Effect of Nooglutil on Benzodiazepine Withdrawal Syndrome and Binding of ^{3}H-Spiperone with D_2 Receptors in Rat Striatum

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A new nootropic preparation nooglutil (N-(5-oxynicotinoyl)-L-glutamic acid), a positive modulator of AMPA receptors for glutamate, administered intraperitoneally in a dose of 70 mg/kg reduced anxiety of rats in the Vogel conflict test after 24-h withdrawal from chronic diazepam treatment (4 mg/kg intraperitoneally for 45 days). Nooglutil (5 nM-750 μM) had no effect on *in vitro* binding of ^{3}H-spiperone in intact rats. Systemic administration of 50 mg/kg nooglutil *in vivo* increased the dissociation constant and density of D_2 receptors. Increasing the dose to 100 mg/kg abolished this effect. Our findings suggest that nooglutil produces an indirect effect on the brain dopaminergic system under normal and pathological conditions and this effect is probably mediated via the glutamatergic system.

Key Words: *nooglutil; diazepam; withdrawal syndrome; D_2 receptors*

The dopaminergic system plays an important role in the development of dependence on narcotic analgesics, depressants, psychostimulators, and alcohol [1,11,14]. Withdrawal after chronic treatment with benzodiazepine tranquilizers leads to behavioral changes associated with dysfunction of the dopaminergic system [11,14].

A new nootropic preparation nooglutil (N-(5-oxynicotinoyl)-L-glutamic acid) exhibits pronounced antiamnesic and antihypoxic activities [2,15]. The effects of nooglutil are determined by its interaction with the glutamatergic system and positive modulation of receptors for (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA receptors) [2,4]. The involvement of the dopaminergic system into the mechanisms of the corrective effect of nooglutil after prenatal alcoholization was experimentally proved [3, 8]. Published data show that D_2 receptors are involved in the development of drug dependence [11].

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Here we studied the effect of nooglutil on behavioral characteristics of rats during diazepam withdrawal. The effect of nooglutil on the dopaminergic system in intact animals was evaluated at the level of D_2 receptors. Presynaptic localization of these receptors determines their autoreceptor regulatory functions [7].

MATERIALS AND METHODS

Experiments were performed on male outbred rats weighing 200-300 g. The rats were kept in a vivarium and had free access to water and food.

For evaluation of behavioral manifestations of withdrawal syndrome, diazepam (4 mg/kg) suspended in Tween 80 was injected intraperitoneally for 45 days. Control animals received 0.9% NaCl in Tween 80. Nooglutil suspended in Tween 80 was injected intraperitoneally in a dose of 70 mg/kg 24 h after diazepam withdrawal. The animals were tested 40 min after treatment. The degree of anxiety was evaluated in the Vogel conflict test with some modifications [5]. The number of punished responses was determined.

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In experiments with systemic administration, nooglutil in Tween 80 was injected intraperitoneally in doses of 50 and 100 mg/kg 30 min before decapitation. Control animals received an equivalent volume of distilled water with Tween 80.

Binding capacity of D_2 receptors in membrane preparations isolated from rat striatum was evaluated by a modified radioreceptor assay [6]. The reaction mixture (300 µl) contained 30 µl labeled ligand, 90 µl suspension of membrane proteins (final concentrations of 0.8-1.0 mg/ml), and 30 µl nooglutil (5 nM-750 µM, *in vivo* experiments). Haloperidol (10 µM, evaluation of nonspecific binding) and ^{3}H-spiperone (0.1-2.0 nM, 23 Ci/mmol, Amersham) served as D_2 receptor ligands.

The reaction was stopped by rapid filtration of samples through GF/F fiberglass filters (Whatman) on an Automash 2000 device (Dynatech). The samples were washed with 1 ml 50 mM Tris-HCl buffer (4°C, pH 7.7). Filters were dried on air and placed in scintillation vials with 7 ml standard toluene scintillator. Radioactivity was measured on a RackBeta scintillation spectrometer (LKB) with an efficiency of not less than 30%.

Primary data were processed using Ligand software. Binding capacity of receptors was evaluated by the dissociation constant (K_d), a parameter inversely related to receptor affinity for the ligand, and the number of binding sites (B_{max}). Protein content in membrane suspensions was measured by the method of Lowry after treatment with sodium deoxycholate.

The results were analyzed by Mann—Whitney *U* test (Statistica software).

RESULTS

Diazepam withdrawal increased animal anxiety in conflict situation and reduced punished responding compared to the control (16.8 and 25.6, respectively, $p<0.05$). Nooglutil increased the number of punished responses to 36 ($p<0.05$ compared to animals with withdrawal syndrome). Hence, the test preparation reduced anxiety in animals with diazepam abstinence.

In intact rats nooglutil had no effect on *in vitro* binding of ^{3}H-spiperone to D_2 receptors. The preparation slightly increased specific binding compared to the control, but this effect was unstable.

Nooglutil in a dose of 50 mg/kg increased K_d and the number of ^{3}H-spiperone binding sites by 2 times compared to the control ($p<0.05$, Table 1). Increasing the dose of nooglutil to 100 mg/kg practically abolished this effect (we observed only minor and insignificant changes in K_d and B_{max}).

Thus, nooglutil reduces anxiety in rats with diazepam withdrawal. It should be emphasized that nooglutil does not change the number of punished responses in intact animals. Therefore, nooglutil selectively modulates anxiety associated with benzodiazepine withdrawal.

Preparations with nootropic activity are effective in various diseases of the central nervous system, including functional disturbances in the dopaminergic system [2,3,8,15]. Our results on the selective antianxiety effect of nooglutil in rats with diazepam abstinence are consistent with published data.

Nooglutil has no effect on *in vitro* binding of labeled ligand to D_2 receptors, which suggests that this preparation does not directly interact with this receptor subtype. However, nooglutil *in vivo* increases the number of D_2 receptors and decreases their affinity for the ligand. These data suggest that nooglutil indirectly modulates functional activity of the dopaminergic system.

Previous studies demonstrated the interaction between the dopaminergic system and the system of excitatory amino acids [9,12,13]. It was hypothesized that the key role in the interaction between glutamatergic and dopaminergic systems is played by D_2 receptors localized on the presynaptic membrane of corticostriatal neurons [9]. Three ionotropic subtypes of glutamate receptors are present as heteroreceptors on terminals of dopaminergic nigrostriatal neurons [13]. Administration of glutamatergic substances into the striatum and substantia nigra stimulates dopamine release in the striatum. AMPA and kainate are most effective in this respect [12]. Nooglutil possesses pronounced AMPA-potentiating activity. This preparation in a concentration of 4 µM promotes an increase in the maximum amplitude of the excitatory postsynaptic potential in dentate gyrus neurons in response to electrical stimulation of the perforant pathway [4]. The involvement of AMPA receptors in the mechanism of

TABLE 1. Binding Parameters of D_2 Receptors in Rat Striatum (*M*±*s*)

Parameter	Control	Nooglutil, mg/kg	
		50	100
K_d, nM	0.397±0.030	0.835±0.170*	0.45±0.05
B_{max}, fmol/mg protein	364.4±69.6	643.8±73.3*	292.6±92.6

Note. *p<0.05 compared to the control.

antiamnesic action of nooglutil was demonstrated on the model of amnesia using specific antagonist of AMPA receptors glutamic acid diethyl ester in the conditioned passive avoidance paradigm [2]. In our experiments systemic treatment with nooglutil in doses producing the AMPA-potentiating effect also modulated D_2 receptors. These changes are probably related to the indirect effect of nooglutil on D_2 receptors associated with positive modulation of the glutamatergic system.

Indirect modulation of the dopaminergic system with another nootropic preparation aniracetam was previously reported [10]. It was hypothesized that stimulation of dopaminergic transmission in the mesocorticolimbic pathway after systemic administration of aniracetam results from activation of nicotinic cholinergic neurons in the ventral segmental region.

The ability of nooglutil to modulate binding capacity of D_2 receptors is of considerable importance, because these receptors play a role in the development of benzodiazepine dependence [11] and regulate dopaminergic and glutamatergic systems [7,9,13].

REFERENCES

1. I. P. Anokhina, A. G. Veretinskaya, G. N. Vasil'eva, and I. V. Ovchinnikov, *Fiziol. Chel*., **26**, No. 6, 74-81 (2000).
2. T. A. Voronina, T. L. Garibova, I. V. Khromova, *et al*., *Medicines for Humans* [in Russian], Moscow (1997), Vol. IV, pp. 135-148.
3. G. I. Kovalev, E. A. Budygin, R. R. Gainetdinov, *et al*., *Byull. Eksp. Biol. Med*., **116**, No. 7, 56-58 (1993).
4. I. V. Komissarov, *Synaptic Ionotropic Receptors and Cognitive Activity* [in Russian], Donetsk (2001).
5. G. M. Molodavkin and T. A. Voronina, *Eksp. Klin. Farmakol*., **58**, No. 2, 54-56 (1995).
6. T. V. Proskuryakova, O. B. Petrichenko, N. V. Pankratova, *et al*., *Neirokhimiya*, **17**, No. 2, 115-122 (2000).
7. K. S. Raevskii, *Vestn. Ros. Akad. Med. Nauk*, No. 8, 19-24 (1998).
8. S. S. Trofimov, R. U. Ostrovskaya, N. M. Smol'nikova, *et al*., *Eksp. Klin. Farmakol*., **55**, No. 1, 18-21 (1992).
9. L. Kerkerian and A. Nieoullon, *Exp. Brain Res*., **69**, 424-430 (1988).
10. K. Nakamura, M. Shirane, and N. Koshikawa, *Brain Res*., **897**, Nos. 1-2, 82-92 (2001).
11. C. Nath, R. C. Saxena, and M. B. Gupta, *Clin. Exp. Pharmacol. Physiol*., **27**, No. 3, 167-171 (2000).
12. A. Svensson, A. Carlsson, and M. L. Carlsson, *J. Neural. Transm*., **90**, 199-217 (1992).
13. F. I. Tarazi and R. J. Baldessarini, *J. Neurosci. Res*., **55**, No. 4, 401-410 (1999).
14. J. W. van der Laan, L. Eigeman, and C. J. van't Land, *Arch. Int. Pharmacodyn. Ther*, **326**, 13-21 (1993).
15. T. A. Voronina, *Sov. Med. Rev. Sect. G. Neuropharmacology. London*, **2**, 51-106 (1992).