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Modulatory effect of agents active in the presynaptic dopaminergic system on the striatal dopamine transporter

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Abstract

We have investigated the effects of agents active in the presynaptic dopaminergic system on the characterization of the rat striatal dopamine transporter. The dopamine transporter was characterized by high-affinity [^{3}H]GBR 12935 (1-[2-diphenylmethoxy)-ethyl]-4-(3-phenylpropyl)-piperazine) binding to a membrane preparation and by [^{3}H]dopamine uptake into striatal synaptosomes. Subchronic treatment with reserpine (2.5 mg/kg, 4 days), a monoamine depletor, caused a significant decrease in both [^{3}H]GBR 12935 binding (20%) and [^{3}H]dopamine uptake (51%). In contrast, amantadine (a dopamine releaser) treatment (20 mg/kg, 21 days) induced an increase (28%) in the maximal number of [^{3}H]GBR 12935 sites. Chronic levo-dopa (dopamine precursor) treatment combined with carbidopa (50 mg/kg and 5 mg/kg respectively, 21 days) as well as benztropine (dopamine uptake inhibitor) treatment (10 mg/kg, 21 days) did not affect the striatal dopamine transporter characteristics. The present results showed that the striatal dopamine transporter is sensitive to changes in dopaminergic neurotransmission caused by agents that do not interact directly with the dopamine carrier.

Keywords: Dopamine transporter; [^{3}H]GBR 12935 binding; L-DOPA; Reserpine; Amantadine; Benztropine

1. Introduction

The re-uptake of neurotransmitters is the major mechanism for their inactivation after their release into the synaptic cleft. Neurotransmitter uptake sites can be regulated by changes in the level of the relevant neurotransmitter at the synaptic cleft. That is, a low extracellular neurotransmitter level can lead to down-regulation of the presynaptic uptake sites and vice versa. For example, the choline uptake sites in the hippocampus are regulated by changes in the choline level (Lowenstein and Coyle, 1986), and the norepinephrine transporters in the frontal cortex are regulated by the synaptic norepinephrine level (Lee et al., 1983).

There are only a few studies of the regulatory effect of drugs acting on the dopamine system on the expression of the neuronal dopamine transporter.

Previous studies have shown that the repeated administration of reserpine (monoamine depletor) reduces the in vivo [^{18}F]GBR 13119 binding to dopamine uptakes sites (Kilbourn et al., 1992), whereas the administration of L-deprenyl (monoamine oxidase-B inhibitor), GBR 12909, and L-dopa (levo-dopa) increases the density (B_{max}) of mazindol binding sites (Wiener et al., 1989; Ikawa et al., 1993). On the other hand, the administration of dopamine uptake inhibitors in rats does not significantly affect either dopamine transporter density or maximal dopamine uptake rate (V_{max}) (Kula and Baldessarini, 1991; Allard et al., 1990; Boulay et al., 1994).

[^{3}H]GBR 12935 is a widely used ligand for labelling the dopamine uptake site. The present study was designed to evaluate the effects of treatment with drugs that affect dopamine neurotransmission on the pharmacodynamic characteristics of the presynaptic dopamine carrier. To this aim rats were treated with the following agents: reserpine, a monoamine depletor; amantadine, a dopamine-releasing drug used for treatment of Parkinson's disease, L-dopa, the precursor of dopamine and benztropine, an anticholinergic drug. The latter agent binds (albeit weakly) to the dopamine uptake complex (Vaugeois et al., 1990).

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2. Materials and methods

2.1. Materials

[^{3}H]GBR 12935 (1-[2-diphenylmethoxy)-ethyl]-4-(3-phenylpropyl)-piperazine) (53 Ci/mmol) and [^{3}H]dopamine (30.6 Ci/mmol) were purchased from New England Nuclear, Boston, MA, USA.

Reserpine, amantadine, benztropine, L-dopa and D-dopa (dextro-dopa) were purchased from Sigma Chemcial Co., St. Louis, MO, USA. Carbidopa and mazindol were generously donated by Teva, Israel and Sandoz Pharmaceuticals, New Jersey, USA.

2.2. Drug treatments

Charles River (CD strain) male rats (150–200 g) were injected intraperitoneally (i.p.) with amantadine (20 mg/kg), L-dopa (50 mg/kg) together with carbidopa (5 mg/kg), or benztropine (10 mg/kg) once daily for 21 days. Reserpine (2.5 mg/kg) was administered for a period of 1 day (acute treatment) and a period of 4 days (subchronic treatment). Five to nine rats were included in each group.

2.3. [^{3}H]GBR 12935 binding to striatal membranes

Brain membranes were prepared as previously described (Janowsky et al., 1986). Briefly, striatal tissue (male Charles River rats, CD strain, 150–200 g) was dissected over ice and immediately placed into a chilled glass homogenizing vessel containing 10 volumes of ice-cold 0.32 M sucrose. The tissue was homogenized 8 times with a Teflon pestle and centrifuged for 10 min at $1000 \times g$. The supernatant fraction was then cetrifuged for 20 min at $17\,000 \times g$. The resulting pellet (P2) was resuspended in 200 volumes of 50 mM Tris HCl buffer (pH 7.4) containing 120 mM NaCl, using a Brinkman Polytron. The final protein concentration was 50–100 μg/ml. [^{3}H]GBR 12935 binding was carried out at 24°C, as described previously (Janowsky et al., 1986). The incubation mixture contained 100 μl striatal (5–10 μg protein) membranes, 50 μl [^{3}H]GBR 12935 (0.5–8 nM) and 350 μl buffer (50 mM Tris HCl, pH 7.4, containing 120 mM NaCl and 0.01% bovine serum albumin). Following a 45-min incubation, the incubate was quickly diluted in 3 ml ice-cold buffer and filtered under vacuum through glass-fiber filters (Whatman GF/C). The filters were washed 3 times with 3 ml ice-cold buffer and counted in a liquid scintillation cocktail (hydro-Luma, Lumac) using a scintillation counter (Tri-carb 300c, Packard). Non-specific binding was determined in the presence of 50 μM mazindol. Scatchard plots were made and both B_{max} of the binding sites and K_d were determined by a least squares linear regression analysis.

2.4. [^{3}H]Dopamine uptake into striatal synaptosomes

[^{3}H]Dopamine uptake into striatal synaptosomes was determined according to Coyle and Snyder (1969). Striatal tissue was homogenized (8 strokes) with a Teflon pestle in 20 volumes of ice-cold 0.32 M sucrose and centrifuged for 10 min at $1000 \times g$. Aliquots (50 μl) of the supernatant (S1) were added to tubes containing 0.85 ml oxygenated ice-cold buffer (119 mM NaCl, 3.9 mM KCl, 11.1 mM glucose, 0.65 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.16 mM EDTA, 0.1 mM pargyline and 19 mM phosphate buffer, pH 7.4). Non-specific uptake was determined in the pres-

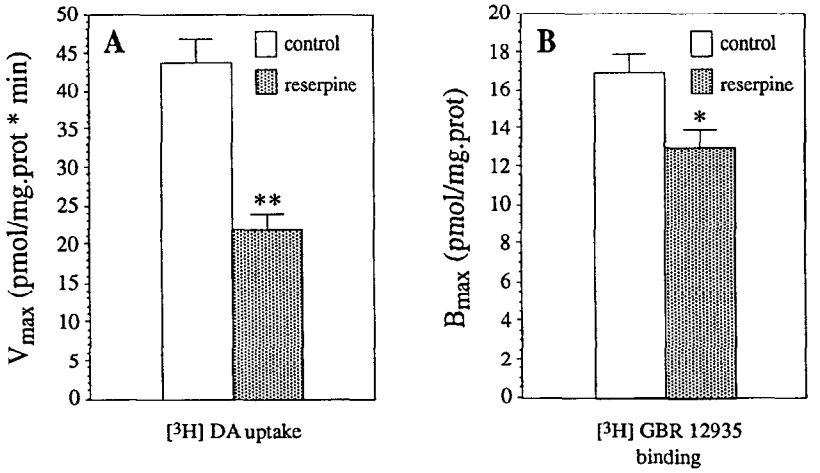

Fig. 1. The effect of chronic reserpine treatment on the striatal dopamine transporter. Rats were treated ($n = 7$) with reserpine (2.5 mg/kg) for 4 days and were compared with vehicle-treated controls ($n = 8$). [^{3}H]GBR 12935 binding (0.5–8.0 nM) to striatal membranes was evaluated using Scatchard analysis. Non-specific binding was determined in the presence of 50 μM mazindol. [^{3}H]Dopamine uptake (6.25×10^{-8}–7.5×10^{-7} M) into striatal synaptosomes was determined using a Eadie-Hofstee analysis. Non-specific uptake was determined in the presence of 50 μM mazindol. The results are expressed as means ± S.E.M. * $P < 0.05$, ** $P < 0.01$ vs. control.

ence of 50 μM mazindol. The tubes were preincubated for 10 min in a 37°C bath. Thereafter 100 μl [^{3}H]dopamine (6.25×10^{-8}–7.5×10^{-7} M) was added and the incubation was continued for another 3 min. Incubation was terminated by adding mazindol (50 μM final concentration) and by rapidly cooling the tubes on ice. The incubate was filtered under vacuum through glass-fiber filters (Whatman GF/C). The filters were washed 3 times with 3 ml of ice-cold buffer and counted as described before. K_m and V_{max} values were obtained using linear Eadie-Hofstee least squares fit of the data.

2.5. Statistical analysis

The unpaired Student's t-test was used for inter-group comparisons. All results are expressed as means ± S.E.M.

3. Results

One day of reserpine treatment did not affect [^{3}H]GBR 12935 binding to striatal membranes [reserpine ($n = 5$) vs. control ($n = 5$); B_{max}: 8.0 ± 0.4 vs. 7.9 ± 0.5 pmol/mg protein; K_d: 2.5 ± 0.4 vs. 2.5 ± 0.3 nM]. Subchronic treatment with reserpine (2.5 mg/kg, 4 days, i.p.) resulted in catalepsy, severe akinesia, ptosis and weight loss. The reserpine-induced monoamine depletion was associated with a significant (20%) decrease in the density of [^{3}H]GBR 12935 binding sites in the striatal membranes of the reserpine-treated rats as compared to the controls (16.6 ± 0.9 vs. 13.0 ± 0.7 pmol/mg protein; $P < 0.05$) (Fig. 1), and an increase (32%) in the affinity of [^{3}H]GBR 12935 to its binding sites (3.4 ± 0.8 vs. 2.6 ± 0.3 nM; $P < 0.05$). A parallel but more prominent decrease (51%) in the uptake (V_{max}) of [^{3}H]dopamine (43.3 ± 3.5 vs. 20.8 ± 1.4 pmol/mg protein/min; $P < 0.01$) in the striatal synaptosomes occurred following 4 days of reserpine treatment (Fig. 1) with no change in K_m values (310 ± 50 vs. 330 ± 130 nM).

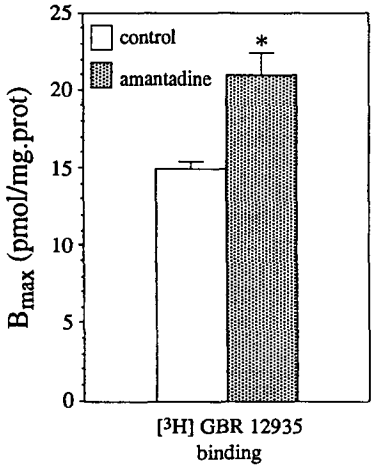

Fig. 2. The effect of chronic amantadine treatment on [^{3}H]GBR 12935 binding to the striatal dopamine transporter. The rats were treated ($n = 5$) with amantadine (20 mg/kg) for 21 days, and were compared with vehicle-treated controls ($n = 5$). [^{3}H]GBR 12935 binding was evaluated as described in Materials and methods. * $P < 0.05$ vs. control.

Table 1
The effect of L-dopa treatment on the dopamine transporter in the striatum

Drug		[^{3}H]Dopamine uptake V (pmol/mg protein/min)	[^{3}H]GBR 12935 binding B (pmol/mg protein)
Control	($n = 6$)	31.2 ± 3.3	5.2 ± 0.5
D-dopa	($n = 5$)	34.6 ± 4.6	5.4 ± 0.4
L-dopa	($n = 7$)	35.7 ± 2.3	5.1 ± 0.2

Rats were treated for 21 days with a combination of either L-dopa or D-dopa (50 mg/kg) with carbidopa (5 mg/kg). [^{3}H]Dopamine uptake (5×10^{-7} M) was measured in striatal synaptosomes, while [^{3}H]GBR 12935 binding (2 nM) was measured in striatal membranes. Since no difference was obtained with single-point [^{3}H]GBR 12935 binding and [^{3}H]DA uptake measurements for L-DOPA and D-DOPA, we did not go on with binding and uptake saturation analyses.

Amantadine treatment (20 mg/kg, 21 days) induced an increase (41%) in [^{3}H]GBR 12935 maximal binding capacity (15.2 ± 0.2 vs. 21.4 ± 1.3 pmol/mg protein; $P < 0.05$) (Fig. 2), but had no significant effect on the affinity of [^{3}H]GBR 12935 to its binding sites (2.7 ± 0.8 vs. 3.5 ± 1.8 nM, N.S.). Specific [^{3}H]GBR 12935 binding and specific dopamine uptake were not affected by treatment with either L-dopa (the active enantiomer) or D-Dopa (the inactive enantiomer) (Table 1). Benztropine treatment (10 mg/kg, 21 days) also did not affect the [^{3}H]GBR 12935 maximal binding capacity to striatal membranes (control 16.8 ± 2.8 pmol/mg protein; benztropine 16.4 ± 3.5 pmol/mg protein $n = 7$ for each group) or the K_d values (2.1 ± 0.5 nM vs. 1.8 ± 0.2 nM).

4. Discussion

The present study showed that the dopamine carrier is sensitive to changes in synaptic dopamine content. Reserpine treatment, which depletes dopamine (by 90% according to Neisewander et al., 1991), caused a significant decrease in maximal [^{3}H]GBR 12935 density as well as in maximal dopamine uptake into the synaptosomes. A similar effect was observed by Kilbourn et al. (1992) who reported that repeated reserpine administration reduces in vivo [^{18}F]GBR 13119 binding to the dopamine uptake sites. Using this method, however, they could not determine whether the decrease was caused by a change in the affinity of the ligand to the dopamine transporter, a change in the density of the uptake sites, or both. We demonstrated that the decrease in the density of the transporter is accompanied by an increase in the affinity of the ligand to its binding site. This mechanism may allow a sufficient

dopamine uptake after the reduction in the number of binding sites.

This effect is apparently caused by the reduction in dopamine concentration in the synaptic cleft and the direct effect of dopamine on the transporter, since one day of treatment did not change either the maximal density of the GBR 12935 binding sites or the affinity of GBR 12935 to the dopamine transporter. The greater reduction of dopamine uptake (51% decrease in V_{max}) than of GBR 12935 binding (20% decrease in B_{max}) reflects the suppresion of the transporter function beyond the reduction in transporter density, as labelled by [^{3}H]GBR 12935.

On the other hand, treatments that increase the extracellular synaptic dopamine level yielded conflicting results. Amantadine, an antiparkinsonian drug that induces dopamine release, caused a significant increase in maximal [^{3}H]GBR 12935 binding sites. However, treatment with L-dopa, the active precursor of dopamine, combined with carbidopa (DOPA decarboxylase inhibitor), did not affect either specific [^{3}H]GBR 12935 binding or specific dopamine uptake. It has been reported that chronic treatment with L-dopa reduces rather than elevates extracellular dopamine (Brannan et al., 1991). Up-regulation of [^{3}H]mazindol binding sites after treatment with L-dopa together with benseride was demonstrated by Ikawa et al. (1993). However, these researchers used a high dose of L-dopa (4-fold greater than the dose we used) and this may explain the discrepancy between results. The negative findings in our study may have been caused by the supersensitivity effects of chronic L-dopa on the dopamine receptors, which inhibited dopamine release from the nerve terminals (Klawans et al., 1977).

The mechanism by which amantadine increases dopamine release is unclear and may be associated with its binding to the MK-801 binding site in the NMDA receptor (Jackisch et al., 1992). The increase in the density of the [^{3}H]GBR 12935 binding sites following amantadine treatment can also be explained as a compensatory mechanism to enhance dopamine uptake and to preserve the normal synaptic dopamine level. L-Deprenyl, which increases the dopamine level by monoamine oxidase-B inhibition, also caused an increase in the density of striatal [^{3}H]mazindol binding sites but had no effect on K_d (Wiener et al., 1989). Such increases in the expression of the dopamine transporter prevent the excess accumulation of dopamine at the synaptic cleft. Our results concerning benztropine are in accordance with those of previous studies which did not demonstrate any alteration of the striatal dopamine transporter following treatment with dopamine uptake inhibitors (Kula and Baldessarini, 1991; Allard et al., 1990; Boulay et al., 1994).

In summary, the present study showed that the dopamine transporter is sensitive to some changes in dopaminergic neurotransmission. It appears that the expression of the dopamine transporter is affected by agents that modulate the synaptic dopamine content without direct interaction with the dopamine carrier, but is relatively insensitive to agents that interact directly with the dopamine transporter.

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