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Central effects of nafadotride, a dopamine D_3 receptor antagonist, in rats. Comparison with haloperidol and clozapine*

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Abstract:
The aim of this study was to examine behavioral and biochemical effects of nafadotride, the new dopamine D_3 receptor antagonist, and to compare it with haloperidol (dopamine D_2 receptor antagonist) and clozapine (predominate dopamine D_4 receptor antagonist). Each drug was injected to adult male Wistar rats intraperitoneally, each at a single dose and for 14 consecutive days. Thirty minutes after single or last injection of the examined drugs, the following behavioral parameters were recorded: yawning, oral activity, locomotion, exploratory activity, catalepsy and coordination ability. By HPLC/ED methods, we determined the effects of the examined antagonists on the levels of biogenic amines in striatum and hippocampus: dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline (NA). Additionally, DA and 5-HT synthesis rate was determined in striatum and 5-HT in hippocampus. The results of the study indicate that nafadotride, the dopamine D_3 receptor antagonist, has a behavioral and biochemical profile of action different from that of haloperidol but partially similar to that of clozapine.

Key words:
nafadotride, haloperidol, clozapine, behavior, brain biogenic amines, rats

Introduction

Dopamine (DA) receptors in the central nervous system attract significant scientific interest due to their possible involvement in several psychiatric and neurodegenerative disorders. Initially, DA receptors were divided into D_1 and D_2 subtypes, on the basis of their different action on adenylate cyclase activity [20]. In the 1990s, a third receptor subtype designated as D_3 was cloned and classified as a subtype of the DA D_2 receptor family [48, 49]. The D_3 receptor is localized primarily in the limbic brain structures, including nucleus accumbens [25, 32, 48, 49], and is expressed both pre- and post-synaptically [24, 30]. The DA D_4 receptor has also been recently cloned [54].

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DA D_2 receptors couple to multiple-effector systems, including the inhibition of adenyl cyclase activity, suppression of Ca^{2+} currents, and activation of K^+ currents [4]. The effector systems to which D_3 and D_4 receptors couple, have not yet been unequivocally defined.

DA D_1 and D_2 receptors have been implicated in the pathophysiology of Parkinson's disease and schizophrenia. A correlation exists between the average clinical dose of a neuroleptic and its affinity for brain DA receptors, as evaluated in the inhibition binding studies with the D_2 antagonist ^{3}H-spiperone [45]. Because long-term administration of typical neuroleptics to humans or to experimental animals can lead to development of extrapyramidal side-effects (including parkinsonian-like movement disorders and tardive dyskinesia), a group of antipsychotic drugs, referred to as "atypical neuroleptics", was developed.

The first atypical neuroleptic introduced into clinical practice was clozapine [8, 19], which has higher affinity for the DA D_4 receptor *vs*. D_2 receptor. Clozapine, in contrast to "typical" antipsychotics, has low propensity to produce extrapyramidal side effects.

DA D_3 and D_4 receptors raised great interest, because of their distribution in brain, and because they represent potential targets for new groups of antipsychotic and neuroleptic drugs [39, 41, 48]. Among these drugs, several new DA D_3 antagonists were synthesized, like nafadotride [26, 43].

The aim of the present study was to examine behavioral and biochemical effects of the new central DA D_3 receptor antagonist, nafadotride, and to compare its effects with those of haloperidol (DA D_2 receptor antagonist) and clozapine (predominate DA D_4 receptor antagonist) in rats.

Materials and methods

Animals

Adult 2–3 months old male albino Wistar rats were used in this study. The animals were housed six per cage at 22 ± 1°C, with an alternating 12 h/12 h light/dark cycle. Rats had free access to standard food pellets (Murigran, Animal Food Works, Motycz, Poland) and filtered tap water. Care of the animals was under the control of the Animal Facility of the Medical University of Silesia. All procedures used in these studies were approved by the Local Ethics Committee.

Chemicals

The L-aromatic acid decarboxylase inhibitor NSD 1015 (m-hydroxybenzylhydrazine dichloride) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Haloperidol and clozapine were purchased from Polfa Pharmaceutical Company (Warszawa, Poland). Nafadotride was kindly provided by Dr. P. Sokoloff, Unité de Neurobiologie et Pharmacologie, Centre Paul Broca de L'INSERM, Paris, France.

Schedule of drug injections

Single injection

Rats were divided into four groups. The first group (control) was injected with a single dose of saline (0.9% NaCl) at 1.0 ml/kg *ip*. The second group was injected with haloperidol at 0.5 mg/kg *ip*, the third group was given nafadotride at 0.25 mg/kg *ip*, and the fourth group received clozapine at 5.0 mg/kg *ip*. Immediately after the injection, each rat was individually placed in a transparent cage in a quiet well-ventilated and well-illuminated room to acclimatize to the laboratory environment. After 30 min, behavioral assessment was undertaken.

Multiple 14-day injections

Rats were divided into four groups as above. The first group (control) was injected once daily with saline (0.9% NaCl) at 1.0 ml/kg *ip* for 14 consecutive days. The second group was injected for 14 consecutive days with haloperidol at 0.05 mg/kg per day *ip*, the third group was given nafadotride at 0.025 mg/kg per day *ip*, and the fourth group was administered clozapine at 0.5 mg/kg per day *ip*. All injections were performed daily between 9 and 10 a.m. Immediately after the last (14th) injection, each rat was placed individually in a transparent cage in a quiet well-ventilated and well-illuminated room to acclimatize to the laboratory environment. After 30 min, behavioral assessment was undertaken.

Doses of haloperidol and clozapine for multiple applications approximated that used in humans; for single injection, the respective doses were approximately 10 times higher.

Behavioral study

Yawning behavior and oral activity

After 30 min of acclimation, each rat was observed for the next 60 min, and numbers of yawns and oral movements were counted [5, 21].

Irritability

After completing the above-described observations, the irritability was assessed by a scored test according to Nakamura and Thoenen [33].

Locomotor activity

Locomotor activity was determined on separate groups of rats (given a single injection or 14-day treatment). After 30 min of acclimation, each rat was observed for 10 min to determine the total time (s) that rats spent walking and sniffing. Simultaneously, grooming time (s) was recorded as well as numbers of rearings.

Exploratory activity

After the 10-min observation of locomotor activity, each rat was placed individually in the center of a flat wooden platform, 100 cm square, surrounded by a 40 cm high fence, to prevent escape. The platform had 4 rows of 4 holes each, 7 cm in diameter, and 20 cm apart. The number of times (during a 3-min period) that each rat stuck its head beneath the intramural line, into any hole, was counted and recorded [14].

Locomotor coordination

After completing 3-min observation of exploratory activity, each rat was placed on a wooden bar, 3 cm in diameter. The bar rotated longitudinally at 5 rpm, and the length of time (in seconds) each rat managed to stay on the rotating bar was recorded. The maximum time was 300 s. This test was carried out on each rat three times, with one-minute intervals between tests, and the mean time was calculated per rat.

Cataleptogenic activity

After completing 3-min assessment of locomotor coordination, each rat was placed on a wire mesh screen measuring 25 × 50 cm with 1 × 1 cm squares, and inclined by 60^{o} to the horizontal plane. The time (in seconds) for each rat to move any paw along at least one screen division within 60 s (maximal catalepsy time) was recorded. Measurements were performed 3 times with 10-min intervals. The final number was the sum of the three measurements [23].

Each examined group consisted of 8 rats.

Biochemical study

Assay of biogenic amines

Separate groups of rats injected once or for 14 days were used. Thirty minutes after a single or the last injection, rats were decapitated by guillotine, and their brains were immediately excised and placed on ice. The corpus striatum and hippocampus were separated, placed on dry ice, weighed, and stored at –70^{o}C, pending assay of the biogenic amines. Brain specimens were homogenized in ice-cold 0.1 M trichloroacetic acid, containing 0.05 mM ascorbic acid. After centrifugation (5000 × g for 5 min), the supernatants were filtered through 0.2 μm cellulose membranes (Titan MSF Microspin Filters Scientific Resources Inc., Eatontown, Great Britain) and the supernatant was injected onto the HPLC/ED column. Dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline (NA) were assayed in the striatum and hippocampus by an HPLC/ED technique [28]. Results are expressed as ng/g of wet tissue.

Assay of L-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP)

This experiment was performed on separate groups of rats injected once or for 14 consecutive days with the examined substances. Thirty minutes after a single or the last injection, rats were injected with the aromatic acid decarboxylase inhibitor, NSD-1015 (100.0 mg/kg *ip*) [7], and decapitated 30 min later for excision of the corpus striatum and hippocampus. Brain specimens were stored at –70^{o}C until assayed.

L-DOPA and 5-HTP were assayed in the striatum and 5-HTP in the hippocampus according to Magnusson et al. [28]. The level of the above amino acids in examined brain's part expressed indirectly the DA and 5-HT synthesis rate [7].

Each examined group consisted of 5 rats (tissues).

Statistical analysis

Analysis of variance (ANOVA) and Student's *t*-test were used to evaluate the differences between drug-treated groups and saline-treated groups of rats.

Results

Behavioral study (Tab. 1)

A single injection of haloperidol, clozapine or nafadotride did not influence irritability in rats. Conversely, a 14-day treatment regimen of haloperidol increased irritability, while a 14-day treatment regimen of nafadotride reduced irritability, as compared to the control group.

A single challenge dose of clozapine significantly reduced the numbers of yawns, while haloperidol and nafadotride did not affect yawning behavior. The 14-day treatment regimen of haloperidol nonsignificantly reduced the numbers of yawns, while the 14-day treatment regimen of nafadotride greatly increased yawning number as compared to the control group.

Oral activity decreased after either single and multiple injections of clozapine, and increased after 14 daily injections of haloperidol, as compared to control group.

Haloperidol at a single dose reduced locomotor time, while repeated (14 daily injections) nafadotride increased locomotor time.

Grooming time decreased after single and multiple injections of either haloperidol or clozapine, while nafadotride was without effect.

Rearings were completely abolished in animals treated with single or multiple doses of haloperidol. Conversely, single and multiple injections of nafadotride increased the number of rearings, as compared to controls.

Haloperidol injected at a single and multiple doses reduced the number of "peepings" in the exploratory test, and the effect of a single dose was greater than

Tab. 1. Effect of single and multiple injections of haloperidol, nafadotride and clozapine on the behavior of rats

No	Parameter of behavior	Examined substances							
		Saline		Haloperidol		Nafadotride		Clozapine	
		Single	Multiple	Single	Multiple	Single	Multiple	Single	Multiple
1	Irritability (scores)	1.00 ± 0.27	1.13 ± 0.13	0.75 ± 0.25	2.25* ± 0.53	1.38 ± 0.18	0.63* ± 0.18	1.13 ± 0.23	1.63 ± 0.26
2	Number of yawns	4.13 ± 0.55	6.75 ± 1.28	2.50 ± 1.22	3.38 ± 1.48	3.75 ± 1.00	14.00* ± 4.39	0.25* ± 0.16	5.13 ± 1.17
3	Number of oral movements	27.75 ± 5.30	23.25 ± 3.90	21.25 ± 4.66	38.00* ± 6.11	23.63 ± 4.11	26.13 ± 3.66	11.00* ± 1.99	9.63* ± 1.14
4	Locomotor activity (seconds)	102.50 ± 38.90	62.25 ± 24.78	5.00* ± 2.79	81.50 ± 34.55	127.00 ± 27.52	209.13* ± 44.18	79.00 ± 44.77	147.63 ± 51.06
5	Gromming (seconds)	66.25 ± 29.44	103.63 ± 32.30	4.25* ± 3.84	10.25* ± 4.91	40.25 ± 21.96	80.00 ± 41.06	10.00* ± 6.44	46.25 ± 18.30
6	Number of rearings	0.38 ± 0.26	0.25 ± 0.16	0.00* ± 0.00	0.00* ± 0.00	1.88 ± 0.67	2.50* ± 1.05	0.50 ± 0.50	0.63 ± 0.63
7	Number of peepings	19.25 ± 3.69	14.88 ± 3.20	1.50* ± 0.50	10.50* ± 1.20	17.50 ± 1.65	14.38 ± 2.52	11.00* ± 2.42	18.75 ± 2.55
8	Coordination (seconds)	226.88 ± 47.88	181.25 ± 50.35	55.13* ± 18.64	62.00* ± 28.88	177.13 ± 47.32	152.63 ± 46.87	300.00 ± 0.00	266.13 ± 33.88
9	Catalepsy (seconds)	5.61 ± 1.51	5.88 ± 0.97	102.50* ± 20.33	26.50* ± 6.56	10.13* ± 1.69	11.25 ± 2.77	26.00* ± 9.25	15.13* ± 3.04

Mean SEM, n = 8; * $p < 0.05$ as compared to the respective control groups (saline)

Tab. 2. Effect of single and multiple injections of haloperidol, nafadotride and clozapine on the level of biogenic amines (ng/g of wet tissue) in the striatum and hippocampus of rats' brain

		Examined substances							
		Saline		Haloperidol		Nafadotride		Clozapine	
		Single	Multiple	Single	Multiple	Single	Multiple	Single	Multiple
Striatum	DA	8057.519 ± 387.673	8353.267 ± 429.717	9224.498 ± 416.411	7395.502 ± 702.551	8665.344 ± 296.513	8369.024 ± 541.257	9118.352 ± 680.276	7232.901 ± 390.537
	DOPAC	852.131 ± 30.380	855.807 ± 28.917	1148.263* ± 55.488	1198.816* ± 36.006	1017.3696 ± 77.0912	745.826 ± 35.114	1028.402 ± 83.040	582.192 ± 15.284
	HVA	852.473 ± 63.545	842.230 ± 16.176	1215.898* ± 64.451	1240.399* ± 207.670	940.501 ± 87.365	889.816 ± 32.832	915.872 ± 68.840	848.466 ± 31.133
	5-HT	251.561 ± 16.623	372.016 ± 20.528	261.248 ± 17.892	332.430 ± 11.076	283.997 ± 14.125	383.172 ± 24.471	317.430 ± 24.074	362.440 ± 23.960
	5-HIAA	340.479 ± 11.328	357.168 ± 24.245	418.865 ± 54.355	311.511 ± 15.727	520.225 ± 23.717	299.309 ± 12.122	552.092 ± 51.528	312.133 ± 21.721
	NA	221.684 ± 42.647	268.002 ± 39.578	207.677 ± 21.097	255.912 ± 26.118	266.331 ± 26.550	349.315 ± 85.511	258.194 ± 30.365	437.686 ± 116.690
Hippocampus	5-HT	262.728 ± 25.419	280.430 ± 12.324	210.967* ± 25.419	282.005 ± 16.557	230.030 ± 9.695	330.918 ± 37.775	219.469 ± 10.092	336.957 ± 23.021
	5-HIAA	217.392 ± 10.519	300.341 ± 23.178	195.068 ± 10.340	299.293 ± 29.225	203.590 ± 14.233	320.168 ± 55.491	198.595 ± 8.878	316.036 ± 15.565
	NA	438.358 ± 19.658	426.461 ± 9.335	406.184 ± 10.031	486.900* ± 19.741	431.120 ± 29.254	556.318* 30.183	382.853 ± 15.002	467.866 ± 36.038

Mean ± SEM, n = 5; * $p < 0.05$ as compared to the respective control groups (saline)

that of multiple treatments. A single clozapine injection reduced the numbers of peepings, while repeated clozapine treatments (daily for 14 days) increased number of peepings. Nafadotride was without effect.

Haloperidol at single and multiple doses reduced coordination ability of rats (i.e., time on the rotarod) as compared to control. Conversely, clozapine increased coordination ability, while nafadotride was without effect.

Haloperidol and clozapine, injected once or in a multiple dose regimen, each produced catalepsy. Nafadotride only slightly increased catalepsy in rats after a single treatment, but had no effect after multiple injections.

Biochemical study

Biogenic amines level (Tab. 2)

Haloperidol increased the levels of DOPAC and HVA in the striatum after a single injection, as compared to the control. Nafadotride and clozapine nonsignificantly increased the level of 5-HIAA in the striatum after a single injection. All three examined substances did not alter the levels of DA and NA in the striatum after a single injection, as compared to the respective controls.

Fourteen consecutive daily injections of haloperidol also increased the DOPAC and HVA levels in the striatum as compared to control. All three examined substances, however, did not alter the striatal tissue levels of DA, 5-HT, 5-HIAA and NA after multiple treatments.

Haloperidol reduced the 5-HT level in the hippocampus after a single injection. A single injection of haloperidol, clozapine or nafadotride failed to alter the level of 5-HIAA and NA in the hippocampus.

Fourteen consecutive daily injections of nafadotride or haloperidol increased the NA level only in the hippocampus, and was without effect on 5-HT and 5-HIAA levels. DA level in the hippocampus was on the border of detection (results were not presented).

Tab. 3. Effect of single and multiple injections of haloperidol, nafadotride and clozapine on L-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (ng/g of wet tissue) in the striatum and hippocampus of rats' brain

Part of the brain	Amino acid	Examined substances							
		Saline		Haloperidol		Nafadotride		Clozapine	
		Single	Multiple	Single	Multiple	Single	Multiple	Single	Multiple
Striatum	L-DOPA	1200,3 ± 109.3	1170.9 ± 98.3	3218.9* ± 275.7	2750.4* ± 457.9	1513.1 ± 140.2	1098,1 ± 176.0	1316.3 ± 123.7	1079.1 ± 120.7
	5-HTP	157.2 ± 24.1	159.9 ± 20.3	87.3* ± 16.6	142.4 ± 8.6	160.6 ± 14.7	148.2 ± 20.3	110.4 ± 11.9	160.6 ± 21.8
Hippocampus	5-HTP	108.2 ± 5.4	131.4 ± 14.5	101.7 ± 3.5	149.3 ± 23.8	127.2 ± 10.4	127.4 ± 11.5	110.1 ± 12.6	131.6 ± 9.8

Mean SEM, n = 5; L-DOPA – L-dihydroxyphenylalanine, 5-HTP – 5-hydroxytryptophane, *$p < 0.05$ as compared to the respective control groups (saline)

L-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP) levels (Tab. 3)

Single and multiple injections of haloperidol increased L-DOPA level in the striatum of rats. Conversely, only a single injection but not multiple treatments with haloperidol reduced the 5-HTP level in striatum.

Single or multiple treatments with haloperidol, clozapine, or nafadotride did not significantly alter 5-HTP level in the hippocampus.

Discussion

Nafadotride is a selective antagonist of the DA D_3 receptor, which is presented predominately in limbic structures, mostly in the nucleus accumbens [44, 58]. In other brain structures, the density of the DA D_3 receptors is 2–3 times lower *vs*. D_2 receptors [11, 16]. In contrast, DA D_2 receptors are situated mainly in the nucleus accumbens, caudate putamen, olfactory tubercle and substantia nigra [11]. D_4 receptors are situated mainly in the hippocampus, hypothalamus, frontal cortex and midbrain [11] and their density is also much lower than that of DA D_2 receptors. It is of interest that in schizophrenic patients there is an increased number of DA D_3 and D_4 receptors in brain (*vs*. untreated healthy individuals), and that the number of D_3 and D_4 receptors normalizes when schizophrenics are treated with antipsychotic drugs [18, 37, 46].

The DA D_3 receptor can be localized presynaptically (autoreceptor), acting by autofeedback inhibition to reduce DA exocytosis [44, 55]. Detailed studies reveal opposite roles for the DA D_2 and D_3 receptors in locomotor activity, learning and memory. The effects depend, in large part, on the specific agonists or antagonists used in the studies [47, 52, 57]. Generally, classic neuroleptics reduce locomotor activity in mammals [50, 51], and we confirmed this in the present study.

Synthesis of 7-OH-DPAT, a selective DA D_3 receptor agonist, provides the opportunity to localize the distribution of D_3 receptors in brain [27] and to determine the function of the D_3 receptor [12]. 7-OH-DPAT stimulates D_3 receptors and inhibits endogenous DA synthesis [15]. Compared to the positive locomotor effects of the DA D_2 agonist quinpirole, 7-OH-DPAT inhibited locomotion in rats [52, 56, 57] – in agreement with our prior results [36].

Subsequent to cloning of the DA D_3 receptor by Sokoloff et al. [48], numerous antagonists, with high or low affinity for D_3 receptors, were synthesized and tested. Among these are AJ76, UH232 [53, 56] and nafadotride [43], which have high affinity and high specificity. A major objective is to identify a new generation antipsychotic drug with high efficacy.

Nafadotride has 10–20 times higher affinity for the D_3 *vs*. the D_2 receptor [17]. At a dose of 1.0 mg/kg, nafadotride selectively blocks the D_3 receptor [47]. At a high dose, nafadotride also blocks DA D_2 receptors. Clifford and Waddington [9] and Sautel et al. [43] found that nafadotride, in contrast to sulpiride (D_2 receptor antagonist), increased locomotor activity, grooming,

learning, and memory in rats; and induced climbing behavior in mice. Interestingly, a similar effect on locomotor activity was confirmed in the present experiment. When administered daily to rats for 14 consecutive days, nafadotride increased locomotion and rearings, but not grooming. At very high doses (100.0 mg/kg), nafadotride reportedly induced catalepsy [43], but we failed to confirm this in our experiment, either after single or multiple injections (i.e. in opposition to haloperidol and clozapine). It must be added that we examined also the effect of another D_3 receptor antagonist, U-99194A, on behavior in rats [6], and found that U-99194A blocked locomotor activity and yawning behavior induced by 7-OH-DPAT (D_3 receptor agonist). U-99194A also induced a moderate degree of catalepsy and enhanced haloperidol-induced catalepsy. However, U-99194A did not alter DA and DOPAC release in the striatum of rats, as assessed by *in vivo* microdialysis and *in vivo* voltametry [6].

Interestingly, in the present experiment, long-term application of nafadotride induced yawning behavior. We interpret this as possible D_3 receptor priming, analogous to the observed increase in oral activity after 14-day haloperidol treatment. Significantly, long-term nafadotride treatment failed to induce oral activity, a characteristic symptom of DA D_2 receptor blockade in rats [22], comparable to extrapyramidal effects seen in humans treated prolongably with classic neuroleptics.

Nafadotride increased DA turnover in the nucleus accumbens, striatum and brain cortex in the rat, but to a much lesser extent than haloperidol [2]. Others found that UH232 and AJ76 increased DA and DOPAC levels in microdialysates of striatum and nucleus accumbens [40, 59]. In the present experiment, we determined biogenic amine levels in striatum and hippocampus, as well as, indirectly, DA and 5-HT turnover in the striatum and 5-HT in the hippocampus by L-DOPA and 5-HTP assay, but we did not perform *in vivo* microdialysis. Nafadotride applied in single and multiple doses did not influence DA, DOPAC, HVA and 5-HT levels in striatum, but increased NA content of hippocampus, following 14 daily injections. Nafadotride did not alter L-DOPA level in the striatum after single and multiple injections.

In summary, we have compared behavioral and biochemical effects of nafadotride with clozapine, a prominent D_4 receptor antagonist. Clozapine, an atypical neuroleptic with 10 times higher affinity for the DA D_4 *vs*. D_2 receptor [1, 13], is an effective antipsychotic drug which does not induce extrapyramidal effects [34]. In laboratory studies on animals, clozapine and several other D_4 antagonists induced moderate catalepsy [34], as we confirmed in the present study. Clozapine did not block amphetamine- and apomorphine-induced stereotyped behavior, confirming that clozapine does not alter DA exocytosis in the corpus striatum. In contrast, clozapine blocked amphetamine- and apomorphine-induced hyperlocomotion [34]. However, in DA D_4 receptor knock-out mice, clozapine failed to block apomorphine-induced hyperlocomotion [42]. Clozapine, at the doses ranging from 2.0 to 20.0 mg/kg, did increase DA and DOPAC levels in *in vivo* microdialysates of rat brain [10, 40]. Others found no effect of clozapine on DA release [29, 31, 35, 38]. In the present experiment, clozapine reduced 5-HTP level in the striatum after a single injection only. It appears that the serotonergic system is involved in the biological actions of clozapine [3].

From our experiment, we conclude that the pharmacological (behavioral and biochemical) profile of nafadotride action is different from that of haloperidol, but partially similar to that of clozapine.

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