Neuromodulatory Mechanism Underlying the Effect of the Atypical Dipeptide Neuroleptic Dilept

M. V. Retyunskaya, L. S. Guzevatykh, N. A. Bondarenko, T. A. Gudasheva, R. U. Ostrovskaya, and T. A. Voronina

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We studied the effects of a new dipeptide neuroleptic Dilept (N-caproyl-L-prolyl-L-tyrosine methyl ester) on activity of neurotransmitter systems in the brain. Dilept possessed antidopamine, glutamate modulatory, and cholinomimetic properties. These data indicate that Dilept is of potential efficacy in relieving positive and negative symptoms of schizophrenia.

Key Words: *Dilept; dipeptide; neuroleptics; neurotransmitters; schizophrenia*

The therapy of mental disorders accompanying schizophrenia is an urgent problem of psychiatry. Classic neuroleptics used in clinical practice are efficient in eliminating productive symptoms of the disease. Repeated treatment with these preparations is followed by the appearance of extrapyramidal symptoms and aggravates negative symptoms of the disease. Atypical neuroleptics cause less pronounced side effects. However, their antipsychotic activity is often in sufficient. Schizophrenia is accompanied by hyperreactivity of the dopaminergic (DAergic) system in limbic structures of the brain [4] and dysfunction of central neurotransmitter systems. For examples, glutamatergic dysfunction develops during schizophrenia [8]. In some patients with schizophrenia hyperactivation of the glutamatergic system in various structures of the hippocampus and cortex is accompanied by progressive death of neurons [6]. An imbalance in glutamatergic neurotransmission is a major cause of cognitive disorders during schizophrenia. The appearance of psychotic symptoms is followed by activation of serotoninergic transmission. The antipsychotic effect of most atypical neuroleptics is determined by antidopamine and antiserotonin properties [11]. Recent studies showed that deficiency in cholinergic neurotransmission in the limbicofrontal brain system [5] and, particularly, in nicotinic cholinoceptors [10] plays an important role in the development of negative symptoms of schizophrenia.

The peptidergic concept describing the action of neurotropic preparations and developed by T. A. Gudasheva allowed synthesizing peptide analogues of β-turn structure of the active site of neurotensin (neuropeptide with antipsychotic activity) and atypical neuroleptic sulpiride [9]. Further experiments were performed with N-caproyl-L-prolyl-L-tyrosine methyl ester (Dilept), which possesses pronounced activity. Dilept does not cause side effects typical of most antipsychotic drugs (*e.g.*, catalepsy, sedation, and muscle relaxation). Dilept is a promising atypical neuroleptic not producing extrapyramidal disorders [1].

The neuromodulatory mechanism underlying influence of Dilept was studied using analytic compounds for major neurotransmitter systems (DA-, glutamate-, serotonin-, and cholinergic) involved in the development of psychopathological disturbances during schizophrenia.

MATERIALS AND METHODS

Experiments were performed on male C57BL/6 mice and male outbred albino rats weighing 20-22 and 220-250 g, respectively. The animals were kept in a vivarium under standard conditions and had free access to water and food. Previous studied showed that Dilept

Institute of Pharmacology, Russian Academy of Medical Sciences. ***Address for correspondence:*** rita_o@postman.ru. Ostrovskaya R. U.

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in a dose of 0.8 mg/kg displays maximum activity [1]. A freshly prepared suspension of Dilept and Tween 80 was injected intraperitoneally.

Sensitivity of the DAergic system to Dilept was studied in tests of apomorphine-induced verticalization and haloperidol-induced catalepsy. To evaluate individual emotionality the mice were tested for the reaction to novelty. We recorded the duration of freezing behavior in a metal-grid chamber (35×35 cm). By freezing behavior to novelty, the animals were divided into 2 groups. Group 1 included the animals with a first-movement latency of not more than 1 sec (highly reactive mice, HRM). The animals whose freezing behavior lasted more than 1 sec comprised group 2 (low reactive mice, LRM).

Antidopamine activity of Dilept in HRM and LRM was studied in the test of apomorphine-induced verticalization. The animals received Dilept or solvent in an equivalent volume (physiological saline and Tween 80) 15 min before apomorphine administration. Apomorphine in a dose of 2 mg/kg was injected subcutaneously immediately before placing the animal into cylindrical wire chambers (diameter 13 cm, height 16 cm). Recording of vertical activity started 10 min after apomorphine administration and continued for 1 h at 2-min intervals. The degree of verticalization was determined by a 4-point scale (number of limbs put on the vertical wall) [2]. The total score of verticalization was estimated for each animal over the period of observations.

Haloperidol (Gedeon Richter) in a dose of 1 mg/kg was injected intraperitoneally to produce catalepsy. The degree of catalepsy (ability to maintain posture) was determined in the staircase test by a 4-point scale 30, 60, 90, and 120 min after haloperidol administration. We evaluated the ability of mice to hold 1 or 2 forelimbs on steps (2.5 and/or 5.0 cm in height) for 10 sec.

For evaluation of the possible effects of Dilept on the glutamatergic system we used two types of pharmacological compounds: noncompetitive NMDA receptor blocker ketamine and glutamate decarboxylase inhibitor thiosemicarbazide inducing accumulation of neuronal glutamate. The ability of Dilept to improve learning impaired by ketamine was determined in the test of conditioned passive avoidance response (CPAR). Experiments were performed in a 2-compartment device (Lafayette Instrument Co.) consisting of a dark chamber with an electrode floor and a platform fixed at a height of 1 m and illuminated with a 60-W lamp. The rat was placed on a platform and the latency of the first entry into the compartment with electrode floor was recorded. Unavoidable electroshock (5 electrical stimuli, 0.45 mA, 1 sec) was delivered after 3-min exploration of the chamber. CPAR performance was evaluated after 24 h. The latency of transition into the dark compartment was recorded (testing). Passive avoidance of performance was assessed by the difference between latencies of the first entry into the dark compartment during testing and learning (DLP). Ketamine in a dose of 30 mg/kg was injected intraperitoneally 5 min before learning. The test compound or solvent in an equivalent volume was administered 15 min before ketamine injection. The solvent was given 2 times to animals of the passive control group.

In mice receiving Dilept or solvent the severity (4-point scale) and latency of seizures produced by thiosemicarbazide (7.5 mg/kg subcutaneously) and animal death were evaluated. Thiosemicarbazide not only increases glutamate content, but also reduced γ-aminobutyric acid (GABA) concentration in the brain. We studied the effect of Dilept on the latency of seizure and death produced by the $GABA_A$ receptor blocker bicuculline (3 mg/kg). The animals received Dilept 15 min before bicuculline administration.

The effect of Dilept on the serotoninergic system was evaluated by modulation of the head-twitch response produced by serotonin precursor 5-hydroxytryptophan (5-HTP). Dilept was injected intraperitoneally 15 min before 5-HTP (150 mg/kg).

The effect of Dilept on the central muscarinic cholinergic system was determined in mice with tremor produced by arecoline (25 mg/kg subcutaneously). The mean duration of tremor was estimated (sec). The effect of Dilept on the central nicotinic cholinergic system was determined by modulation of seizures produced by nicotinic receptor agonist nicotine hydrotartrate (25 mg/kg subcutaneously). The severity of tremor and clonic seizures was evaluated using a 5-point scale. We summarized points that were recorded over 20-min observations. Dilept or solvent in an equivalent volume was administered 15 min before treatment with arecoline or nicotine.

The results were analyzed by Mann—Whitney *U* test (Statgraf software). The differences were significant at $p<0.05$.

RESULTS

Preliminary study showed that the degree of apomorphine-induced verticalization in C57BL/6 mice varies from 26 to 70 points. We hypothesized that the sensitivity to this dopaminomimetic depends on the initial emotional reactivity of animals. The mice were divided into groups depending on the reaction to novelty. The degree of apomorphine-induced verticalization in LRM was higher than in HRM. In these animals the mean degree of verticalization was 69.71±4.86 and 34.67±6.45 points, respectively. Study of sensitivity of the DAergic system to the DA receptor antagonist

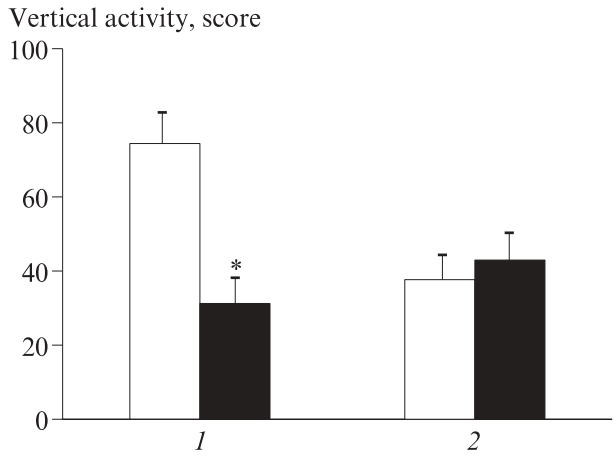

Fig. 1. Effect of Dilept on apomorphine-induced verticalization in low reactive (*1*) and highly reactive mice (*2*). Light bars: apomorphine hydrochloride (control). Dark bars: Dilept and apomorphine hydrochloride. *$p<0.05$ compared to the control.

haloperidol showed that the severity of catalepsy in LRM is higher than in HRM. The mean degree of catalepsy in HRM and LRM was 2.50±0.25 and 3.69±0.16 points, respectively, 90 min after treatment. These data suggest that in LRM the sensitivity of the DAergic system to DA receptor agonists and antagonists is higher than in HRM.

The effect of Dilept on the DAergic system was studied in the test of apomorphine-induced verticalization. Dilept decreased the degree of verticalization more pronounced in LRM, but had no effect on HRM with low initial verticalization (Fig. 1). In some HRM the degree of verticalization slightly increased after Dilept administration.

In the CPAR test ketamine produced an amnestic effect, which manifested in shortening of DLP compared to control animals (28.7±7.9 and 117±13 sec, respectively). Dilept decreased the severity of amnesia and increased DLP to 62.7±12.7 sec (Fig. 2).

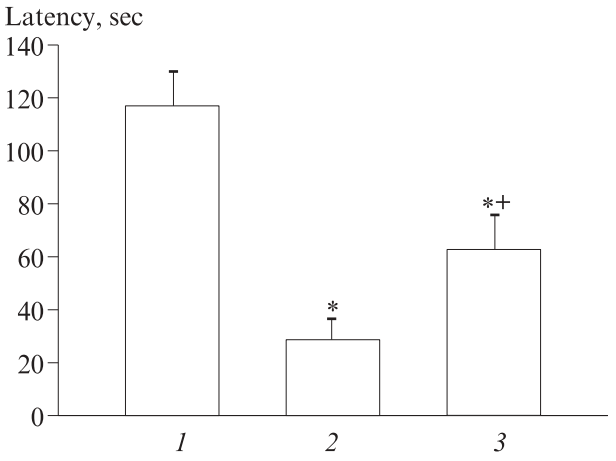

Fig. 2. Effect of Dilept on the impairment of learning in the test of conditioned passive avoidance response produced by ketamine: control (*1*), ketamine (*2*), and Dilept and ketamine (*3*). $p<0.05$: *compared to the control group; +compared to active control animals receiving ketamine.

Dilept markedly relieved thiosemicarbazide-induced seizures, decreased mortality rate (56.2 *vs*. 93.7% in the control), and delayed the time of the animal death (164.9±19.6 *vs.* 114.6±7.8 min in the control). The severity of bicuculline-induced seizures remained practically unchanged after treatment with Dilept.

The study of the effect of Dilept on head-twitch response induced by 5-HTP showed that this preparation has no effect on hyperkinesia. It can be hypothesized that Dilept does not modulate activity of the serotoninergic system.

The mean duration of tremor produced by the muscarinic receptor agonist arecoline increased by 48.8% in animals receiving Dilept ($p<0.05$). Dilept increased the degree of nicotine-induced tremor by 37% compared to the control ($p<0.05$).

Our results indicate that a new promising dipeptide neuroleptic Dilept modulates the state of various neurotransmitter systems. D_2 receptor blockade causes side extrapyramidal effects [12]. We found that Dilept possesses moderate antidopamine activity. Moreover, this substance produce no cataleptic and myorelaxing effects [1]. These data suggest that Dilept would produce a mild antipsychotic effect not accompanied by the development of extrapyramidal disorders. Our previous experiments showed that atypical neuroleptic sulpiride displays similar activity in the test of apomorphine-induced verticalization. However, this effect was observed after treatment with sulpiride in doses 20-fold exceeding those of Dilept. Moreover, administration of sulpiride in doses slightly surpassing the effective concentration caused catalepsy.

Glutamate agonistic properties of Dilept manifesting in antagonism to the effect of noncompetitive NMDA receptor blocker ketamine suggest its potential efficacy in relieving negative symptoms of schizophrenia. Dilept had antiglutamate activity in the test of thiosemicarbazide-induced seizures, which is consistent with the ability of this preparation to reduce the severity of neurocytotoxic damage to cultured cerebellar cells produced by glutamate in high concentrations. Glutamate modulatory properties of Dilept are of considerable importance, since disturbances in glutamatergic transmission play a role in the pathogenesis of schizophrenia. Hypoactivity of this system contributes to the development of cognitive deficit [6], while hyperactivity is accompanied by neurotoxic changes [8].

The cholinergic system of the hippocampus and cortex plays an important role in memory. The number of muscarinic [5] and nicotinic receptors [10] in these brain structures decreases during schizophrenia. The

positive effect of nicotine on cognitive function is related to modulation of DAergic systems in the cortex and hippocampus [7,10]. Neuroleptics possess cholinolytic activity [3], which aggravates the symptoms of cognitive deficit. Dilept acts as the agonist of muscarinic and nicotinic cholinoceptors and, therefore, is of potential efficacy in relieving negative symptoms of schizophrenia.

Thus, we analyzed the effect of Dilept on various neurotransmitter systems and found that this substance possesses antidopamine, glutamate modulatory, and cholinomimetic properties. Modern notions about the multifactor nature of neuromodulatory disturbances during schizophrenia suggest that Dilept is a promising atypical neuroleptic. The efficiency of Dilept in relieving positive symptoms of schizophrenia is probably associated with its antidopamine activity. Cholinomimetic and dopamine agonistic properties of Dilept determine its efficiency in eliminating negative symptoms of schizophrenia. The decrease in neurotoxic activity of excess glutamate contributes to the neuroprotective effect of Dilept.

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