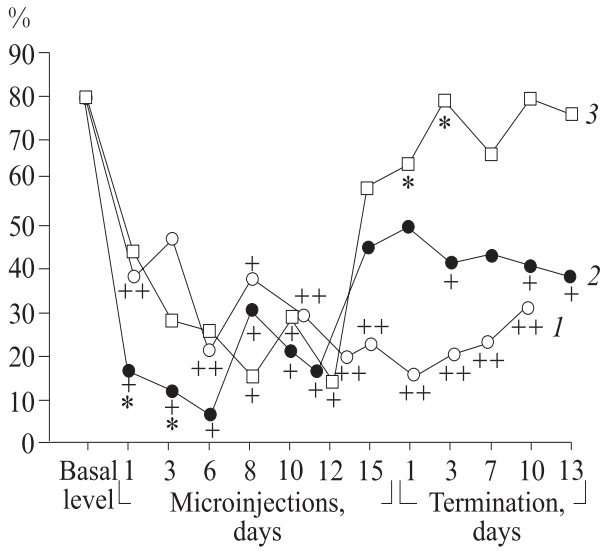

Fig. 1. Accuracy of conditioned active avoidance response in a shuttle box. Chronic administration of 2 μg picrotoxin (*1*), 2 μg picrotoxin and 5 μg glutamate (*2*), and 2 μg picrotoxin and 5 μg MK-801 into the rostral neostriatum (*3*). Ordinate: ratio between the numbers of executed conditioned reactions and presented conditioned stimuli. *p<0.01 compared to *1*; ^{+}p=0.01-0.05 and ^{++}p<0.01 compared to the control.

ment of hyperkinesis in 50% animals. In other animals this compound significantly decreased the duration of hyperkinesis and relieved symptoms of generalized disorder (Table 1). Conditioned response was impaired in these rats (similar to PT treatment). It should be emphasized that the latency of the conditioned response decreased, but not increased. As differentiated from group 1 and 2 rats, the response accuracy in group 3 animals rapidly returned to normal after termination of microinjections (Fig. 1, *3*). Group 3 rats did not exhibit increased locomotor activity, which is usually observed after PT administration.

Our results indicate that modulation of the glutamaergic system modifies the effect of chronic $GABA_A$ receptor blockade on normal (conditioned and free) and pathological behavior (hyperkinesis). The neostriatal glutamatergic system is presented by efferent endings that originate from the neocortex. Autoradiography revealed high density of NMDA glutamate receptors in NS and their irregular distribution in the basal ganglia [12]. Close morphological and functional relationships were revealed between glutamatergic and nigrostriatal dopaminergic endings in NS. We found no published data on the projection of glutamatergic endings to nigrostriatal GABAergic neurons and relationship between receptor systems of these two amino acid neurotransmitters. Therefore, it is impossible to propose a mechanism for reciprocal influence of test preparations.

Death of GABAergic neurons (interneurons and projecting cells) in NS is a pathoanatomical signs for HC. The neocortex, paleostriatum, and hippocampus are involved in the pathological process at the onset and progression of several forms of HC [9]. Persistent clinical signs of the disease are observed after loss of one-third of striatal neurons. Degeneration of more than 50% cells is accompanied by the development of severe HC. Postmortem examination of patients with chronic HC found no more than 5-10% cells in the subcortical nucleus [13]. The pathogenesis of this disease is characterized by stages of molecular-genetical, molecular-cellular, and neurochemical rearrangements. A considerable number of CAG-repeats appear in the IT15 gene due to hereditary mutation in the chromosomal locus 4p16.3. These changes are followed by synthesis of a modified protein huntidin (huntingtin) that contains toxic polyglutamate domains [2]. This protein alters intracellular metabolic reactions, blocks the oxidation-reduction processes in mitochondria, and activates apoptosis in neurons [10].

Toxic properties of glutamate were described at the end of 1970s [8]. In this period the etiopathogenesis of HC was not deciphered. Excitotoxicity of glutamate in relation to neurons is realized via synaptic and non-synaptic NMDA receptors [11] and affects energy metabolism in neurons. None of clinical neurochemical studies revealed hyperactivity of the glutamatergic system at the onset of the disease. Indirect evidences were obtained in experiments on rodents [10]. Preparations with glutamatergic activity were low effective in preventing the major manifestations of HC [7]. At the same time, there are unambiguous data that progressive degeneration of neurons in the cortex and subcortical nuclei [5] is accompanied by death of striatal cells carrying membrane NMDA receptors [4]. The label for AMPA and kainate receptors (not for NMDA glutamate receptors) disappears in cortical layer VI, which is particularly sensitive to adverse effects of glutamate [14]. Tyrosine hydroxylase activity increases in the cortex and subcortical area during HC [6]. The nigrostriatal astroglia responds to this process by compensatory activation of glutamate transporters [4]. Therefore, the pathogenesis of HC involves complex glutamatergic structures. The model of PT-induced striatal hyperkinesis reproduces symptoms that are usually observed in manifestation of the disease. The processes of excitation and inhibition in the basal ganglia are disorganized during this stage of the disease. Our study showed that the glutamatergic system plays an important role in the pathogenesis of these disturbances.

REFERENCES

1. V. M. Varshavskaya, O. N. Ivanova, and A. F. Yakimovskii, *Ros. Fiziol. Zh.*, **88**, No. 10, 1317-1323 (2002).

2. S. N. Illarioshkin, *Conformational Diseases of the Brain* [in Russian], Moscow (2003), p. 248.
3. A. F. Yakimovskii, *Byull. Eksp. Biol. Med*., **114**, No. 1, 7-9 (1993).
4. T. Arzberger, K. Krampfl, S. Leimgruber, and A. Weindl, *J. Neuropathol. Exp. Neurol*., **56**, No. 4, 440-454 (1997).
5. B. G. Jenkins, H. D. Rosas, Y. C. Chen, *et al*., *Neurology*, **50**, No. 5, 1357-1365 (1998).
6. M. Lesort, W. Chun, J. Tucholski, and G. V. Johnson, *Neurochem. Intern*., **40**, No. 1, 37-52 (2002).
7. L. V. Metman, M. J. Morris, C. Farmer, *et al*., *Neurology*, **59**, No. 5, 694-699 (2002).
8. T. L. Perry and S. Hansen, *Ibid*., **40**, No. 1, 20-24 (1990).
9. H. D. Rosas, W. J. Koroshetz, Y. I. Chen, *et al*., *Ibid*., **60**, No. 10, 1615-1620 (2003).
10. Ch. A. Ross and R. L. Margolis, *Neuropsychopharmacology: The Fifth Generation of Progress*, Eds. K. L. Davis et al., New York (2002), pp. 1817-1830.
11. R. Sattler, Z. Xiong, W. Y. Lu, *et al*., *J. Neurosci*., **20**, No. 1, 22-23 (2000).
12. J. C. Villares and J. C. Stavale, *Exp. Neurol*., **172**, No. 2, 391-404 (2002).
13. J. P. Vonsattel, R. H. Myers, T. J. Stevens, *et al*., *J. Neuropathol. Exp. Neurol*., **44**, No. 6, 559-577 (1985).
14. M. V. Wagster, J. C. Hedreen, C. E. Peyser, *et al*., *Exp. Neurol*., **127**, No. 1, 70-75 (1994).