Effects of Neurotensin Dipeptide Analog Dilept on Dopamine Metabolism and Synthesis in the Nucleus Accumbens of Wistar Rats

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We studied the effects of neurotensin dipeptide analog Dilept (N-caproyl-L-prolyl-L-tyrosine methyl ester) on dopamine metabolism and synthesis in the nucleus accumbens of Wistar rats. Dilept increased the levels of dopamine and its metabolites (homovanillic acid and dioxyphenylalanine) and stimulated dopamine turnover in this structure. Dilept accelerated dopamine synthesis under conditions of pulsed activity blockade in dopaminergic neuron by injection of γ-butyrolactone combined with inhibition of aromatic acid decarboxylase with 3-hydroxybenzylhydrazine. The spectrum of pharmacological activities of Dilept towards the dopaminergic system of the nucleus accumbens was similar to that of atypical neuroleptics and neurotensin (endogenous antipsychotic).

Key Words: *Dilept; neurotensin; dopamine; nucleus accumbens; γ-butyrolactone model*

According to the dopaminergic hypothesis of schizophrenia, manifestation of positive and negative symptoms of this disease is related to hyperactivity of the dopaminergic neurons in the mesolimbic system paralleled by reduction of their functioning in the prefrontal cortex [11]. An important role in mesolimbic system dysfunction is played by changes in dopaminergic innervation of the nucleus accumbens. It seems that activation of the latter leads to inhibition of the corresponding cortical system and hence, to manifestation of schizophrenia symptoms [13]. A characteristic effect of classical and atypical antipsychotic drugs is stimulation of dopamine (DA) metabolism in the mesolimbic system. This effect is usually regarded as a compensatory response in this brain system unfolding by the feedback mechanism as a result of blockade of presynaptic DA receptors of the D_2 subtype [4]. This effect is intrinsic of neurotensin neuropeptide. Some effects of this substance, *e.g.* acceleration of DA turnover in the nucleus accumbens and neostriatum, prevention of the effects of dopaminomimetics, potentiation of barbiturate effects, hypothermia, suggest that neurotensin can be regarded as an endogenous neuroleptic [8]. Because of complex structure of neurotensin determining its poor bioavailability for the brain, its effects can be reproduced only after injection of this tridecapeptide into the cerebral ventricles or its delivery to certain structures [5]. This precludes clinical use of neurotensin as an antipsychotic agent. An original approach to the search for highly active peptide drugs developed at V. V. Zakusov Institute of Pharmacology consists in creation of substituted dipeptides simulating active center of the endogenous peptide and a nonpeptide drug with appropriate activity. Neurotensin-like active neuroleptics have been modeled on the basis of prolyl-tyrosine dipeptide, because this sequence corresponds to the central fragment of β-turn of the main neurotensin metabolite NT_{8-13} and is topologically similar to sulpiride (atypical neuroleptic) [7]. Of the N-acyl-prolyl-tyrosine series, N-caproyl-L-prolyl-

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L-tyrosine methyl ester (Dilept) was selected, which demonstrated activity by the standard parameters of neuroleptic effect [3]. The results suggested that under conditions of systemic treatment Dilept reproduced the behavioral effects characteristic of neurotensin injected into the brain ventricles. Neurochemical analysis of Dilept effects seemed to be appropriate for detecting the mechanisms of this phenomenon. In addition to evaluation of changes in the initial DA system activity in the nucleus accumbens, the functional model of the neuronal pulsed activity arrest with γ-butyrolactone (GBL) was used. This substance blocked pulsed activity of nucleus accumbens DA neurons projecting to the substantia nigra, sharply stimulated DA synthesis, and inhibited DA release into the intersynaptic space (which increases binding of presynaptic D_2 receptors with presumable ligands). Additional injection of 3-hydroxybenzylhydrazine (3-HOBH; aromatic amino acid decarboxylase inhibitor) in the GBL model demonstrated the effects of the studied substance on the rate of dioxyphenylalanine (DOPA; DA precursor) synthesis, determined by tyrosine hydroxylase activity. Activity of this enzyme was inversely related to the signals from DA presynaptic receptors [12].

We studied the effects of Dilept on the initial levels of DA and its metabolites in the nucleus accumbens in Wistar rats and evaluated the effects of Dilept on dopamine synthesis in a GBL model.

MATERIALS AND METHODS

The study was carried out on 48 male Wistar rats (250-300 g) and consisted of two experimental series. In series I we evaluated the effects of Dilept on the content of catecholamines and their metabolites in the nucleus accumbens of Wistar rats. The rats were divided into 4 groups, 8 per group. Animals of groups 1 and 2 were intraperitoneally injected with Dilept (0.8 mg/kg) and rats of groups 3 and 4 received an equivalent volume of saline. The animals were decapitated 30 min (groups 1 and 3) or 60 min (groups 2 and 4) after injection of the drug or saline. The nucleus accumbens was removed on ice, frozen in liquid nitrogen, and homogenized in 500 μl isolation medium containing 0.1 n $HClO_4$ with 0.5 nmol/ml dioxybenzylamine (internal standard). The samples were then centrifuged at 10,000*g* for 15 min. The supernatant was used for measurements of DOPA (DA precursor) by HPLC with electrochemical detection [1].

In series II, we evaluated the effect of Dilept on DA synthesis in the nucleus accumbens of Wistar rats in a GBL model. The rats were divided into 2 groups, 8 per group. Group 1 animals were intraperitoneally injected with Dilept (0.8 mg/kg) 60 min before decapitation and group 2 rats were injected with an equivalent volume of saline. The animals were intraperitoneally injected with GBL (750 mg/kg) and 3-HOBH (100 mg/kg), respectively, after Dilept or saline 35 and 20 min before decapitation. The nucleus accumbens was removed and processed similarly as in series I.

The data were statistically processed using Statistica 6.0 software. The significance of differences was evaluated by Student's *t* test.

RESULTS

The levels of DA and its metabolites 3,4-dioxyphenylacetic (DOPAA) and homovanillic (HVA) acids increased 30 min after Dilept injection in a dose of 0.8 mg/kg reaching 118±3, 126±5, and 125±3% of the parameters in control rats (injected with saline).

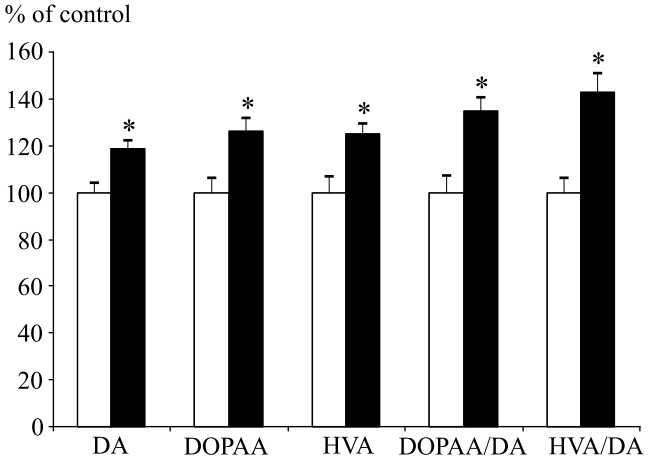

Fig. 1. Effect of Dilept on the content of DA and its metabolites in the nucleus accumbens of Wistar rats 30 min after its injection. Ordinate: content of DA and its metabolites. Light bars: control (0.9% NaCl). Dark bars: Dilept. *p<0.05 in comparison with the control.

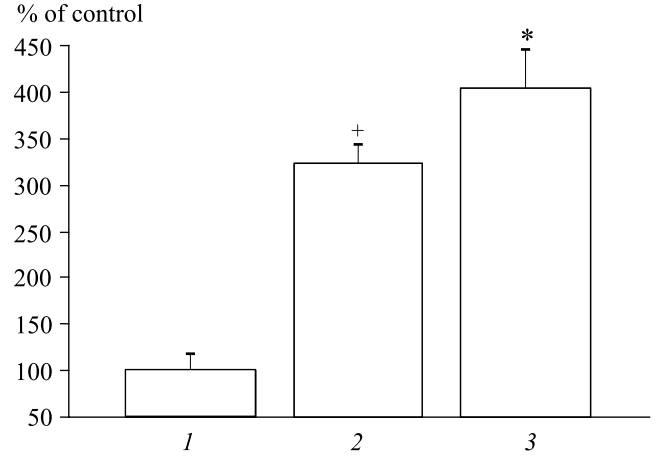

Fig. 2. Dilept effect on DOPA content in the nucleus accumbens of Wistar rats under conditions of neuronal pulsed activity arrest in the presence of 3-HOBH. Ordinate: DOPA content. *1*) control, taken for 100% (3-HOBH+saline); *2*) changes caused by GBL injection following 3-HOBH; *3*) Dilept effects in the presence of GBL and 3-HOBH. p<0.05 in comparison with: *1, +2.

TABLE 1. Effect of Dilept Injected 60 min before Decapitation on the Content of DA and Its Metabolites in the Nucleus Accumbens of Wistar Rats (*M±SEM*; % of control)

Substance	DA	DOPAA	HVA	DOPAA/DA	HVA/DA
Control	100.000±6.373	100.000±6.266	100.000±5.909	100.000±5.622	100.000±9.423
Dilept	106.000±4.859	96.596±5.038	95.926±10.315	127.214±3.588*	82.743±13.159

Note. *$p<0.05$ in comparison with the control (Student's *t* test).

Complex indexes HVA/DA and DOPAA/DA characterizing the velocity of DA utilization reached 143±8 and 135±7%, respectively (Fig. 1).

The DOPAA/DA ratio increased in comparison with the control 60 min after Dilept injection (Table 1).

Dilept accelerated DA turnover in the nucleus accumbens. Significant changes in the levels of DA and its metabolites were detected mainly 30 min after the drug injection, while 60 min postinjection the differences were significant only for the DOPAA/DA ratio. Typical neuroleptics, for example, haloperidol, increase DA turnover in the nucleus accumbens by more than 50% [2,10], while atypical antipsychotic agents, such as sulpiride, increase this parameter by only 15-20% [2]. The effect characteristic of atypical neuroleptics was described also for neurotensin (endogenous neuroleptic) [10]. Hence, Dilept stimulates the velocity of DA turnover to a degree characteristic of atypical antipsychotics and neurotensin.

The increase of DOPA content under conditions of the GBL model to a detectable level made it possible to evaluate changes in DA synthesis after Dilept injection. The DOPA level measured after injection of saline following 3-HOBH was taken for 100%.

Injection of GBL in combination with the aromatic acid inhibitor caused an elevation of DOPA content in the nucleus accumbens, which was in complete agreement with published data [12]. The content of DOPA in our experiments increased to 324±20%. Injection of Dilept led to a still greater increase of this parameter (to 405±41%; Fig. 2), this indicating stimulation of DA synthesis.

The compensatory increase of DA synthesis in the dopaminergic neuron by the feedback mechanism could be a response to blockade of DA D_2 receptors [14]. This shift led to stimulation of DA metabolism. GBL blocks the effects of some substances on DA release and metabolism, and hence, the GBL model is convenient for evaluation of the capacity of these substances to modulate DOPA accumulation. The DA agonists reduced DOPA level under conditions of GBL model, while antagonisms stimulated its accumulation in the nucleus accumbens [12]. Neurotensin also stimulated DA synthesis under conditions of the GBL model [10]. In addition, this neuropeptide blocked the DA D_2 receptors [4], thus increasing tyrosine hydroxylase activity [9]. Neurotensin elevated the DOPAA/DA and HVA/DA ratios in the nucleus accumbens [6,10]. This suggested that neurotensin stimulated DA utilization in the mesolimbic system.

Hence, it seems that Dilept stimulation of DA synthesis and metabolism is one of the mechanisms of its neuroleptic-like effect. It is also probable that Dilept stimulation of DA synthesis is explained by its antagonistic effect on the presynaptic D_2 receptors, resulting in elevation of tyrosine hydroxylase activity. However, these hypotheses should be verified.

Our data on Dilept capacity to elevate the content of DA and its metabolites in the nucleus accumbens and stimulate DA synthesis under conditions of the GBL model indicate similarity of the neurochemical mechanisms of the effects of this dipeptide and atypical antipsychotic drugs, as well as of neurotensin.

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