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DEFICIENCY OF GABA-ERGIC INHIBITION DUE TO EARLY POSTNATAL CYCLOHEXIMIDE TREATMENT CORRECTED BY PYRACETAM

Yu. V. Burov, R. U. Ostrovskaya, N. M. Smol'nikova, S. S. Trofimov, and G. M. Molodavkin

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Important indications for the use of pyracetam* are disturbances of memory functions in children exposed in the antenatal or early postnatal period to toxic influences. Since we know that proteins and nucleic acids play an important role in learning processes, and that pyracetam has a favorable effect on these processes, to analyze the mechanism of action of this drug early postnatal interference with protein metabolism is particularly interesting. In young rats the period between the 6th and 8th days after birth is characterized by a rapid increase in the number of neurons and synapses in the cerebral cortex [5, 7], and by maximal rates of protein, RNA, and DNA synthesis [9, 10]. The degree of development of GABA receptors toward the end of the first week is 25% of that in adults [6]. However, the increase in activity of the GABA shunt enzymes, glutamate decarboxylase and succinic semialdehyde dehydrogenase [13], which takes place at the 6th-8th days of ontogeny, creates conditions for maturation of the GABA system.

In this investigation we studied the effect of inhibition of protein synthesis during this period on the learning capacity of adult animals and electrophysiological parameters of GABA-ergic inhibition and we analyze the effects of pyracetam on these processes.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats. From the progeny of 10 females, on reaching the age of 7 days, 30 young rats were selected and divided into three equal groups randomly. Animals of group 1 received isotonic NaCl solution from the 7th through the 14th days after birth (control). The remaining rats were given cycloheximide (CHX), an inhibitor of protein synthesis, in a dose of 0.6 mg/kg on the 7th day [12, 13], after which some of the animals received isotonic NaCl solution from the 8th through the 14th days (group 2), and the other rats received pyracetam on the same days in a dose of 200 mg/kg (group 3). All substances were injected subcutaneously. The effect of CHX and pyracetam was assessed on the basis of several tests. From the 7th through the 14th days of life and at the end of the experiments the increase in body weight was noted. The remaining experiments were conducted on grown animals, starting from the age of 3 months. The motor activity was first estimated with the "Opto-Varimex" apparatus (USA), and 7 days later the animals' behavior was studied in an open field test on five successive days. Another 14 days later the ability of the rats to learn a conditioned active avoidance reflex (CAAR) was studied in a shuttle box, the animals receiving 60 combinations daily for 3 days. The conditioned acoustic stimulus was presented for 5 sec alone, and later for 5 sec accompanied by reinforcement by painful electrical stimulation (interval between combinations 5-60 sec). The number of avoidances and the number of interstimulus responses for every 10 consecutive presentations was recorded. Another 7 days later the rats were tested by a modified conditioned passive avoidance reflex (CPAR) method in a chamber consisting of two compartments: a lit compartment, initially avoided by the rat, and a dark compartment, initially preferred, in which the rat received painful stimulation. The degree of

*A Soviet GABA analog.

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preservation of the memory trace was judged after 24 h. To assess the state of GABA-ergic inhibition in the cerebral cortex at the end an acute experiment was carried out in which the recovery cycles of the primary response of the sensomotor cortex to sciatic stimulation were recorded [2]. Paired pulses, the intervals between which varied from 80 to 900 msec, were used for stimulation. The responses were averaged in the course of the experiment on a Nokia LP 4940 analyzer, using results of application of 50 pairs of stimuli. The ratio of the amplitude of the second (testing) and the first (conditioning) responses was calculated for each interval. The experiments were conducted simultaneously on two rats, one from the control group, the other from the group of rats receiving CHX alone or CHX + pyracetam. Differences between the parameters for animals in the different groups were assessed by the Wilcoxon–Mann–Whitney nonparametric test. At the end of each physiological experiment intravenous "titration" of the threshold dose of bicuculline (expelled from an injector at a constant rate of 0.04 mg/kg/min) was carried out, during which this blocker of GABA receptors caused the appearance of seizure discharges on the EEG.

Postnatal exposure of the animals to CHX took place in summer, and the tests were carried out in the fall and winter.

EXPERIMENTAL RESULTS

Comparison of the time course of changes in body weight of the animals of all three groups showed that during the first 5 days after a single injection of CHX the rats put on weight more slowly than the control animals: animals of group 2 weighed 18.9% less than the rats of group 1. Pyracetam reduced this difference to 0.4%. Otherwise the general state of the animals of group 2 showed no significant change. By the 3rd month of life the general state and weight of the animals of all three groups were identical. The study of behavior in the open field test (Fig. 1) on the first day of the investigation on animals of group 2 revealed some increase in horizontal activity and a decrease in the investigative reaction. Comparison of a particular parameter on the first day and on subsequent days showed a decline in all parameters of activity and, in particular, of the orienting-investigative reaction, connected with loss of interest in the environment as a result of preservation of a definite memory trace of being in that particular situation. CHX reduced the degree of habituation. Pyracetam weakened the effect of CHX.

Weakening of the ability of adult rats which had received CHX in the early postnatal period to fix memory traces also was demonstrated in the experiments with CPAR.

Most (80%) animals of the control group, during testing of preservation of the memory trace of painful stimulation applied the day before did not enter the dangerous darkened compartment, or spent a much shorter time in it than before formation of the reflex. CHX increased the relative number of animals which visited the dark compartment, i.e., animals which did not remember the painful stimulation applied the day before. On entering this compartment, the animals spent just as long there as before learning (a decrease in the value of Δt, Fig. 2B). The number of animals not entering the dangerous compartment was reduced in this case (Fig. 2A). Pyracetam weakened the amnesic effect of CHX relative to both parameters.

During investigation of the speed of learning on the shuttle nox model at first sight paradoxical effect was observed: CHX increased the total number of runs, including those of conditioned-reflex nature. However, later analysis showed that CHX sharply increased the frequency of interstimulus responses. This increase in the number of purposeless runs may be the result of an increase in total motor activity, which was confirmed by recording behavior on the Opto-Varimex apparatus. Pyracetam reduced the number of interstimulus reactions during learning in a shuttle box and restored parameters of total motor activity to normal.

The recovery cycle of the primary responses in rats of the control group was characterized by a phase of inhibition of the test response with intervals of 80-125 msec between stimuli, and of the phase of its facilitation with intervals of 125-250 msec between stimuli (Fig. 3). The first phase evidently reflects activity of the intracortical GABA-ergic system of recurrent inhibition, the second phase reflects activity of the thalamo-cortical cholinergic system. CHX had a marked effect on GABA-ergic inhibitory processes in the cerebral cortex, which was expressed as weakening of depression of the test response when intervals between stimuli were 80-125 msec. Another indication of the deficiency of GABA-ergic inhibition was increased sensitivity to the action of bicuculline: the threshold dose in which it caused seizure discharges on the EEG was 0.1 mg/kg in the control animals and 0.05 mg/kg in rats receiving CHX. Pyracetam compensated these disturbances, as was shown by increased depression

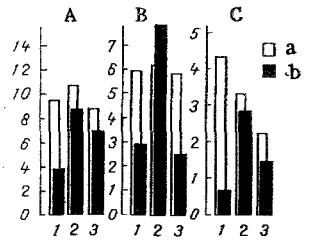

Fig. 1

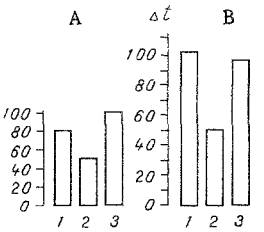

Fig. 2

Fig. 1. Time course of changes in behavior of rats in open field test for 5 days. Abscissa, groups of animals. A) Number of squares crossed (horizontal activity), B) vertical standing movements, C) number of inspections of holes (investigative activity). White and black columns denote 1st and 5th days of study of rats' behavior respectively in the open field test.

Fig. 2. Ability of rats receiving isotonic NaCl solution (1), CHX (2), or CHX + pyracetam (3) to form CPAR. A) Number of animals (in %) not entering dangerous dark compartment 24 h after learning; B) difference in time (Δt, sec) spent by rat in dark compartment before and 24 h after learning. *P < 0.01 compared with group 2.

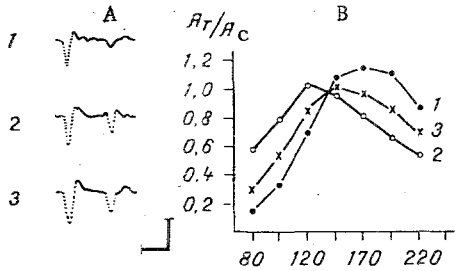

Fig. 3. Changes in recovery cycles of primary response of rat sensomotor cortex under the influence of CHX and corrective effect of pyracetam after early postnatal administration of these substances. Abscissa, intervals between stimuli (in msec); ordinate, ratios of amplitudes of testing and conditioning responses. A) Primary responses with intervals of 80 msec between stimuli: 1) Isotonic NaCl solution, 2) the same after injection of CHX, 3) the same after successive injections of CHX and pyracetam. Calibration 500 μV, 50 msec; B) recovery cycles of primary responses of rat sensomotor cortex. Legend as to Fig. 3A.

of the test response and an increase in the dose of bicuculline to 0.12 mg/kg. In some animals, the threshold dose at which pyracetam, in a single injection, increased the intensity of GABA-ergic inhibition also was determined at the end of the experiment: it was 500 mg/kg for the animals of group 1 and 200 mg/kg for those of group 2. In the presence of a deficiency of inhibition induced by CHX, sensitivity to both GABA-negative and GABA-positive factors was thus enhanced.

It follows from the data described above that when injection of CHX is used during the period of brain development that is critical for protein, nucleic acid, and GABA metabolism, CHX is active in small doses that would be ineffective in adult animals [8]. Although the inhibitory effect of CHX lasts for not more than 48 h [11], it provokes the development of late disturbances of learning and general behavior. One reason for this may be a deficiency of inhibitory GABA-ergic processes which was revealed in the cerebral cortex. Pyracetam, if injected after CHX, normalizes parameters of behavior and abolishes the deficiency of GABA-ergic inhibition. This action of pyracetam is evidently connected with the enhancement of inhibitory GABA-ergic processes in the cerebral cortex under its influence, which was demonstrated previously in experiments on intact adult animals [3]. Inhibition of the effect of CHX on behavioral responses may also be due to the ability of pyracetam, described in the literature [4] to quicken the rats of RNA and protein synthesis. Behavioral disturbances arising in the progeny after injection of CHX resemble the syndrome of delayed mental development with intellec-

tual insufficiency and with motor disinhibition observed in children [1]. The technical approach used in this investigation is adequate for the study of the mechanism of action of nootropic drugs, and data on a deficiency of inhibitory processes draw attention to the use of GABA-ergic drugs as a promising method of correcting disturbances of mental functions in children.

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