PHYSIOLOGY

Effect of Zinc Chloride on Picrotoxin-Induced Hyperkinesis Depends on Its Concentration in Solution Injected into Rat Neostriatum

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The hyperkinekic effect (increase in spontaneous activity and development of choreomyoclonic hyperkinesis of the extremities and body) of picrotoxin injected into the rostral neostriatum of rats in a dose of 2 μg was reduced if the drug was injected together with $ZnCl_2$ in a concentration of 0.1 μg/μl. $ZnCl_2$ in a concentration of 1 μg/μl did not modulate the effects of picrotoxin, while in a concentration of 3 μg/μl it increased spontaneous motor activity in the open field test without affecting the symptoms of choreomyoclonic hyperkinesis.

Key Words: *neostriatum; picrotoxin; zinc chloride; hyperkinesis*

Disturbances in calcium homeostasis in the neostriatum (NS) constitute the basis of Huntington chorea, a hereditary degenerative disease of the brain [1,3]. Therefore, modern studies are focused on the search of effective means preventing or at least minimizing hyperactivity of calcium processes in NS neurons. On the model of picrotoxin-induced choreomyoclonic hyperkinesis (experimental symptomatic analog of Huntington chorea) we showed high efficiency of magnesium ions injected into NS before or simultaneously with picrotoxin (PT) [4,5]. Zinc is also considered as a calcium channel antagonist [6,7]. Here we present the results of the use of zinc chloride ($ZnCl_2$) injected into rat NS and evaluate its capacity to reduce hyperkinetic activity of PT.

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MATERIALS AND METHODS

Experiments were carried out on 35 male Wistar rats weighing 250 g. The rats were narcotized with hexenal and polyethylene microinjection cannulas were implanted bilaterally in the area of rostral NS (coordinates: 1.0-2.0 mm rostrally from bregma, 2.0-2.5 mm laterally from the midline, and 6.0-6.5 ventrally to the skull surface). The cannulas in control rats (n=8) were filled with physiological saline; in rats of three experimental group, the saline contained $ZnCl_2$ in different concentrations instead of $NaCl_2$ and 2 μg PT per 1 μl saline (Serva). The rats of experimental groups 1 (n=7), 2 (n=9), and 3 (n=11) received $ZnCl_2$ in concentrations of 3, 1, and 0.1 μg/μl, respectively. Additionally, previous data [3-5] obtained on 8 animals with bilateral microinjections (by the method described elsewhere [2,3]) of PT alone (2 μg/μl saline) into NS were used. The preparations were injected daily for 15 days starting from day 3 after surgery. Motor activity in the open field (OF) test was evaluated 15-20 min after microinjections: the number of crossed squares

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over 3 minwas determined. Before the surgery, the reaction to novelty in OF was quenched and two control tests were performed.

Morphological analysis verified localization of implanted cannulas in the rostral NS. Parameters of spontaneous motor activity in OF on a certain day of microinjections were compared with those in the same group before implantation of cannulas and with those in the control group on the same day of the experiment. Parameters of hyperkinesis in rats receiving PT were compared with the results of previous experiment with administration of PT alone [2,3]. The data were processed statistically using nonparametric 2-tailed exact Mann–Whitney test and ANOVA.

RESULTS

In rats of the control group, the number of crossed squares in OF throughout the period of microinjections was 11.33-26.33, which significantly ($p<0.01$) surpassed the corresponding value before surgery 1.56±0.41 (Fig. 1). Microinjections of PT into NS induced hyperkinetic syndrome in experimental rats; it has two components: choreomyoclonic hyperkinesis of the head, extremities, and body and increase in spontaneous motor activity [2,3]. Hyperkinesis manifested in imperative movements of the forepaws and head. The latency of hyperkinesis (time from microinjection to the first signs of hyperkinesis) was 9.4±4.2 min. During the next 20-30 min, the amplitude of movements increased, hyperkinesis involved both forepaws, head, and body (generalization), and then the intensity of hyperkinesis decreased. The duration of hyperkinesis was on average 88.4±27.7 min. The intensity and duration of hyperkinesis decreased during the second week of the experiment; after cessation of microinjections hyperkinesis was not observed. The increase in spontaneous motor activity was observed throughout the experiment, but was most pronounced during the first week.

The effect of zinc on parameters of PT-induced motor hyperactivity depended on $ZnCl_2$ concentration in the injected solution. In rats receiving PT and 3 μg $ZnCl_2$, the parameters of hyperkinesis did not differ from those observed in rats receiving PT alone. In rats receiving PT solution containing 1 μg $ZnCl_2$, the latency of hyperkinesis increased to 12.2±1.9 min, but its duration increased to 106.8 min, on average.

To detect the increase in spontaneous motor activity, we compared to the number of crossed squares per day in groups receiving PT with 1 or 3 μg $ZnCl_2$ (Fig. 1). Significant differences between the groups were observed only on day 3 of microinjections ($F_{2,20}=6.379$, $p=0.007$): the number of crossed squares in the control group and groups receiving PT with 1 and 3 μg

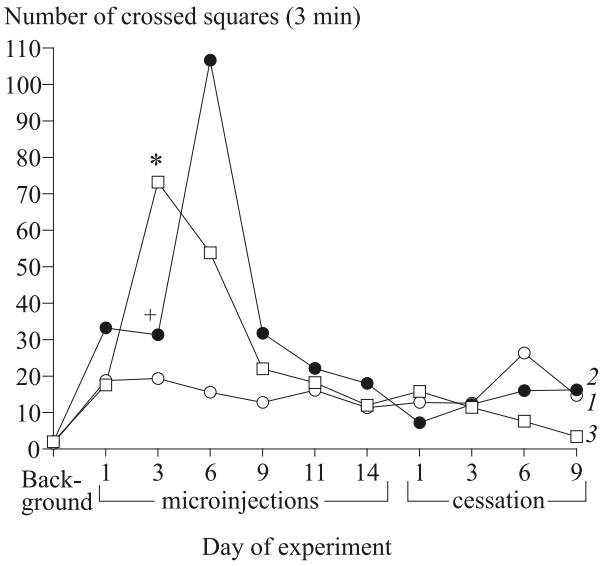

Fig. 1. Spontaneous motor activity in OF in rats receiving microinjections of physiological saline (*1*), 2 μg PT+1 μg $ZnCl_2$ (*2*), and 2 μg PT+3 μg $ZnCl_2$ (*3*). Mean values for each group are presented. Background values: before microinjections; **p*=0.006 compared to *1*, +*p*=0.033 compared to *3*.

$ZnCl_2$ was 19.3±6.6, 31.3±10.3, and 73.2±14.7, respectively. Significant differences in this parameter were observed between the control and PT+3 μg $ZnCl_2$ groups ($p=0.006$) and PT+1 μg $ZnCl_2$ and PT+3 μg $ZnCl_2$ groups ($p=0.033$). The maximum numbers of crossed squares in animals receiving PT+1 μg $ZnCl_2$ (106.7±49.0) and PT+3 μg $ZnCl_2$ (73.2±14.7) were noted on days 6 and 3 of microinjections, respectively. After that, motor activity sharply decreased (days 9-14).

Considerable inhibition of hyperkinesis was noted only in animals receiving $ZnCl_2$ in the lowest dose (0.1 μg): the latency increased to 19.0±4.5 min, the duration tended to decrease, reproducibility did not exceed 45%, and in none of the animals generalization of hyperkinesis was observed. Motor hyperactivity in OF was increased only on day 1 of microinjections and was equal to that in the control group.

Thus, zinc ions were ineffective in blocking the effect of PT in doses equal to those of $MgCl_2$, which were sufficient to prevent PT-induced syndrome [4,5]. Moreover, spontaneous motor activity in rats receiving high doses of $ZnCl_2$ even increased. Only the lowest concentration of $ZnCl_2$ was effective against choreomyoclonic hyperkinesis. It is known that zinc is taken up by glutamatergic neurons via specific transporter systems, packed in vesicles, and released into the synaptic cleft, where it modulates synaptic transmission [7]. Depending on its concentration, zinc can either activate, or block glutamate AMPA receptors relative insensitive to magnesium ions [6]. It can be hypothesized that the relationships between the neuronal

substrate of NS and magnesium ions differ from its relationships with zinc, which is seen from our results.

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