PHYSIOLOGY

Effect of Repeated Microinjections of Bicuculline into Rostral and Caudal Neostriatum and Globus Pallidus on Avoidance Reaction in Rats

A. F. Yakimovskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 12, pp. 613-615, December, 1999
Original article submitted April 27, 1999

Repeated bilateral microinjections of bicuculline (5 μg), an antagonist of A-type GABA-receptors into the rostral and caudal neostriatum and globus pallidus impair realization of avoidance conditioned behavior in rats, but produce no motor disturbances (hyperkineses) similar to those produced by intrastriatal injection of picrotoxin. The most pronounced albeit compensated disturbances in avoidance behavior were found after blockade of the GABAergic system in the rostral neostriatum. In rats subjected to similar blockage in the globus pallidus the disturbances persisted after termination of bicuculline microinjections.

Key Words: *neostriatum; globus pallidus; GABAergic system; bicuculline; conditioned avoidance*

Early experiments with repeated microinjections (MI) of picrotoxin into neostriatum (NS) proved the role of the striatal GABAergic system in the pathogenesis of extrapyramidal hyperkineses and elaborated a controllable and reversible experimental model of these hyperkineses [4]. Their structural and functional features were demonstrated and different contribution of the rostral and caudal NS, as well as globus pallidus (GP) into the genesis of these hyperkineses was shown [6]. GABAergic system of NS is known to have a complex organization with heterogeneous reception apparatus [1,7]. The role of different subsystems (types of receptors) of the NS GABAergic system in the genesis of extrapyramidal hyperkineses remains unclear. In this paper the role of GABA receptors is studied using bicuculline MI into NS and GP performed under the same conditions as in our previous experiments.

Department of Normal Physiology, I. P. Pavlov State Medical University; Laboratory of High Nervous Activity, I. P. Pavlov Institute of Physiology, Russian Academy of Medical Sciences, St. Petersburg

MATERIALS AND METHODS

The study was carried out on 36 male Wistar rats (body weight 200-250 g) with conditioned active avoidance response (CAAR) in a shuttle box [4,5]. Polyethylene cannulas filled with sterile apyrogenic physiological saline (control) or bicuculline (Serva) were implanted bilaterally to caudal NS or GP under Hexenal narcosis. Stereotactic coordinates for rostral NS were 0.1-1.5 mm rostrally to the bregma, 2.0-2.5 mm laterally to the median skull line, and 6.0-6.5 mm ventrally to the skull surface. The coordinates for caudal NS were: 0.5-0.8 mm caudally to the bregma, 1.0-1.5 mm laterally to the median line, and 6.0-6.5 mm ventrally to the skull surface. The corresponding coordinates for GP were 0.8-1.0, 1.8-2.8, and 7.0-7.5 mm. The procedure of MI was described previously [5]; the volumes for each MI were 0.75 μl into rostral and caudal NS and 0.5 μl into GP. The dose of bicuculline was 5 μg. Each group comprised 5-7 rats. All experiments were started 2-3 days postoperation. The drugs were injected daily during 3 weeks. The rats were tested 3 times a

0007-4888/99/0012-1190$22.00

week with 1-2-day intervals. Spontaneous motor activity was tested in an open field 15-20 min postinjection during 5 min and then parameters of CAAR were measured. Tests were continued during 2-3 weeks after the end of MI course.

The rats were sacrificed under Hexenal narcosis to perform histomorphological control. The results were statistically analyzed with Student's t test only for rats with proper bilateral localization of MI into NS and GP.

RESULTS

No behavioral changes were observed in control rats receiving MI of physiological saline into the rostral NS (Fig. 1, *a*). By contrast, the first injections of bicuculline into the rostral NS virtually completely disturbed conditioned activity, which then gradually recovered, but was completely restored only after termination of MI. It was impossible to measure the latency of CAAR under conditions of its low realization. General motor activity remained unchanged.

MI of physiological saline into caudal NS significantly impaired the accuracy of reflex realization. After 3 MI it decreased to 50.0±28.3% of the initial value (84.3±9.2%, p=0.05). The latency of CAAR did not change significantly, while spontaneous motor activity in the open field significantly decreased in this group. Bicuculline MI into caudal NS less markedly decreased CAAR than MI into rostral NS. Bearing in mind the same tendency in the control group, these shifts were significant only at the end of bicuculline course (Fig. 1, *b*). In these rats CAAR latency and spontaneous motor activity did not change.

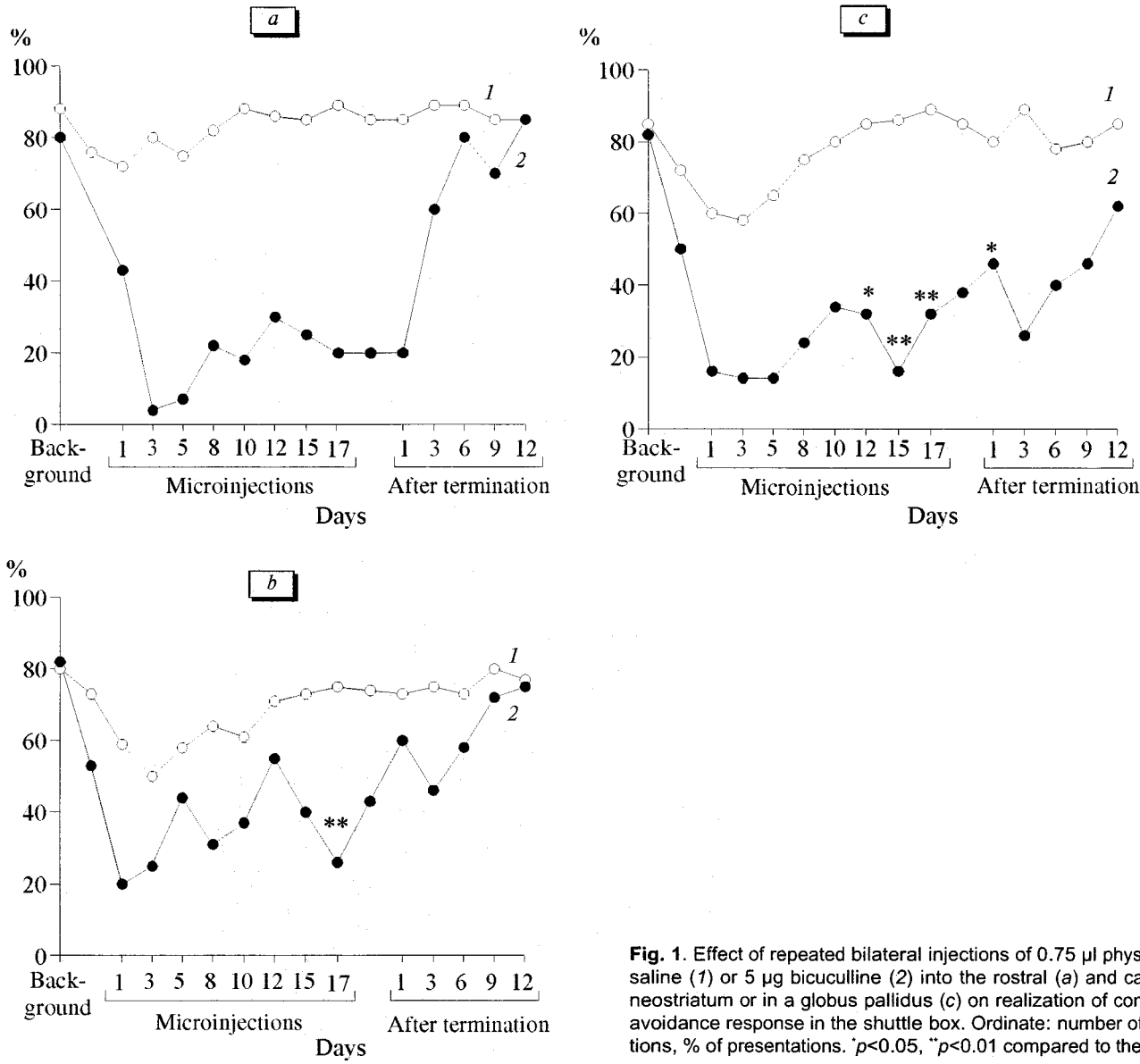

Fig. 1. Effect of repeated bilateral injections of 0.75 μl physiological saline (*1*) or 5 μg bicuculline (2) into the rostral (*a*) and caudal (*b*) neostriatum or in a globus pallidus (*c*) on realization of conditioned avoidance response in the shuttle box. Ordinate: number of realizations, % of presentations. $^{*}p<0.05$, $^{**}p<0.01$ compared to the control.

After the first week of MI of physiological saline into GP we observed a distinct (in some days significant) impairment of CAAR realization, although in the following days this parameter did not differ from the control (Fig. 1, *c*); other behavioral parameters did not change significantly. Bicuculline MI into GP produced sustained CAAR disturbances: during the entire course of MI, CAAR realization in this group did not exceed 40-50% (Fig. 1, *c*). After termination of MI conditioned activity recovered very slowly and did not reach the initial level to the end of observation period (3 weeks). There were no clear shifts in spontaneous activity in this group.

Taking into consideration previous data [4], in addition to visual observation, complex behavioral tests were performed to reveal possible masked disturbances of motor activity (catalepsy, pathological freezing, *etc.*). None of these tests revealed motor disturbances in rats receiving bicuculline MI.

The data obtained show that choreic myoclonic hyperkinesis of the body and extremities (similar to manifestations of Huntington's or chorea minor in rheumatic attack in men), which was provoked in rats by MI of picrotoxin in NS, is not reproduced by bicuculline MI. At the same time, the effect of both GABA blockers on CAAR realization was similar: they partially or completely inhibited conditioned activity. There were differences in the effect of bicuculline on CAAR when this agent was injected into various striatal structures. Behavioral aberrations were most pronounced when bicuculline was injected into the rostral NS, but these disturbances were compensated. By contrast, injection of this agent into GP produced less pronounced inhibition of conditioned skills, but it did not restore completely after termination of MI. These data confirm similar role of these structures of the neo- and paleostriatum in the regulation of motor activity, but attest to different degree and character of involvement of various pools of striatal GABAergic system in the regulation of CAAR [2].

This study does not corroborate the role of A-type GABA-receptor striatal subsystem in the genesis of hyperkineses thereby stressing the leading role of B-subtype receptors in this process. Evidently, the mechanisms of these phenomena are more complex and based on some shifts in the intertransmittory striatal interrelationships. We observed similar hyperkineses during activation of enkephalinergic opiate system of NS [3]. It can be hypothesized that the blockade of B-GABA subsystem in the rostral NS is a trigger event in succession of transmittory shifts in NS inducing hyperkineses.

REFERENCES

1. K. S. Raevskii and V. P. Georgiev, *Transmittory Amino Acids: Neuropharmacological and Neurochemical Aspects* [in Russian], Leningrad (1986).
2. T. A. Leontovich, *Ros. Fiziol. Zh.*, **83**, No. 1-2, 44-52 (1997).
3. A. F. Yakimovskii, *Fiziol. Zh. SSSR*, **77**, No. 1, 22-28 (1991).
4. A. F. Yakimovskii, *Byull. Eksp. Biol. Med.*, **114**, No. 1, 7-9 (1993).
5. A. F. Yakimovskii, *Ros. Fiziol. Zh.*, **84**, No. 9, 906-912 (1998).
6. A. F. Yakimovskii, *Byull. Eksp. Biol. Med.*, **126**, No. 10, 381-383 (1998).
7. G. J. Kilpatrick, M. S. Muhyaddin, P. J. Roberts, and G. N. Woodruff, *Br. J. Pharmacol.*, **78**, Suppl., 6 (1983).