Dopamine Autoreceptors of Subtype D_3 Regulate Mainly Dopamine Release in the Basal Ganglia of Rat Brain

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By means of intracerebral microdialysis it is shown that a selective agonist of the dopamine D_3 receptors, 7-hydroxy-N,N-di-n-propyl-2-aminotetralin, causes a dose-dependent decrease of dopamine release but not its synthesis, which confirms the preeminent involvement of D_3 dopamine autoreceptors in presynaptic regulation of the release of this transmitter in the basal ganglia of rat brain.

Key Words: *microdialysis; D_3 dopamine receptors; 7-OH-DPAT*

Interest in the mechanisms of dopaminergic neurotransmission in the brain springs from the well-known role of this transmission in the execution of motor, cognitive, and neuroendocrine functions [6] as well as from its possible involvement in the pathogenesis of a number of important neuropsychic illnesses such as Parkinson's disease [6], Alzheimer's disease [7], schizophrenia, and some others [6,10].

Dopamine (DA) receptors belong to a large family of metabotropic receptors for transmitters and hormones which are functionally conjugated with G-protein, and according to their molecular properties they are subdivided into suptypes D_1 (D_1 proper and D_5) and D_2 (D_2, D_3, and D_4) [6]. Of the five known DA receptor subtypes only the D_2 and D_3 receptors are localized presynaptically, and thus they may be involved in autoreceptor regulation of DA neurotransmission, acting on the impulse activity of the DA neurons, and influencing the synthesis, release, and metabolism of DA [6,10]. The preeminent distribution of the D_3 DA receptors in the limbic structures as well as their specific molecular and pharmacological properties [6,10] make it of interest to study their functional role. The D_3 receptor is characterized by a high affinity of binding with DA, its agonists, and atypical (noncataleptogenic) neuroleptics, whereas the D_2 receptor shows a higher affinity for typical cataleptogenic neuroleptics [6,10]. Atypical and typical neuroleptics have been previously shown to have a diverse effect on the processes of release and synthesis/metabolism of DA in the dorsal striatum [1,3,4,9,14] and nucleus accumbens [14] of the rat brain. Moreover, we established a correlation between the ability of atypical and typical neuroleptics to exert a preeminent effect on DA release or its metabolism in the dorsal striatum of freely moving rats and the relative affinity of these substances for the D_3 and D_2 DA receptors, respectively [4]. These data permitted us to hypothesize that the autoreceptors of the D_2 suptype are involved in the regulation of synthesis/metabolism, while the D_3 autoreceptors modulate chiefly DA release [1,3-5]. To verify the above hypothesis a selective agonist of the D_3 receptors, 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT) [8], was used in this study.

Our goal was to study the influence of 7-OH-DPAT on DA release and synthesis in the dorsal striatum and nucleus accumbens in freely moving rats using the method of intracerebral microdialysis.

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MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 200-250 g. The animals were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally) and placed in a stereotaxic apparatus (David Kopf Instruments). A transcerebral microdialysis membrane (Hospal, type AN-69, permeability up to 15,000 D) with an internal tungsten pin was implanted in the dorsal striatum or nucleus accumbens according to coordinates AP+2, V-4 and AP+2.5, V-6 in relation to the bregma, respectively. After the operation the animals were placed in cages with food and water *ad libitum*. Twenty-four hours postoperation perfusion with Ringer's solution (composition in mM: NaCl 147, $CaCl_2$ 1.5, KCl 4, pH 6.0) was performed using a syringe pump (Braun Perfusor VI) at a rate of 2.7 μl/min. Collection of the dialysate samples was started one hour after the biginning of perfusion and performed every 20 min. When 3-4 basal samples had been obtained the substance of interest was administered intraperitoneally. The content of DA and L-3,4-dihydroxyphenylalanine (L-DOPA) was measured using high performance liquid chromatography with electrochemical detection. For DA measurement on a reverse-phase column 0.1 M phosphate-citrate buffer containing 1.1 mM sodium octane sulfonate, 0.1 mM EDTA, and 9% acetonitrile (pH 3.7) was used as the mobile phase. Recording was performed with the aid of a glass-carbon working electrode at 0.8 V. For L-DOPA determination the same column and mobile phase with a modification (2.2 mM sodium octane sulfonate, pH 3.7) were used. For the study of DA biosynthesis *in vivo* 3-hydroxybenzylhydrazine (3-HBH) was added to the perfused Ringer's solution to a concentration of 10^{-5} M as an inhibitor of decarboxylase of L-aromatic acids [15]. Under these conditions of perfusion the extracellular content of L-DOPA reached a stable value within 2 h. The baseline level in dialysates of the dorsal striatum was 191±0.7 fmol/20 min for DA and 3.2±0.648 pmol/20 min for L-DOPA and in those of the nucleus accumbens 110.4±16.9 fmol/20 min for DA and 2.087±0.351 pmol/20 min for L-DOPA. The content of DA and L-DOPA in the basal samples was taken as 100% (control). The effects of 7-OH-DPAT were estimated in relation to the basal level of DA and L-DOPA. The results of the experiments were processed statistically using Student's *t* test.

RESULTS

As shown in Fig. 1, 7-OH-DPAT caused a dose-dependent drop of the DA extracellular level both in the nucleus accumbens and in the dorsal striatum in the freely moving rats. In this case the effect in the nucleus accumbens was noted at lower 7-OH-DPAT doses, the threshold dose of agonist at which a reliable drop of the extracellular DA level was found being 0.005 mg/kg, whereas in the dorsal striatum a similar effect was obtained only at a dose of 0.05 mg/kg. Administration of 7-OH-DPAT at 0.25 and 1 mg/kg resulted in the maximal drop of the DA concentration (to 30% in relation to the control) both in dialysates of the dorsal striatum and in those of the nucleus accumbens.

Figure 2 illustrates the effect of 7-OH-DPAT on the extracellular content of L-DOPA in the nucleus accumbens and dorsal striatum in the freely moving rats. A fall in the extracellular concentration of L-DOPA, which is considered to be a reflection of the rate of DA synthesis under conditions of 3-HBH (10^{-5} M) perfusion [15], was noted at higher 7-OH-DPAT doses (as compared to the influence on DA release), no reliable difference being noted between the effects on the different brain structures. When 7-OH-DPAT was injected in a dose of 0.05 mg/kg, the extracellular concentration of L-DOPA did not change significantly either in the dorsal striatum or in the nucleus accumbens. A reliable (20%) reduction in the L-DOPA level was only noted when the agonist was administered in a dose of 0.1 mg/kg, and at just 0.25 mg/kg the L-DOPA decrease was maximal (as much as 40% in comparison with the baseline level). An increase of the 7-OH-DPAT dose to 1 mg/kg did not lead to a further drop of the extracellular level of L-DOPA in the rat brain structures.

In an additional experimental series using a quantitative microdialysis "point of no net flux" method [11] we calculated the free interstitial 7-OH-DPAT concentration in dialysates of the dorsal striatum of freely moving rats. The maximal concentration of the substance measured 20 min after its intraperitoneal administration in a dose of 6 mg/kg was 1918±239 nM. On the basis of the linear relationship found between the concentration of a substance in a brain dialysate and the dose [5] it may be assumed that the intracerebral 7-OH-DPAT concentration after administration in a dose of 0.05 mg/kg will not exceed 13 nM, which is significantly below the value of the affinity for DA binding with D_2 receptors (the corresponding values of K_i are 0.78 nM for D_3 and 61 nM for D_2 [8]). Thus, the decrease of DA release in the dorsal striatum and nucleus accumbens caused by 7-OH-DPAT in doses below 0.05 mg/kg, at which the intracerebral concentration of the substance is significantly lower than the indexes of affinity for binding with D_2 receptors, is logically to be attributed to the selective effect of the agonist

on D_3 autoreceptors. The more pronounced effect of 7-OH-DPAT on DA release in the nucleus accumbens in comparison with the striatum may be due to the preferential distribution of D_3 receptors in the limbic system [6,8,10]. These data agree with the results of a study in which the effects of DA ago-

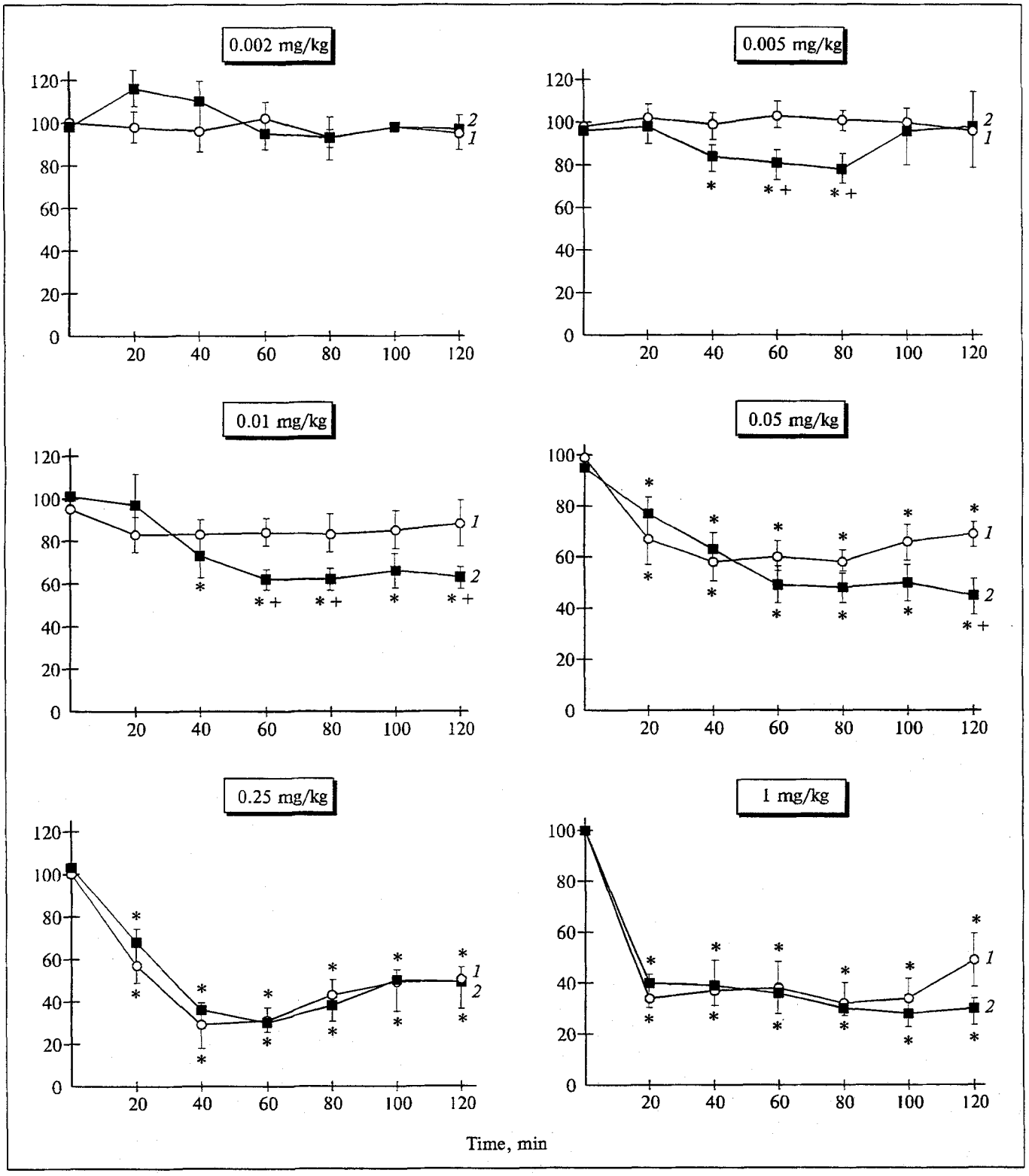

Fig. 1. Effect of different doses of the selective agonist of dopamine (DA) D_3 receptors 7-OH-DPAT on DA release in the dorsal striatum (*1*) and nucleus accumbens (*2*) in freely moving rats ($n=5$). Ordinate: DA level, % in relation to baseline. Here and in Fig. 2: $p<0.05$: *compared to control, +differences between the effects in the dorsal striatum and nucleus accumbens (Student's *t* test).

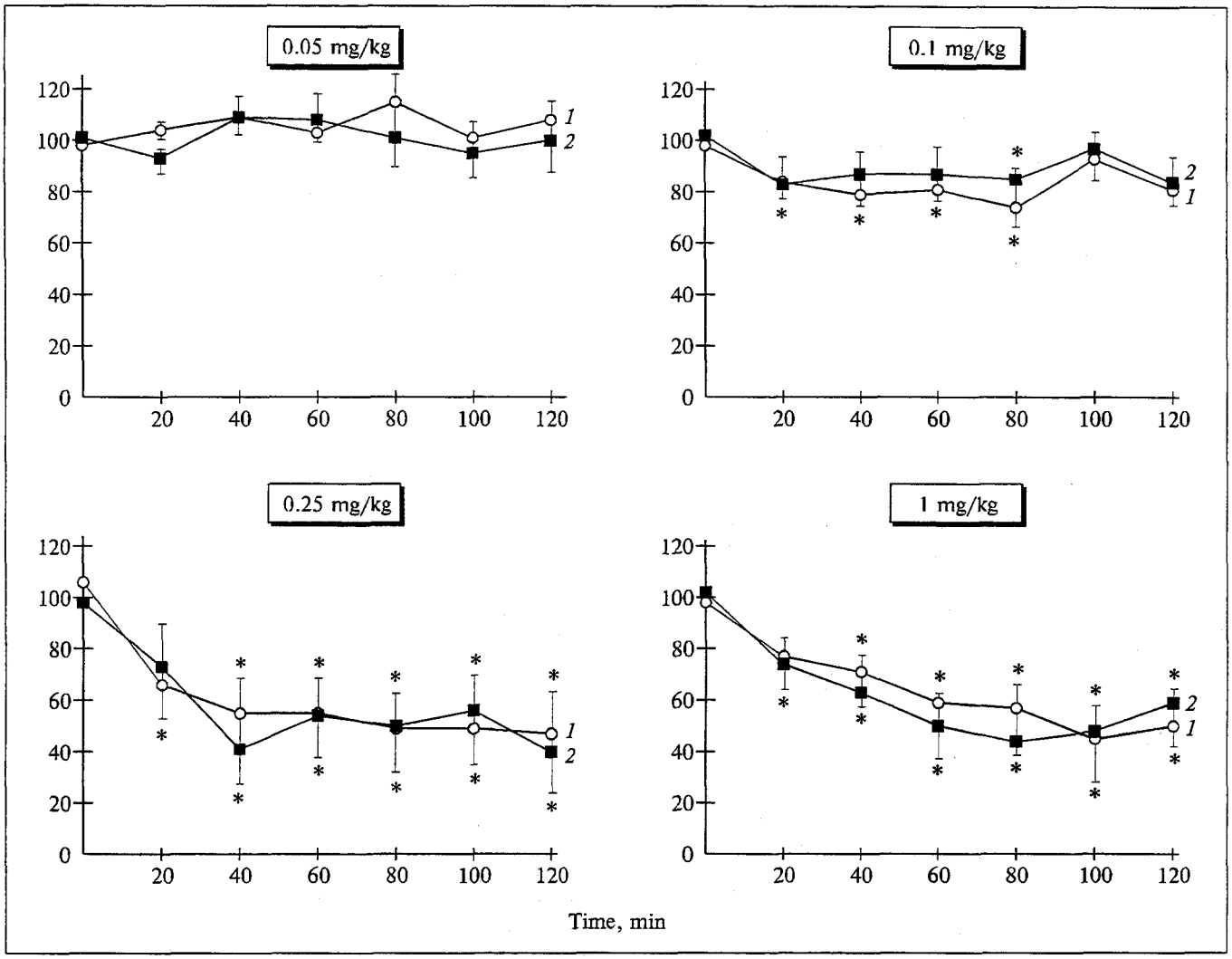

Fig. 2. Effect of different doses of the selective agonist of dopamine (DA) D_3 receptors 7-OH-DPAT on the extracellular content of L-DOPA in the dorsal striatum (*1*) and nucleus accumbens (*2*) in freely moving rats after perfusion with Ringer's solution with the addition of 10^{-5} M 3-HBH ($n=5$). Ordinate: L-DOPA level, % in relation to baseline.

nists on DA release in the basal ganglia of rat brain were studied *in vitro* [13].

It is instructive to note that beginning from a dose as low as 0.005 mg/kg, 7-OH-DPAT caused a marked inhibition of motor activity in rats, which correlated well with the decrease of DA release precisely in the nucleus accumbens but not in the dorsal striatum of rat brain (Fig. 1). These results confirm the original autoreceptor theory [2], according to which inhibition of the locomotor activity of animals induced by DA agonists is considered to be a result of a drop of the synaptic level of the transmitter in the limbic structures of the brain mediated in turn by stimulation of the autoreceptors which regulate DA release. The latter, as we previously assumed, belong to receptors of the D_3 subtype [1,3-5].

It is the D_2 autoreceptors which appear to play a key role in the presynaptic regulation of DA synthesis [1,4,5,12], since 7-OH-DPAT causes inhibition of DA synthesis in the basal ganglia of rat brain only in doses of 0.1 mg/kg and higher, at which the effect is realized via both the D_2 and D_3 autoreceptors of DA [5].

Thus, the results of this study along with the data obtained previously [1,3-5,9] confirm the role of the D_3 autoreceptors in the regulation of DA release rather then its synthesis/metabolism in the basal ganglia of rat brain.

The presumed involvement of the D_3 receptors in the pathogenesis of schizophrenia [6,10], Alzheimer's disease [7], and narcomania [6] may be associated with the key functional role of the D_3 subtype of autoreceptors in the presynaptic regulation of DA release in the basal ganglia.

REFERENCES

1. R. R. Gainetdinov, T. D. Sotnikova, T. V. Grekhova, and K. S. Raevskii, *Dokl. Rossiisk. Akad. Nauk*, **337**, No. 6, 821-823 (1994).

2. A. Carlsson, in: *Pre- and Postsynaptic Receptors*, E. Usdin *et al.*, eds., New York (1975), pp. 49-63.
3. R. R. Gainetdinov, M. B. Bogdanov, V. S. Kudrin, and K. S. Rayevsky, *Neuropharmacology*, **33**, 215-219 (1994).
4. R. R. Gainetdinov, T. V. Grekhova, T. D. Sotnikova, and K. S. Rayevsky, *Eur. J. Pharmacol.*, **261**, 327-331 (1994).
5. R. R. Gainetdinov, T. D. Sotnikova, T. V. Grekhova, and K. S. Rayevsky, *Soc. Neurosci. Abstr.*, **20**, 1355 (1994).
6. J. A. Gingrich and M. G. Caron, *Annu. Rev. Neurosci.*, **16**, 299-321 (1993).
7. E. Gurevich, M.-P. Kung, H. Kung, and J. N. Joyce, *Soc. Neurosci. Abstr.*, **20**, 1169 (1994).
8. D. Levesque, J. Diaz, C. Pilon, *et al.*, *Proc. Natl. Acad. Sci. USA*, **89**, 8155-8159 (1992).
9. K. S. Rayevsky, R. R. Gainetdinov, T. V. Grekhova, *et al.*, *Soc. Neurosci. Abstr.*, **19**, 1065 (1993).
10. P. Sokoloff, B. Giros, M.-P. Martres, *et al.*, *Nature*, **347**, 146-151 (1990).
11. L. Stahle, in: *Microdialysis in Neuroscience*, T. E. Robinson *et al.*, eds., Amsterdam (1991), pp. 155-174.
12. K. Svensson, A. Carlsson, R. M. Huff, *et al.*, *Eur. J. Pharmacol.*, **263**, 235-243 (1994).
13. S. Yamada, H. Yokoo, and S. Nishi, *Brain Res.*, **648**, 176-179 (1994).
14. N. Waters, S. Lagerkvist, L. Lofberg, *et al.*, *Eur. J. Pharmacol.*, **242**, 151-163 (1993).
15. B. H. C. Westerink, J. B. De Vries, and R. Duran, *J. Neurochem.*, **54**, 381-387 (1990).

Piracetam as a Corrector of the Delayed Learning Disabilities Caused by Prenatal Alcoholization: the Importance of Times of Treatment

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The use of piracetam from the 8th to the 20th day of postnatal development prevents disruption of the elaboration of the conditioned bilateral avoidance reflex in the shuttle box in mature male offspring of rats which had received ethanol intragastrally from the 1st to the 20th day of gestation. When used for 13 days one month prior to conditioning, piracetam acts with less efficiency on mature animals, while the same duration of treatment just before conditioning does not affect the impairment of conditioned reflex elaboration.

Key Words: *alcoholization; active avoidance; correction; piracetam; ontogenesis*

Alcohol intake during pregnancy causes various disorders in the offspring, including intellectual disturbances which require treatment [2,8,13]. Among the pharmacological agents for the correction of mental disorders of different genesis in children, nootropics, and in particular piracetam, are widely used [11]. However, usually treatment is started only when there are already manifestations of developed pathology and so it does not always have a positive outcome. Preventive therapy would be more effective [3,7]. In experiments on animals the possibility of correcting cognitive disorders which are the delayed result of prenatal alcoholization was demonstrated with the aid of substances with nootropic activity administered during the early postnatal period [9, 10,12]. The goal of the present study was to compare the efficacy of nootropic agents used in the early postnatal period and in mature animals for the correction of learning and memory deficiencies induced by *in utero* alcoholization. The original

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