Cholinopositive Effect of Dilept (Neurotensin Peptidomimetic) as the Basis of Its Mnemotropic Effect

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Dilept eliminated learning disturbances in the extrapolation avoidance test, caused by chronic injections of scopolamine alone and in combination with madopar to rats. Dilept improved the dynamics of training and parameters of spatial memory impaired by olfactory bulbectomy (operation causing hypofunction of the central cholinergic system). The detected cholinepositive effect of dilept together with pronounced dopaminergic activity necessitate its further development as a drug effective in positive and negative symptoms of schizophrenia and psychotic manifestations of Alzheimer's disease.

Key Words: *dilept; neurotensin; dipeptides; schizophrenia; Alzheimer's disease*

Persuasive evidence on the role of neurotensin as an endogenous neuroleptic and low enzymatic resistance and poor bioavailability of this tridecapeptide for the brain necessitates active search for its agonists effective in systemic treatment. An original approach to the search for highly active oligopeptides, developed at Institute of Pharmacology, consists in creation of minor molecules of di- and tripeptides characterized by better biological stability and higher bioavailability for the brain. Pro-Tyr dipeptide was taken for the base for creation of active neurotensin-like neuroleptics, because this sequence represents the "head" of β-turn of the neurotensin active fragment (NT_{8-13}) and is structurally similar to sulpyride, an atypical neuroleptic [11]. Caproyl-prolyl-tyrosine methyl ether (dilept), a tripeptide analog of neurotensin, was selected from the series of acyl-prolyl-tyrosines for detailed studies; this compound is effective after systemic treatment and its activity is appreciably higher than that of sulpyride. It is noteworthy that even in doses surpassing the doses of neuroleptic 250-500 times this agent exhibits no cataleptogenic, myorelaxant, and sedative effects, which suggests that the drug has no extrapyramidal side effects.

Deficiency of the central cholinergic (CE) system is the main pathogenetic component in the development of cognitive disorders in Alzheimer's disease [14] and within the framework of the negative symptom complex of schizophrenia [9]. Neuroleptics used for the treatment of psychopathological symptoms often produce central cholinergic receptor blocking effects, which leads to further progress of cognitive disorders. It was previously shown that in contrast to other known neuroleptics dilept, along with the antipsychotic effect, exhibited a positive mnemotropic effect [7]. Analysis of the mechanisms of mnemotropic activity of dilept showed its capacity to attenuate the amnestic effect of CE receptor blockers. The study of the effects of this drug on the model of chronic CE deficiency will be a new step in these investigations carried out under conditions of acute blockade of cholinoreceptors.

We analyzed activity of dilept on a model of chronic CE deficiency induced by prolonged treatment

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with scopolamine (central muscarinic receptor blocker) [5,6] or by olfactory bulbectomy (BE) [3,13].

MATERIALS AND METHODS

Experiments with chronic blockade of CE receptors were carried out on male Wistar rats (250-280 g) kept under standard vivarium conditions with natural illumination and free access to water and food. The deficiency of the central CE system was induced by long treatment with muscarinic receptor antagonist scopolamine [5,6].

Experimental animals were randomly divided into 3 groups, 18 per group (Fig. 1). During 20 days the animals were daily intraperitoneally injected with: saline in equivalent volume (group 1) or scopolamine in a dose of 1 mg/kg (groups 2 and 3). On days 21-29 rats from groups 1 (passive control) and 2 (active control) were injected with saline, while group 3 animals were injected with dilept (0.8 mg/kg, fresh suspension in Twin-80).

On day 30 behavioral effects of CE deficiency were studied in the extrapolation avoidance test (EAT) and the efficiency of correction of the developing disorders with dilept was evaluated. This form of behavior characterizes exploratory activity, when the animals have to find heretofore unknown variants of adaptive behavior (diving) [4]. The device consisted of a vessel (35 cm in diameter, 40 cm high) filled with 22°C water (17.5 cm deep). A glass cylinder (9 cm in diameter, 22 cm high) was fixed in the center of the cylinder, its lower end was plunged into water to a depth of 2.5 cm. The rat was placed into the cylinder with its tail down and its behavior was monitored for 2 min. The capacity of L-DOPA (100 mg/kg, madopar; a combination of L-DOPA and benzeraside, aromatic amino acid peripheral decarboxylase inhibitor) to prevent the realization of avoidance was demonstrated previously;

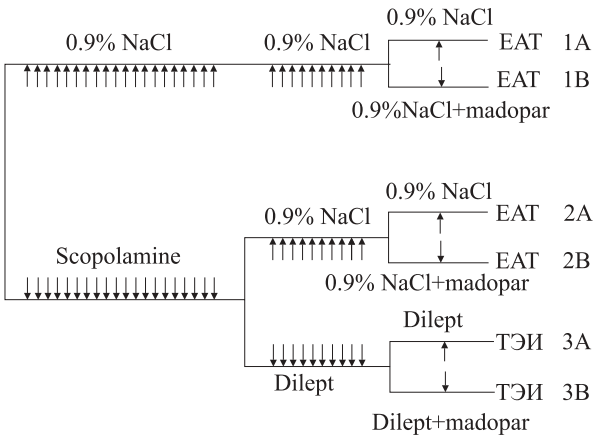

Fig. 1. Scheme of experiments with chronic blocking of cholinergic receptors by scopolamine.

neuroleptics of different structures [4], including dilept [8], were shown to eliminate this disorder.

In order to evaluate the effect of CE deficiency on the capacity to dive and on the intensity of dopamine damage, and to clear out the possible effects of dilept on the functional aftereffects of CE deficiency, each of the three groups was divided into subgroups: A) rats receiving no madopar before EAT and B) rats injected with madopar 1 h before EAT. The animals of subgroups 1B and 2B received the last injection of 0.9% NaCl and subgroup 3B animals were injected with dilept 10 min before madopar. The number of diving animals and the latent period (LP) of diving were recorded. Based on this latter parameter, the mean coefficient of efficiency (K_{EF}) was calculated using the following formula, reflecting the rate of finding the correct way out from the acute stress situation:

$$K_{EF} = \frac{1}{LP} \times 100\%$$

The significance of differences in the number of diving animals was evaluated by χ^2 test, the differences in the efficiency coefficient by the Mann—Whitney test.

Experiments with olfactory BE were carried out on 6-month-old male NMRI mice kept at 21-23°C with free access to water and food. The mice were divided into 4 groups. Bilateral extirpation of the olfactory bulbs (BE) by their aspiration through a trephination hole [3,13] was carried out in groups 1 and 2. Sham operation in groups 3 and 4 was carried out similarly. Starting from day 15 after the intervention the animals were intraperitoneally injected with 0.9% NaCl (groups 1 and 3) or dilept (0.02 mg/kg; groups 2 and 4) during 21 days and 28 days after surgery the animals were tested for absence of the initial preference of one of the compartments in the Morris water maze. During 6 subsequent days the mice, 1 h after injection of saline (groups 1 and 3) or dilept (groups 2 and 4), were trained to find a rescue platform hidden under water in one of the compartments of the maze; 4 training sessions were carried out daily. LP of finding the platform served as an indicator of learning. On day 7 spatial memory of trained animals was tested without rescue platform; the time spent by animals in the water maze compartments and number of excursions into the compartments was recorded for 1 min. In groups 2 and 4 training and memory testing were carried out 1 h after dilept was injected for the last time.

The statistical significance of differences between the groups was evaluated using Student's *t* test and unifactorial analysis of dispersions (ANOVA) with subsequent LSD post-hoc test.

RESULTS

Chronic treatment with scopolamine (subgroup 2A) led to a significant decrease in K_{EF} in comparison with subgroup 1A (Fig. 2, *b*), this indicating impaired capacity to diving as a result of long-term blockade of CE receptors. Ten-day treatment with dilept after long-term blockade of CE receptors with scopolamine completely abolished the damaging effect of scopolamine on the behavior in EAT (subgroup 3A), this indicating a cholinopositive effect of dilept. Dopaminomimetic madopar disordered the capacity to diving, decreasing the number of animals which solved the problem and reducing the K_{EF} (subgroup 1B). Pretreatment with scopolamine (subgroup 2B) potentiated the damaging effect of the dopaminomimetic. This manifested in decreased number of diving animals (Fig. 2, *a*) and in reducted K_{EF} (Fig. 2, *b*). Ten-day treatment of animals with dilept (subgroup 3B) after chronic treatment with scopolamine led to a reliable recovery of impaired diving capacity in EAT for both parameters (Fig. 2).

Activity of dilept towards CE system was evaluated by its effects on learning and dynamics of spatial memory of control, sham-operated, and BE animals. Factor analysis of LP values showed a significant improvement of the dynamics of learning in BE animals injected with dilept ($F(1.210)=6.99$; $p=0.0087$). Similarly as in previous studies [3,12], BE animals needed more time for reaching the rescue platform in Morris water maze in comparison with controls. Dilept therapy clearly improved the dynamics of learning. For example, on day 4 of learning the time needed for finding the platform was 15.0±2.5 sec for sham-operated mice and 27.0±3.5 sec in BE animals; dilept therapy shortened this parameter to 12.2±3.2 sec in BE mice and to 10.0±2.1 sec in sham-operated animals.

The normalizing effect of dilept was also observed in spatial memory testing in the maze. Sham-operated animals found the compartment with the rescue platform during training sessions (they reached it quickly, spent more time in it, and visited it more often), while in BE animals the capacity to find this compartment was impaired in delayed period after the operation (Table 1, Fig. 3). BE animals lagged behind controls by both memory parameters: number of excursions to the maze compartments and time spent in them. Dilept prevented the loss of spatial memory caused by BE: dilept-treated BE animals did not differ from the control group.

Dilept prevented amnesia caused by a single injection of CE receptor blockers in the conditioned reaction (passive avoidance test) [7]. We used other behavioral tests for evaluating the mnestic effects of this potential antipsychotic: extrapolation avoidance reaction, reflecting the capacity to make a decision in an extreme situation, and behavior in a water maze, characterizing the learning capacity and spatial memory. We should like to emphasize that we used models of chronic CE deficiency largely simulating the mediator situation taking place in such diseases as schizophrenia and Alzheimer's disease.

Stubborn amnesia developing after prolonged treatment and subsequent discontinuation of scopolamine is regarded as a result of prolonged blocking of CE receptors and triggering of the feedback mechanisms, leading at first to increase in the density and affinity of cholinoreceptors and then to CE deficiency caused by accelerated binding of acetylcholine [5,6].

The development of the initial concept of schizophrenia as a dopamine hyperactivity condition gave rise to studies of the role of other mediator systems in recent years. The deficiency of CE transmission is

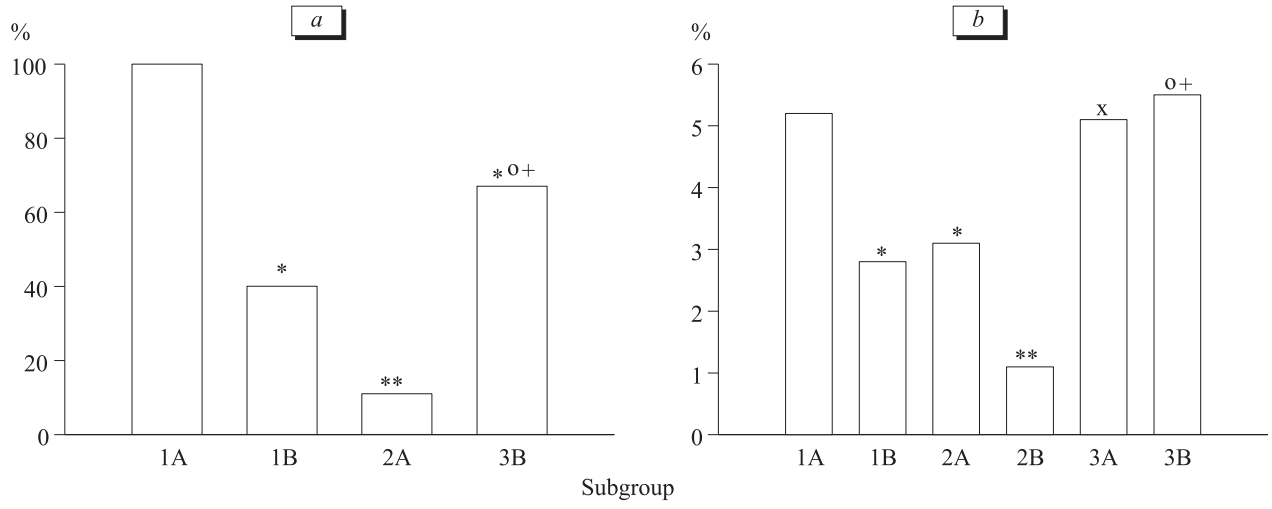

Fig. 2. Dilept prevented the damaging effect of scopolamine and scopolamine in combination with madopar on the extrapolation avoidance reaction of rats. *a*) effect on percentage of diving animals; *b*) effect on efficiency coefficient (value inverse to the latent period of diving). $^{*}p<0.05$, $^{**}p<0.01$ compared to 1A; $^{o}p<0.05$ compared to 1B; $^{+}p<0.05$ compared to 2B; $^{x}p<0.05$ compared to 2A.

considered as one of the main factors of cognitive insufficiency in negative forms of schizophrenia [9]. Therefore the approach used in our study (injection of cholinolytic scopolamine and a dopamine-positive substance madopar) simulates some elements of the mediator imbalance typical of schizophrenia. Hyperactivity of the dopamine system, particularly at the level of D_3 receptors [14], takes place in patients with psychotic manifestations of Alzheimer's disease. The detected effect of dilept (arrest of the damaging effect of scopolamine on the decision making capacity and the absence of damaging effect of dilept on the motor activity and coordination, *i. e.* on the motor component of this reaction) indicate that the positive cognitive effect of this neuroleptic is due to its cholinopositive effect. The dilept-madopar antagonism indicates that dilept is characterized by a combination of cholinopositive and dopaminergic effects. This sum of characteristics is interesting not only because it predicts dilept efficiency in positive and negative symptoms of schizophrenia and psychotic and cognitive disorders in Alzheimer's disease, but also because it is one more argument supporting the modern concepts on the reciprocal relationships between these mediator systems [10].

BE causes a series of morphological, biochemical, and electrophysiological changes, similar to manifestations of Alzheimer's disease. The density of neurons containing cholinacetyltransferase (the main enzyme of acetylcholine synthesis) is lowered in BE mice; neuronal death is observed in the temporal cortex and hippocampus, large-cell preoptic hypothalamic nucleus in the presence of pronounced elevation of the cerebral β-amyloid level [1-3]. A pronounced decrease in the amplitude of long-term potentiation was detected in the hippocampus, which is in line with impaired development of passive and active avoidance reflexes and spatial memory deficiency [12]. Improvement of memory by acetylcholinesterase blockers also attests to an important role of CE deficiency in the development of mnestic disorders in BE [15]. Similarly as with the scopolamine model, dilept abolished the functional aftereffects of BE on learning capacity and memory. The cholinopositive effect of dilept detected on both models of chronic CE deficiency is not associated with acetylcholinesterase suppression: an additional series of experiments carried out at Institute of Evolution Physiology, Russian Academy of Sciences, showed that dilept did not modify activity of this enzyme. This suggests that cholinopositive effect of dilept is due to its agonistic effect at the receptor level or to increased release of acetylcholine. Analysis with PASS software (prediction of activity spectra for substances) at Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, confirmed the probability of these effects.

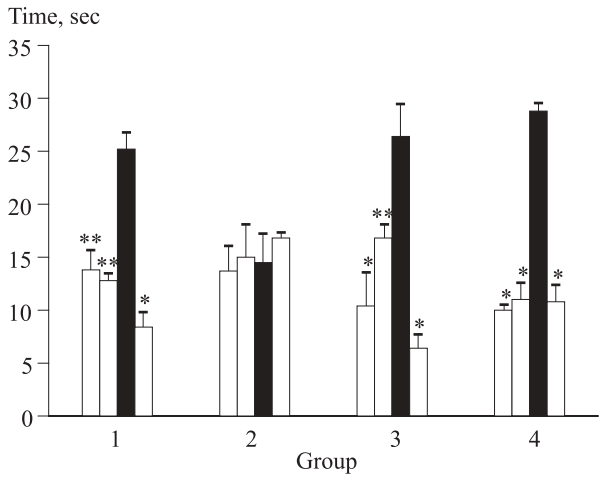

Fig. 3. Effect of dilept on spatial memory of bulbectomied and sham-operated mice, evaluated by the time spent in different compartments of water maze during testing (LSD proc-hoc test). Light bars: indifferent compartments; dark bars: training compartment. *p<0.001, **p<0.01 compared to time spent in the training compartment.

The findings indicate the presence of cholinopositive effect in the spectrum of activities of the new dipeptide neuroleptic. CE system deficiency (along with glutamatergic and neurotensinergic transmission hypofunction) plays an important role in the development of negative symptoms in schizophrenia. As insufficiency of CE transmission is believed to be the main factor of cognitive abnormalities in neurodegenerative diseases, we can predict the efficiency of

TABLE 1. Results of Factor Analysis of Spatial Memory Parameters in Sham-Operated and BE Animals

Group (n=6)	Time spent in compartments		Number of excursions into compartments	
	F(3.20)	p	F(3.20)	p
Sham-operated+0.9% NaCl	9.13	0.001	19.45	0.00006
BE+0.9% NaCl	0.8	0.51	0.84	0.49
BE+dilept	12.9	0.0003	11.03	0.0006
Sham-operated+dilept	49.4	0.0000	14.54	0.00019

dilept in both conditions. In Alzheimer's disease the cognitive disorders are often paralleled by psychotic symptoms. Cholinesterase inhibitors used in such cases are characterized by very high toxicity. Moreover, even if these agents reduce manifestations of cognitive abnormalities, they are absolutely ineffective for the treatment of psychotic symptoms. Standard neuroleptics are usually prescribed for arresting psychotic symptoms in Alzheimer's disease. Even atypical last-generation antipsychotics possess a cholinolytic effect (more pronounced in clozapine and olanzepine and less so in risperidone). It means that by eliminating the psychotic manifestations they can augment cognitive insufficiency.

Our data on the combination of the antipsychotic effect of dilept with its positive effect on mnestic functions based on its dopamin-negative and cholino-positive action together with the low toxicity of this simple peptide open good prospects for its effective clinical use in the treatment of positive and negative forms of schizophrenia and psychotic manifestations of Alzheimer's disease.

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