# Verapamil Aggravates Hyperkinesia Produced by Intrastriatal Administration of Picrotoxin in Rats

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The effects of slow $Ca^{2+}$ channel blocker verapamil and $Ca^{2+}$-binding agent EDTA were studied on the model of choreic hyperkinesia induced by chronic intrastriatal microinjections $GABA_A$ receptor antagonist picrotoxin. Normal and pathological movements were recorded. The test preparations facilitated the effect of picrotoxin on spontaneous and learned behavior. They exerted a permissive effect on picrotoxin-induced hyperkinesia: increase reproducibility and duration of hyperkinesia and decrease the latency of this reaction. Our results indicate that $Ca^{2+}$ channels are involved into the development and progression of hyperkinesia.

**Key Words:** *neostriatum; γ-aminobutyric acid; verapamil; picrotoxin; hyperkinesia*

Studying of the pathogenesis of brain disorders is required to develop the methods for targeted therapy. Huntington's chorea is a hereditary disease of the brain with selective damage to subcortical structures. Etiological characteristics of this disorder were evaluated after deciphering the mechanism of gene mutation [3,7,12]. However, many stages of its pathogenesis remain unclear. One of the leading hypotheses is based on glutamatergic excitotoxicity, which causes death of GABAergic striatal neurons by increasing the concentration of free calcium ions [8,9].

The role of calcium-mediated processes in the pathogenesis of brain dysfunction was studied on the original model of picrotoxin-induced striatal hyperkinesia. We evaluated the effects of slow $Ca^{2+}$ channel blocker verapamil and $Ca^{2+}$-binding agent EDTA on signs of hyperkinesia.

## MATERIALS AND METHODS

Experiments were performed on 37 male Wistar rats (250-300 g) with conditioned avoidance response in a shuttle box. The rats were trained to leave the dark compartment of the chamber within 10 sec after the start of conditioned acoustic stimulation (7000 Hz). Electric current (1 mA) delivered though a wire floor served as the punishment. Each session included 10 presentations of conditioned and unconditioned stimuli delivered at a 20-30-sec interval. Polyethylene cannulas were implanted into the rostral neostriatum (NS) of trained animals under hexenal anesthesia (1-2 mm rostrally from the bregma, 2.0-2.5 mm laterally from the midline, 6.0-6.5 mm ventrally from the skull surface). The cannulas were filled with apyrogenic physiological saline (control group, $n$=9) or pharmacological drugs in physiological saline. The solution (1 μl) for group 1 ($n$=12), 2 ($n$=7), and 3 animals ($n$=9) contained 2 μg picrotoxin (PT, Serva), 2 μg PT and 5 μg EDTA, and 2 μg PT and 0.25 μg verapamil, respectively (procedure of administration was described elsewhere [4]). The study was performed 2-3 days after surgery. The test preparations were administered bilaterally into the rostral NS for 14-16 days. The daily volume of microinjection was 1 μl. The animals were examined 3 times a week at 1-2-day intervals. Spontaneous locomotor activity in the open field (3-min procedure) was recorded 15-20 min

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after administration of the preparation. Shuttle-box behavior was studied in the follow-up period. The location of cannula tips in brain tissue was verified morphologically after the end of the experiment. Cannula tips in all animals were located in the rostral NS. We calculated the arithmetic means, standard errors, and standard deviations. The significance of differences was estimated by Student's *t* test ($p<0.05$).

## RESULTS

The results of experiments with control and PT-treated animals were described previously [5]. Microinjections of physiological saline into NS had little effect on rat behavior. Blockade of $GABA_A$ receptors in NS with PT increased spontaneous locomotor activity, suppressed conditioned response (Fig. 1), and caused hyperkinesia (imperative movements of the limbs and head, generalized movements of the body).

Simultaneous administration of PT and EDTA into NS increased reproducibility of hyperkinesia, which reached 100% over the 1st day of microinjections and exceeded that in group 1 rats during the follow-up period (Fig. 2). Other signs of hyperkinesia did not differ in these rats and PT-treated animals. Spontaneous locomotor activity of experimental rats did not differ from that observed before the surgery. Verapamil significantly aggravated hyperkinesia. Reproducibility of hyperkinesia in these rats was higher than in group 1 animals (similarly to group 2 rats, Fig. 2). The latency of hyperkinesia in rats receiving verapamil was 5.2±3.1 min (*vs.* 9.4±4.2 min in PT-treated animals, $p=0.05$, Fig. 3). The duration of hyperkinesia in verapamil-receiving animals varied from 133 to 50 min (Fig. 3). The stage of generalization was observed in 78% rats (*vs.* 30-40% animals of the verapamil group). Realization of the conditioned response significantly decreased, but recovered by the end of microinjections (as distinct from rats of the PT group, Fig. 1). These animals did not exhibit increased spontaneous locomotor activity, which is usually observed during PT microinjections. Our findings show that verapamil increases the degree of hyperkinesia induced by PT, but facilitates the effect of this compound on spontaneous behavior and conditioned locomotor activity.

Our previous experiments showed that the glutamatergic system of NS is involved in the development of PT-induced hyperkinesia [5]. Activity of this system is realized via the increase in calcium entry through the corresponding ligand-dependent neuronal channels. Combined administration of PT

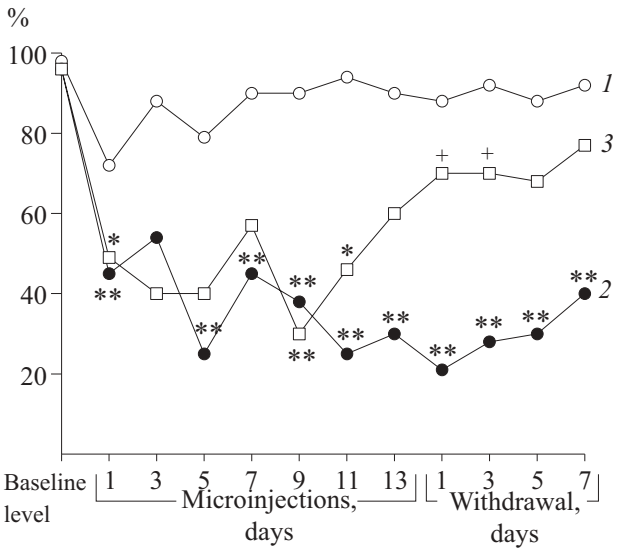


**Fig. 1.** Conditioned avoidance performance in rats in a shuttle box (mean values). Chronic administration of physiological saline (*1*), 2 μg picrotoxin (*2*), and 2 μg picrotoxin and 0.25 μg verapamil (*3*) into the rostral neostriatum. Ordinate: ratio between the number of conditioned responses and total number of presented conditioned stimuli. *$p$=0.01-0.05 and **$p$<0.01 compared to the control; $^{+}p$=0.01-0.05 compared to 2 μg picrotoxin.

and glutamate into NS increased signs of hyperkinesia. However, addition of NMDA receptor blocker dizocilpine (MK-801) to the injected solution significantly decreased the degree of hyperkinesia. It can be hypothesized that the observed effects are associated with modulation of calcium influx. Calcium influx provides physiological function of the neuron, which is realized via the maintenance of membrane charge, regulation of anionic and cationic channels (chemo- and potential-de-

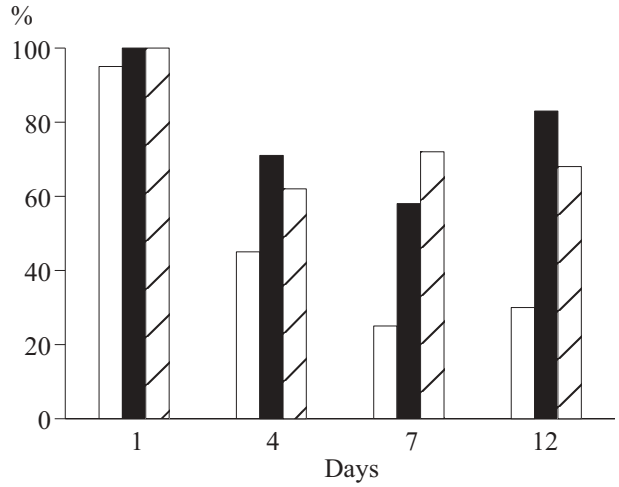


**Fig. 2.** Reproducibility of hyperkinesia during chronic intrastriatal administration of 2 μg picrotoxin alone (light bars) or in combination with 5 μg EDTA (dark bars) and 0.25 μg verapamil (shaded bars). Ordinate: reproducibility of hyperkinesia, percents of the total number of rats per group.

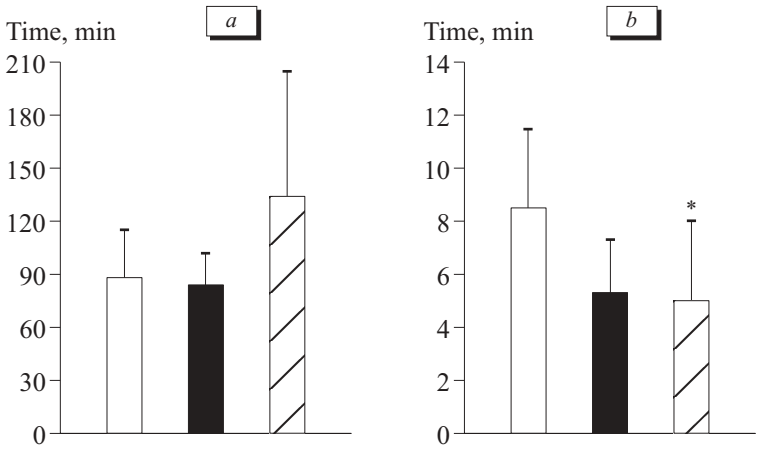


**Fig. 3.** Duration (*a*) and latency (*b*) of hyperkinesia during chronic intrastriatal administration of 2 μg picrotoxin alone (light bars) or in combination with 5 μg EDTA (dark bars) and 0.25 μg verapamil (shaded bars). **p*=0.01-0.05 compared to 2 μg picrotoxin.

pendent), and modulation of transport and enzyme systems [6,9,11,13].

It could be anticipated that addition of potential-dependent $Ca^{2+}$ channel blocker verapamil to the injected solution will decrease the degree of PT-induced hyperkinesia. Verapamil decreases calcium influx into cells by modulating function of other channels. However, verapamil increased the degree of hyperkinesia (similarly to glutamate). Probably, decreased calcium influx into cells led to activation of calcium-binding proteins that increase membrane permeability for $Ca^{2+}$ [11]. These changes result in opening of other channels and pumps that transport calcium into the neuron. Under normal conditions this mechanism plays a compensatory role. In our experiments this mechanism mediated the pathological reaction. It should be emphasized that verapamil prevents membrane hyperpolarization induced by activation of $GABA_A$ receptors. This effect is associated with blockade of calcium influx, does not depend on the influence of PT, and potentiates the effect of this compound [10].

We hypothesized that modulation of calcium-mediated processes by addition of a calcium-binding agent EDTA to the injected medium (more serious treatment compared to verapamil administration) will be important for the realization of PT-induced changes. However, we revealed only an increase in reproducibility of hyperkinesia with no variations in the latency and duration. Probably, the selection of this preparation was not adequate for our microinjection technique. Hence, EDTA did not produce the proper effect on brain tissue *in vivo*.

A balance between fractions of bound and free calcium ions originating from the extracellular space, mitochondria, and endoplasmic reticulum is important for cell function [6,9]. This balance is unstable, since free calcium ions undergo rapid transformation into the bound form under normal conditions. It can be hypothesized that PT blocks $Cl^-$ channels of $GABA_A$ receptors and impairs ion exchange and $Ca^{2+}$ homeostasis in the striatum via several intermediate (hypothetic) stages. Additional modulation of $Ca^{2+}$ channels more significantly changes the effect of PT than activation or blockade of major neuromediators in the nervous system [1,2,5].

Our results indicate that the pathogenesis of disturbances in subcortical structures includes various and even opposite changes in calcium homeostasis. These changes are difficult to detect by recording physiological parameters and studying clinical correlates. It explains apparent discrepancy in the results of similar experiments. We conclude that calcium-mediated processes play a role in the pathogenesis of choreic hyperkinesia.

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