PHYSIOLOGY

Intrastrial Injection of Magnesium after Picrotoxin Does Not Reduce Motor Hyperactivity in Rats

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Magnesium chloride injected into rat rostral neostriatum before picrotoxin prevents motor hyperactivity induced by this $GABA_A$ receptor antagonist: prevents the increase in spontaneous motor activity in the open field test and reduces reproduction and duration of choreic hyperkinesia. Injection of magnesium 15 min after picrotoxin virtually did not modify the hyperkinetic effects of picrotoxin. The results indicate the role of calcium processes in the appearance of strial picrotoxin hyperactivity and principal possibility of its correction with magnesium only before the cascade of respective calcium ionic transformations is triggered in the brain.

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

Bivalent magnesium, zinc, and cobalt ions are the most effective blockers of calcium channels [6,9]. Our previous studies [1,4,5] showed that one of the mechanisms of hyperkinesia caused in rats by microinjection of picrotoxin (PT; $GABA_A$ receptor antagonist) into the neostriatum (NS) is distortion of neuronal calcium homeostasis. These data agree with the notions on the pathogenesis of Huntington's chorea [2,8] (picrotoxin hyperkinesia is an analog of this condition). Our studies showed high efficiency of microinjections of magnesium ions into NS simultaneously (in the same cannula) with PT [4,5].

We compared the antihyperkinogenic effects of $MgCl_2$ injected in rat NS before or after PT microinjection.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (n=23; 250-300 g). The animals were narcotized with hexenal and polyethylene cannulas were bilaterally implanted (two cannulas through one hole in the skull on each side) into the rostral NS area. The implantation coordinates were as follows: 1.0-2.0 mm rostrally from the bregma, 2.0-2.5 mm laterally from the median line of the skull, and 6.0-6.5 mm ventrally from its surface. In control rats, the cannulas were filled with sterile apyrogenic saline, in experimental animals one of the cannulas in each NS contained PT (Serva) dissolved in saline and the other 1 M solution of $MgCl_2$ in sterile apyrogenic bidistillate. Experimental animals were divided into 2 groups. Group 1 rats (n=8) were injected with PT in NS and after 15 min with $MgCl_2$; in experimental group 2 (n=7) $MgCl_2$ was injected first, and PT after it. As a result of one microinjection (1 μl), 2 μg PT or 1 μmol $MgCl_2$ were injected into NS. Controls (n=8) received two injections of saline at 15-min

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interval. The method of injections is described previously [3,4]. The drugs were injected daily for 14-16 days starting from day 3 after surgery. Testing was carried out 15-20 min after microinjection of the last drug. Spontaneous motor activity was tested in the open field test: the number of crossed squares and rearing episodes were counted over 3 min. The tips of the cannulas were located in the rostral NS in all animals.

The data were statistically processed using Student's *t* test; the differences were considered significant at $p<0.05$. The characteristics of spontaneous motor activity on a certain day of microinjections were compared with the results of testing of the same group of animals before implantation and with the values in control rats on the same day. Parameters of hyperkinesia were compared between two experimental groups of animals and the results of previous experiments with microinjections of PT alone [1,4,5].

RESULTS

Chronic microinjections of saline into NS caused no appreciable changes in normal motor behavior of rats; no motor disorders were detected. A clear-cut increase in motor activity was observed in all animals of experimental group 1 during week 1 of the experiment. The number of passages in the open field increased in group 1 on day 1 of the experiment from 7.7±3.9 (before microinjections) to 21.1±2.3. The number of exploration rearing episodes in 3-min testing increased from 2.3±0.8 to 7.1±0.7. These shifts in motor activity differed significantly ($p=0.05$) from the parameters in control rats. Hyperkinesia was observed in rats of this group from the first days of microinjections, its main parameters coinciding with those described previously for PT alone [4,5]. The duration of hyperkinesia on day 1 of microinjections in this group was 84.2±2.8 min *vs.* 88.4±27.7 min after microinjection of PT alone. Its first manifestations were recorded in this group on minutes 13-14 after PT injection (9.4±4.2 min after injection of PT alone), which means that hyperkinesia developed before $MgCl_2$ microinjection. It manifested by imperative movements of one of the fore paws, chewing movements, and nodding (vertical movements of the head). During subsequent 15-20 min, jerking movements became rhythmic, the amplitude and frequency of head and paw movements increased. In half of animals of this group, hyperkinesia involved both fore paws, head, and trunk (hyperkinesia generalization stage). By minutes 30-40 after PT microinjections, hyperkinesia reached the peak, after which its intensity decreased. During week 2 of the experiment, the severity and duration of hyperkinesia decreased; spontaneous hyperactivity also gradually disappeared. No hyperkinesia was observed after microinjections were discontinuated.

Hence, microinjections of $MgCl_2$ after hyperkinesia development were virtually inessential for its development, while injection of $MgCl_2$ before PT completely prevented the development of motor hyperactivity or reduced it significantly (in rats of experimental group 2). Two rats exhibited none episodes of hyperkinesia and one rat had only one episode on day 2 of the experiment (with a latent period of 15 min and duration of only 25 min). In one more rat hyperkinesia episodes were recorded twice (on days 3 and 4 of microinjections), and in the rest three animals there were recorded 3, 4, and 5 times, respectively, only during week 1 of the experiments. Importantly that hyperkinesia did not generalize in any of these rats. No increase of spontaneous motor activity, characteristic of PT microinjections, was observed in these rats: the mean number of crossed squares in this group was 2.0±1.3 ($p=0.02$ compared to experimental group 1), the number of rearing episodes was 0.4±0.7 ($p=0.05$).

Hence, hyperkinogenic effects of PT decreased significantly, if PT was injected in rat NS after magnesium ions had "worked" there. However, magnesium failed to play its "therapeutic" role, if the PT-induced cascade of ionic and membrane channel shifts was triggered [4]. We should like to emphasize that there is no effective therapy for Huntington's chorea in clinical practice. Attempts at replacing neuroleptics (reducing the intensity of hyperkinesia, but suppressing the cognitive capacity of humans) with drugs with glutamatergic mechanism of action [7] showed their inadequate efficiency. For this reason the search for factors modulating not only manifestations, but also the pathogenesis, *i.e.* drugs inhibiting and, presumably, disrupting the cascade of ionic, molecular, cellular, and neurochemical restructuring in Huntington's chorea is an important problem [4,8].

Magnesium directly blocking calcium channels reduces membrane hyperpolarization [6,9], while the increase in the number of cations in the extracellular space attenuates the effect of PT on alteration of the membrane charge. Magnesium binds calmodulin and can modify sodium flow [9]. Picrotoxin model of strial hyperkinesia proposed by us is sufficiently well studied [4] and seems to reproduce the symptoms characteristic of manifest stage of Huntington's chorea, when stimulatory/inhibitory processes in the basal ganglia are disorganized. However, even under these conditions magnesium

delivered to NS neurons can prevent the development of the chain of pathological processes leading to an increase in neuronal excitability.

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