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Effect of Activation and Blocking of the Dopaminergic System of the Neostriatum on Hyperkinesia Caused by Intrastriatal Injections of Picrotoxin in Rats

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With the aid of chronic selective blocking of the GABA-ergic system of the neostriatum by picrotoxin in rats, we succeeded in reproducing a model of choreomyoclonic hyperkinesia of the paws and the head [4]. An important role in the pathogenesis of hyperkinesias is traditionally attributed to the dopaminergic system of the brain. In this study, the influence of chronic activation and blocking of the dopaminergic system of the neostriatum on the development of the above-mentioned neuromotor dyskinesias is investigated.

MATERIALS AND METHODS

The experiments were carried out on 32 male Wistar rats weighing 250-280 g. Stereotaxically, under hexenal, two polyethylene cannulas were bilaterally implanted into the rostral neostriatum (via a single opening on each side of the skull). One of them was filled with a solution of picrotoxin (Sigma, USA), and the second one contained the preparation acting upon the dopaminergic system. The rats were divided into 6 groups (5-6 animals in each). In group 1, the effect of picrotoxin (dose of one microinjection 5 μg) was studied against the background of preliminary microinjections of physiological saline in the neostriatum, and in groups 2, 3, and 4 its effect was investigated against the background of haloperidol (Gedeon Richter, Hungary, 5 μg), metoclopramide (Germed, Germany, 5 μg), and phenamine (Russian-made, 15 μg), respectively. In group 5, the order was reversed: picrotoxin was injected first and phenamine afterwards; in group 6, both preparations were injected simultaneously. The microinjection technique was described earlier [2,3]; the volume of microinjections was 1.0 μl; the second preparation was administered 10-15 min after the first.

The experimental conditions were the same as those previously described [3,4]. The experiments were carried out over 3 weeks; microinjections were performed practically daily. At the end of the ex-

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periments the rats were killed under hexenal anesthesia, and histological verification was performed: the tips of the cannules were seen to be located in the region of the rostral neostriatum. The results were statistically analyzed using Student's test.

RESULTS

The rats of group 1 (given picrotoxin) exhibited motor disturbances against the background of physiological saline, these disturbances being the same as previously described for single microinjections of the former [4] and manifesting themselves (during the first 2 days of injections) as orofacial dyskinesia transforming into choreomyoclonic hyperkinesia of the paws and head, and of the whole body when generalized. The frequency of rhythmic twitchings was around 50 per minute. The maximum development was observed after 60-70 min, after which the intensity of hyperkinesia dropped sharply. The movements of the paws were the leading ones and resembled high-amplitude choreic hyperkinesia in man. In the 2nd week of experiments, hyperkinesia was marked during 2-3 hours, but in the 3rd week the duration of its manifestation was reduced to 40-60 min. After cessation of the microinjections, hyperkinesia was never observed in the animals.

In the group given haloperidol, hyperkinesia was full-blown (except for the stage of generalization) just in one rat, while in the rest of the animals its separate elements were mainly registered on days 1 to 3. Beginning with the 2nd week, the rats of this group exhibited no signs of hyperkinesia. Preliminary injections of metoclopramide completely prevented the development of hyperkinesias; not a single rat experienced them over the whole cycle of injections.

We had assumed that the opposite effect - activation of the dopaminergic system - might cause enhanced hyperkinesia. However, the results of the experiments in groups 3-6 proved otherwise. The form itself and the nature of the development of involuntary movements for all combinations with phenamine were exactly the same as those for the injections of picrotoxin alone: during the first 2-3 days of the experiments orofacial dyskinesias prevailed, after which the extremities, the head, and the body were involved in hyperkinesia. But whereas this was observed in all rats of group 1, about one-quarter of the animals in each of the "phenamine" groups exhibited no abnormalities. In the other rats the complete picture of hyperkinesia was observed just in the 1st week of the experiments, while beginning on the 7th-8th day only separate elements were for the most part registered, arrhythmic twitches of one of the paws being more often observed. The actual time of realization of involuntary movements was also markedly reduced. Just on days 1-3 of the experiments hyperkinesia was observed during 3-4 hours and then assumed an abortive character: it appeared 25-35 min after the microinjection and lasted for 60-80 min.

In the course of the experiments the neuropharmacological effect of picrotoxin was studied as a function of the effect of a dopamine agonist. At the same time, the experimental results provide evidence that the reverse effects also took place: blocking of the GABA receptors influenced the efficacy of phenamine. For instance, almost no stimulation of the dopaminergic system was observed in the rats of groups 5 and 6 [1]. An increased number of runs around the chamber and the episodes of exploratory stereotypy may be regarded as weak manifestations of it in 2 out of 5 rats of this group. Conversely, in all rats preliminarily injected with phenamine, a pronounced effect of the latter on the dopaminergic system of the neostriatum was registered, being similar to that observed for its single injection. Beginning 7-10 min after microinjection of phenamine, spontaneous motor activity arose in all rats of this group; an increased and even inappropriate response to orienting stimuli and general stress were observed in most of them. Involuntary gnawing and licking of the floor and objects in the chamber were observed for the maximum phenamine effect in three animals. The onset of hyperkinesia occurred 25-30 min after picrotoxin microinjection, and during this period the stereotypic gnawing and licking involuntarily decreased. Such a combination of two pathological states (behavioral stereotypy and hyperkinesia of the extremities) exhausted the rats. Two animals of this group which demonstrated the most marked effect of both preparations died on the 3rd and 5th day of the experiments.

The data obtained confirm the hypothesis of an important role of two transmitter systems of the neostriatum in the development of hyperkinesias and provide evidence of their close functional relationships. GABA release has been shown to be inhibited via the activation of the D_2 dopamine receptors in the neostriatum [7]. The influences mediated by the D_1 receptors are weaker and GABA release may be reduced during their activation [6]. Evidently, it is due to the different effects on the two types of dopamine receptors that the impact of the antagonists of the dopaminergic system used by us on the effects of picrotoxin

differed somewhat. Using the same experimental model, we earlier demonstrated the optimizing effect of metoclopramide on the conditioned response behavior, which shows its advantages over the "stringent" neuroleptic haloperidol [3]. Probably, its antihyperkinetic activity surpasses that of haloperidol: several days were necessary for the chronic effects of the neuroleptic to develop and for the hyperkinesias to vanish, whereas metoclopramide acted immediately.

An important fact revealed during the present research is the fundamental possibility of combining two forms of pathological behavior: exploratory stereotypy as a kind of general behavioral disturbance and neuromotor dyskinesias in the form of choreomyoclonic hyperkinesia of the extremities, head, and body. The former is mainly due to the activating effects of the substantia nigra on the basal ganglia, and the latter is associated with the suppression of the striatal GABA-responsive structures. At the same time, preliminary activation of the dopaminergic system of the neostriatum reduced the hyperkinesia-inducing effects of picrotoxin, the inhibition of hyperkinesia being more marked in rats given phenamine after and not before picrotoxin injections. Perhaps, for such a method of administration, when the cannula is in contact with the brain tissue, the preparation is delivered to the neostriatum not only during microinjection, but also (though in much smaller doses) between injections, due to the natural concentration gradient. Such a "tonic microeffect" on the dopamine terminals may be decisive in lowering the efficacy of picrotoxin. Furthermore, earlier practice in clinical pharmacology has shown the intensity of hyperkinesias to be reduced in a number of cases by low doses of dopaminomimetics, for example, with the aid of L-DOPA [5]. The assumption can be made that it is not simply an increased activity of the dopaminergic system per se that is of importance in the occurrence of hyperkinesias, but a certain level of its functional activity is also necessary.

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