ELSEVIER

European Journal of Pharmacology 403 (2000) 75–80

ejp

www.elsevier.nl/locate/ejphar

Increased dopamine uptake in striatal synaptosomes after treatment of rats with amantadine

Guylène Page [*,1], Magali Peeters[1], Jean-Marie Maloteaux, Emmanuel Hermans

Laboratoire de Pharmacologie, Université catholique de Louvain FARL 54.10, Avenue Hippocrate 54, B-1200 Brussels, Belgium

Received 15 May 2000; received in revised form 18 July 2000; accepted 24 July 2000

Abstract

The aim of the present study was to investigate the effect of short- and long-term treatments with amantadine on the activity of the neuronal dopamine transporter (DAT) in the rat striatum. For this purpose, the [^{3}H]dopamine uptake was measured in striatal synaptosomes prepared from rats treated for 2, 7 and 14 days with amantadine (40 mg/kg; i.p.). After 7 days of treatment, amantadine increased the apparent V_{max} by 30% without modification of the apparent K_m of dopamine uptake whereas no change in these parameters was observed after 2 and 14 days treatment. Binding assays conducted with [^{3}H]GBR-12935 on membranes prepared from animals treated with amantadine revealed no difference in the density and the affinity of striatal DAT binding sites as compared to control. This indicates that the increased dopamine uptake was not reflecting a modification at the level of the DAT expression. The activity of the DAT is regulated by phosphorylation and one may propose that ionotropic glutamate receptors present on presynaptic terminals directly modulate this phosphorylation. An indirect mechanism would involve presynaptic dopamine receptors that control the activity of the DAT in response to the increased dopamine concentration in the synaptic cleft.

Keywords: Glutamate; Dopamine uptake; Parkinson's disease; NMDA receptor

1. Introduction

Amantadine which belongs to the adamantanamine group of compounds was found to act as a non-competitive antagonist at the NMDA receptor complex through binding at the phencyclidine (PCP) binding site inside the receptor-associated ion-channel (Kornhuber et al., 1994; Danysz et al., 1994). It is usually proposed that through this antagonism of glutamatergic inhibitory inputs on presynaptic dopaminergic neurons, amantadine enhances the dopaminergic neurotransmission (Quirion and Pert, 1982; Takahashi et al., 1996).

Although its pharmacological properties are similar to that of MK-801, the most thoroughly investigated non-competitive NMDA receptor antagonist, amantadine is generally well tolerated and largely devoid of psychomimetic effects (Porter and Greenamyre, 1995; Kornhuber and Weller, 1997). Hence, amantadine has been used clinically for the treatment of Parkinson's disease for more than a decade (Schwab et al., 1969; Lang and Blair, 1989).

While the effect of amantadine on dopamine release (Farnebo et al., 1971; Heimans et al., 1972) and synthesis (Scatton et al., 1970; Fisher et al., 1998) have been clearly demonstrated, its mechanism of action through the neuronal dopamine transporter (DAT) activity is still unclear. Many in vitro studies have shown that the doses required to directly affect the dopamine uptake were higher than those used clinically (Baldessarini et al., 1972; Heikkila and Cohen, 1972; Brown and Redfern, 1976). On the basis of the primary role of DAT in controlling the intensity and duration of the synaptic response by regulating the concentration and availability of dopamine to downstream receptors (Giros et al., 1996) it appears important to clarify the effect of amantadine on the dopamine uptake system.

For this purpose, we have investigated the effect of short- and long-term amantadine treatments on the DAT activity by measuring [^{3}H]dopamine uptake in striatal synaptosomes prepared from rats treated with this drug. In parallel, the effect of such treatment on the DAT expres-

* Corresponding author. Tel.: +32-2-764-5317; fax: +32-2-764-5460.
E-mail address: guylene.page@farl.ucl.ac.be (G. Page).
[1] Contributed equally to this study.

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00573-2

sion was studied by measuring the binding of [^{3}H]GBR-12935, a specific dopamine uptake inhibitor.

2. Materials and methods

2.1. Chemicals

The chemicals were purchased from the following sources: [7,8-^{3}H]dopamine (spec. act. 47 Ci/mmol) from Amersham Pharmacia Biotech Belgium (Gent, Belgium), dopamine hydrochloride (Dynatra 200) from Sintesa Prodesfarma group (Belgium), [^{3}H]GBR-12935 hydrochloride (spec. act. 53.5 Ci/mmol) from NEN (Belgium), amantadine hydrochloride from Sigma-Aldrich (St. Louis, MO, USA), nomifensine maleate from Research Biochemicals International (Natick, MA, USA), 1,4-dithiotreitol and bovine serum albumin from ICN Biochemicals (Germany). All other chemicals were of the purest quality available and were purchased from Merck Eurolab (Belgium).

2.2. Animals and treatment

Male Wistar rats, weighing 250–300 g were acclimatised at constant temperature (23 ± 1°C) and relative humidity (45 ± 5%) for at least 1 week on a 12-h light, 12-h dark cycle with access ad libitum to both food and water.

The animals were injected once daily with either vehicle (NaCl 0.9%) or amantadine (40 mg/kg; i.p.) for 2, 7 and 14 days. After 24-h washout period the rats were sacrificed by decapitation. Brains were rapidly removed and the striata dissected on an ice-cooled dish according to Glowinski and Iversen (1966). Striata were weighed and used to prepare synaptosomes or striatal membranes.

Experimental protocols were approved by the local ethical committee and meet the guidelines of the responsible governmental agency (Administration de la Santé Animale et de la Qualité des Produits Animaux, Services Vétérinaires du Ministère, Brussels).

2.3. Preparation of striatal synaptosomes

Synaptosomes were prepared as previously described by Bonnet and Costentin (1989), with minor modifications. Striatum were homogenised in 20 vol. of 0.32 M sucrose using 10 up-and-down strokes of a prechilled Teflon-glass homogenizer. The homogenates were then centrifuged at $1{,}000 \times g$ for 10 min at 4°C to give a nuclear pellet. Supernatants were stored at 4°C and the pellet was resuspended in 20 vol. of 0.32 M sucrose and centrifuged for 10 min at $1{,}000 \times g$. The two supernatants were then pooled and centrifuged at $17{,}500 \times g$ for 30 min at 4°C, after which the supernatant was discarded and the final pellet resuspended in ice-cold Krebs–Ringer buffer (120 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 6 mM glucose, pH 7.6).

2.4. Preparation of striatal membranes

The striata were dissected out on ice and immediately homogenised, using a Teflon-glass homogenizer, in 50 volumes of ice-cold 50 mM Tris–HCl (pH 7.4). The homogenate was centrifuged at $600 \times g$ for 10 min at 4°C, and the supernatant obtained was centrifuged for 10 min at $49{,}000 \times g$. The membranes were washed, resuspended in 100 vol. of ice-cold 50 mM Tris–HCl buffer (pH 7.4) and centrifuged for 10 min at $49{,}000 \times g$ twice. The membranes were resuspended in 20 volumes of ice-cold buffer containing 50 mM Tris–HCl (pH 7.4), 1 mM EDTA, 5 mM $MgCl_2$, and 150 mM NaCl.

2.5. Measurement of dopamine uptake

Dopamine uptake was assayed using tritiated ([^{3}H] dopamine, 10 nM) and non-radiolabelled dopamine to obtain final concentrations in the 25–500 nM range. Synaptosomes (from 3 mg of original tissue) were incubated in 1 ml of Krebs–Ringer buffer with dopamine for 2 min at 37°C. Non-specific dopamine uptake was measured in the presence of 10 μM nomifensine (dopamine uptake inhibitor). Dopamine uptake was stopped by addition of 3 ml ice-cold Krebs–Ringer buffer. The suspension was immediately filtered under vacuum through Whatman GF/C filters. Vials and filters were washed twice with 3 ml of ice-cold Krebs–Ringer buffer. The filters were placed in scintillation vials containing 5 ml of Aqualuma (Lumac, Groningen, Netherlands) before being stored at room temperature overnight. Radioactivity was determined by liquid scintillation spectrometry. Specific [^{3}H]dopamine uptake, defined as the difference between dopamine accumulated at 37°C in the presence and absence of nomifensine, was expressed as pmol/mg protein/min.

Protein content was measured by the method of Peterson (1977) using bovine serum albumin as standard.

2.6. [^{3}H]GBR-12935 binding assay

This binding experiment was performed at 22°C in plastic tubes containing 20 μg protein resuspended in a final volume of 1 ml binding buffer containing 50 mM Tris–HCl (pH 7.4), 1 mM EDTA, 5 mM $MgCl_2$, 150 mM NaCl, 1 mM dithiotreitol, 0.1% sodium metabisulfite and 0.1% bovine serum albumin. This binding was initiated by the addition of [^{3}H]GBR-12935 over a concentration range of 0.1 to 6 nM. Non-specific binding was measured in the presence of 10 μM nomifensine. Incubation was performed for 30 min and terminated by addition of 3 ml ice-cold washing buffer (50 mM Tris–HCl (pH 7.4), 1 mM EDTA, 5 mM $MgCl_2$, 150 mM NaCl). The suspension was immediately filtered under vacuum through Whatman GF/B filters previously soaked for 1 h in 0.5% polyeth-

yleneimine. Vials and filters were washed rapidly twice with 3 ml of ice-cold washing buffer. The filters were placed in scintillation vials containing 5 ml of Aqualuma (Lumac, Groningen, Netherlands) before being stored at room temperature overnight. Radioactivity was determined by liquid scintillation spectrometry.

2.7. Data analysis

Data from the dopamine uptake and [^{3}H]GBR-12935 binding experiments were analysed by non-linear regression using the curve fitting program GraphPad Prism® (GraphPad Software, San Diego, CA, USA). Statistical analysis of the data was performed using SPSS® software (Systat, Chicago). Values of DA uptake and [^{3}H]GBR 12935 binding in vehicle- and amantadine-treated rats were compared using either paired *t*-test (saturation curves) or one-way analysis of variance (ANOVA) followed by Scheffé's test for multiple comparisons between all sets of data (V_{max} and K_m; B_{max} and K_d). The mean difference was considered for $P < 0.05$.

3. Results

3.1. Dopamine uptake

The uptake of increasing dopamine concentrations was measured on rat striatal synaptosomes prepared from amantadine- and vehicle-treated animals (Fig. 1). After 7 days of treatment, saturation curves corresponding to amantadine-treated rats were found to be significantly different from controls ($P < 0.05$ for 25 to 250 nM and $P < 0.005$ for 350 to 500 nM). Whereas the apparent affinity constant K_m was not significantly affected by the treatment with amantadine, the apparent maximal velocity V_{max} was significantly increased by 30% as compared to the control value (Table 1). In contrast, no difference in these kinetic parameters was observed after 2 and 14 days of amantadine treatment (Table 1).

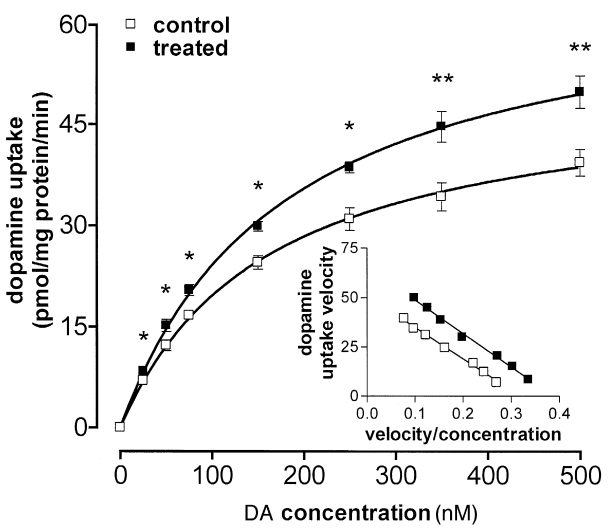

Fig. 1. Effect of a 7-day treatment with amantadine on dopamine uptake in rat striatal synaptosomes. Synaptosomes were incubated for 2 min at 37°C with dopamine over a concentration range of 25–500 nM as described in materials and methods. Results are expressed as the specific dopamine uptake and are the mean ± S.E.M. of four experiments performed in duplicate. Saturation curves corresponding to the amantadine-treated rats were found significantly different from controls: $^*P < 0.05$; $^{**}P < 0.005$ (paired *t*-test). Inset: Eadie–Hofstee analysis of the specific dopamine uptake.

Table 1
Kinetic parameters of dopamine uptake in rat striatal synaptosomes after chronic amantadine treatment

	V_{max} (pmol/mg protein/min)		K_m (μM)	
	Control	Treated	Control	Treated
2 days	48.53 ± 3.61	46.39 ± 3.97	0.111 ± 0.002	0.103 ± 0.005
7 days	51.12 ± 2.36	67.30 ± 2.70[a,b]	0.160 ± 0.018	0.179 ± 0.017
14 days	45.84 ± 6.83	41.14 ± 4.63	0.161 ± 0.033	0.114 ± 0.030

Apparent V_{max} and K_m values were determined from saturation curves of dopamine uptake as shown in Fig. 1, by non-linear regression. The values of V_{max} expressed as pmol/mg protein/min and K_m as μM are the mean ± S.E.M. of four experiments performed in duplicate. The kinetic parameters measured in rat striatal synaptosomes from amantadine- and vehicle-treated rats were compared using a one-way analysis of variance (ANOVA) followed by Scheffé's test.

[a] $P < 0.05$ to 2 days treatment.

[b] $P < 0.05$ to 14 days treatment.

3.2. [^{3}H]GBR-12935 binding

The density of DAT was determined on rat striatal membranes using the potent and selective dopamine uptake inhibitor [^{3}H]GBR-12935. Saturation curves obtained on membranes vehicle-treated animals revealed K_d and B_{max} values in accordance to previous reports from the literature (e.g. Janowsky et al., 1986). As shown in Table 2, none of the treatment with amantadine was found to affect the K_d and B_{max} values of the [^{3}H]GBR-12935 binding.

Table 2
Pharmacological parameters of [^{3}H]GBR-12935 binding to rat striatal membranes after chronic amantadine treatment

	B_{max} (pmol/mg protein)		K_d (nM)	
	Control	Treated	Control	Treated
2 days	5.50 ± 0.35	5.34 ± 0.50	2.19 ± 0.46	2.56 ± 0.26
7 days	6.00 ± 0.49	5.31 ± 0.25	2.92 ± 0.35	2.91 ± 0.27
14 days	5.44 ± 0.43	5.60 ± 0.81	2.45 ± 0.16	3.03 ± 0.46

As described in materials and methods, data obtained from saturation curve of [^{3}H]GBR-12935 specific binding in rat striatal membranes were analysed by non-linear regression. Maximal binding capacity B_{max} (pmol/mg protein) and the affinity constant K_d (nM) are the mean ± S.E.M. of four experiments performed in duplicate. The statistical analysis using a one-way analysis of variance ANOVA followed by Scheffé's test revealed no significant change between amantadine- and vehicle-treated rats.

4. Discussion

The aim of this study was to investigate the effect of short- and long-term amantadine treatments on the activity of the DAT. Our results showed that a treatment with amantadine for 7 days increased the apparent maximal velocity of DAT by 30% without modification of the apparent affinity. In contrast, no change in the density of striatal DAT was observed, indicating that the increased DAT activity did not result from a modification of its expression.

In many studies, it has been suggested that the dopamine release induced by an acute amantadine treatment was the result of NMDA channel blocking properties of this drug (Quirion and Pert, 1982; Mizoguchi et al., 1994; Takahashi et al., 1996). Through this mechanism, amantadine could antagonize the glutamatergic inhibitory inputs on striatal dopaminergic neurons. The enhancement of the dopaminergic neurotransmission by amantadine explains why this drug has been successfully used in Parkinson's disease (Lang and Blair, 1989; Uitti et al., 1996). Another mechanism whereby drugs may increase the extracellular dopamine concentration is the blockade of the neuronal dopamine transporter by which the neurotransmitter is primary cleared from the synaptic cleft. However, the effect of amantadine on the activity of the DAT is still discussed (Baldessarini et al., 1972; Herblin, 1972; Brown and Redfern, 1976; Gianutsos et al., 1985). Most of these in vitro studies showed that amantadine could inhibit the dopamine uptake but authors specified that high concentrations of amantadine were required to produce significant inhibition of dopamine uptake (Fletcher and Redfern, 1970; Heikkila and Cohen, 1972; Brown and Redfern, 1976). At the doses used clinically, it appears unlikely that the central stimulation caused by amantadine could be due to the blockade of DAT activity.

In our study, rats received an i.p. injection of amantadine at 40 mg/kg. Following such an injection, it was demonstrated that the extracellular amantadine concentration in striatal microdialysates was in the range of its K_i-value at the PCP binding site, i.e. 10 μM, (Kornhuber et al., 1995).

The results of the present study clearly show that amantadine treatment resulted in a slowly developing but transient enhancement of the functionality of the DAT. To explain these results, one may propose that, as shown for MK-801 (Snell and Johnson, 1988; Nishimura et al., 1998) and ketamine, another high-affinity non-competitive antagonist of the NMDA receptors (Johnson and Snell, 1985; Nishimura and Sato, 1999), amantadine may directly affect the dopamine uptake. However, this is unlikely since amantadine was found to enhance the dopamine transporter activity whereas MK-801 and ketamine were found to inhibit the uptake probably through their binding to the DAT. Moreover, this enhancement was found to develop slowly and seems not compatible with a direct interaction of amantadine with the dopamine transporter. Finally, Sonders et al. (1997) reported that the high affinity dopamine transporter is insensitive to therapeutically relevant concentrations of amantadine.

Although the importance of PKC and PKA mediated phosphorylation of the DAT in the control of its activity is well established (Vaughan, 2000), little is known about the endogenous neurotransmission inputs of the striatum that could modulate the activity of these kinases and thereby, of the dopamine transporter. The existence of glutamatergic controls of dopaminergic neurons in the striatum is well documented (Cepeda and Levine, 1998) and therefore, one may propose a direct control of the DAT phosphorylation state by glutamate receptors. Through the antagonism at ionotropic glutamate receptors, amantadine could interfere with the endogenous glutamate mediated phosphorylation of the DAT and thereby promotes its activity. However, such mechanism does not appear compatible with the delayed response to amantadine treatment observed in the present study.

Alternatively, the increase in dopamine uptake observed after amantadine treatment could constitute a compensatory mechanism to preserve the physiological synaptic dopamine concentration. This hypothesis was originally proposed by Gordon et al. (1996) who demonstrated that a treatment with amantadine for 21 days increased the expression of the DAT in rat striatal membranes. It is well known that dopamine autoreceptors provide an important inhibitory feedback mechanism for dopamine neurons in response to changes in extracellular levels of dopamine. Both in vivo and in vitro studies using microdialysis or electrochemical methods have found that dopamine D_2-like receptors agonists as quinpirole increased rat striatal DAT activity. This upregulation was blocked by dopamine D_2-like receptors antagonists as sulpiride and raclopride (Meiergerd et al., 1993; Cass and Gerhardt, 1994). On that basis, one may suggest that the increased dopamine uptake observed after amantadine treatment results from an indirect modulation of the DAT activity by presynaptic dopamine D_2 receptors, in response to increased dopamine release. Furthermore, this sustained elevation of dopamine in the synaptic cleft could result in a progressive upregulation of presynaptic dopamine D_2 receptors (Gianutsos et al., 1985), whose putative role in the control of the DAT activity would thereby be enhanced. The regulation of the DAT activity by dopamine D_2 receptor activation could involve the modulation of its phosphorylation by PKA. However, although a role for presynaptic dopamine D_2 receptors in the control of DAT activity has been clearly demonstrated (Meiergerd et al., 1993; Dickinson et al., 1999), the involvement of PKA mediated phosphorylation of the striatal transporter is still controversial (Batchelor and Schenk, 1998; Pristupa et al., 1998). Interestingly, the control of the DAT activity by presynaptic dopamine D_2 receptor was shown to mainly result from a modification of the functionality of the transporter without modification

of its expression (Dickinson et al., 1999). This is in complete accordance with the present study showing the lack of effect of amantadine on the DAT expression measured on striatal membranes. Up to now, we cannot explain the discrepancy between our results and those from Gordon et al. (1996) showing the increased expression of DAT in a similar study (see above). Unfortunately, the modulation of the dopamine uptake was not characterised in this previous study. It is noteworthy that such adaptive process was transiently observed, since experiments performed after 14 days of treatment revealed uptake characteristics similar to those obtained in control animals. Indeed, little is known about the long-term response to amantadine at the level of dopamine release since most microdialysis have been focused on the immediate or short-term effect of amantadine treatment.

This study demonstrated that a long-term treatment with amantadine increases the dopamine transporter activity. This probably reflects a compensatory mechanism to the enhanced release of dopamine in the synaptic cleft and constitutes a relevant example of in vivo regulation of the DAT activity by a drug that does not directly interact with dopaminergic systems.

Acknowledgements

The authors thank H. Lenaert for his excellent technical assistance. This work was supported by the National Fund for Scientific Research (F.N.R.S., Belgium, Convention FRSM 3.4561.98) and by the Belgian Queen Elisabeth Medical Foundation. M. Peeters and E. Hermans are Research Assistant and Senior Research Associate of the National Fund for Scientific Research, respectively.

References

Baldessarini, R.J., Lipinski, J.F., Chace, K.V., 1972. Effects of amantadine hydrochloride on catecholamine metabolism in the brain of the rat. Biochem. Pharmacol. 21, 77.

Batchelor, M., Schenk, J.O., 1998. Protein kinase A activity may kinetically upregulate the striatal transporter for dopamine. J. Neurosci. 18, 10304–10309.

Bonnet, J.J., Costentin, J., 1989. Correlation between [^{3}H]-dopamine specific uptake and [^{3}H]-GBR 12783 specific binding during the maturation of rat striatum. Life Sci. 44, 1759–1765.

Brown, F., Redfern, P.H., 1976. Studies on the mechanism of action of amantadine. Br. J. Pharmacol. 58, 561–567.

Cass, W.A., Gerhardt, G.A., 1994. Direct in vivo evidence that D_2 dopamine receptors can modulate dopamine uptake. Neurosci. Lett. 176, 259–263.

Cepeda, C., Levine, M.S., 1998. Dopamine and *N*-methyl-D-aspartate receptor interactions in the neostriatum. Dev. Neurosci. 20, 1–18.

Danysz, W., Gossel, M., Zajaczkowski, W., Dill, D., Quack, G., 1994. Are NMDA antagonistic properties relevant for antiparkinsonian-like activity in rats? — Case of amantadine and memantine. J. Neural. Transm. 7, 155–166.

Dickinson, S.D., Sabeti, J., Larson, G.A., Giardina, K., Rubinstein, M., Kelly, M.A., Grandy, D.K., Low, M.J., Gerhardt, G.A., Zahniser, N.R., 1999. Dopamine D_2 receptor-deficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum. J. Neurochem. 72, 148–156.

Farnebo, L-O., Fuxe, K., Goldstein, M., Hamberger, B., Ungerstedt, U., 1971. Dopamine and noradrenaline releasing action of amantadine in the central and peripheral nervous system: a possible mode of action in Parkinson's disease. Eur. J. Pharmacol. 16, 27–38.

Fisher, A., Biggs, C.S., Starr, M.S., 1998. Differential effects of NMDA and non-NMDA antagonists on the activity of aromatic L-amino acid decarboxylase activity in the nigrostriatal dopamine pathway of the rat. Brain Res. 792, 126–132.

Fletcher, E.A., Redfern, P.H., 1970. The effect of amantadine on the uptake of dopamine and noradrenaline by rat brain homogenates. J. Pharm. Pharmacol. 22, 957–958.

Gianutsos, G., Chute, S., Dunn, J.P., 1985. Pharmacological changes in dopaminergic systems induced by long-term administration of amantadine. Eur. J. Pharmacol. 110, 357–361.

Giros, B., Jaber, M., Jones, S.R., Wightman, R.M., Caron, M.G., 1996. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379, 606–612.

Glowinski, J., Iversen, L.L., 1966. Regional studies of catecholamines in rat brain. J. Neurochem. 13, 655–663.

Gordon, I., Weizman, R., Rehavi, M., 1996. Modulatory effect of agents active in the presynaptic dopaminergic system on the striatal dopamine transporter. Eur. J. Pharmacol. 298, 27–30.

Heikkila, R.E., Cohen, G., 1972. Evaluation of amantadine as a releasing agent or uptake blocker for H^3-dopamine in rat brain slices. Eur. J. Pharmacol. 20, 156–160.

Heimans, R.L., Rand, M.J., Fennessy, M.R., 1972. Effects of amantadine on uptake and release of dopamine by a particulate fraction of rat basal ganglia. J. Pharm. Pharmacol. 24, 875–879.

Herblin, W.F., 1972. Amantadine and catecholamine uptake. Biochem. Pharmacol. 21, 1993–1995.

Janowsky, A., Berger, P., Vocci, F., Labarca, R., Skolnick, P., Paul, S.M., 1986. Characterization of sodium-dependent [^{3}H]GBR-12935 binding in brain: a radioligand for selective labelling of the dopamine transport complex. J. Neurochem. 46, 1272–1276.

Johnson, K.M., Snell, L.D., 1985. Effects of phencyclidine (PCP)-like drugs on turning behavior, ^{3}H-dopamine uptake, and ^{3}H-PCP binding. Pharmacol. Biochem. Behav. 22, 731–735.

Kornhuber, J., Weller, M., 1997. Psychotogenicity and *N*-methyl-D-aspartate receptor antagonism: implications for neuroprotective pharmacotherapy. Biol. Psychiatry 41, 135–144.

Kornhuber, J., Weller, M., Schoppmeyer, K., Riederer, P., 1994. Amantadine and memantine are NMDA receptor antagonists with neuroprotective properties. J. Neural. Transm. 43, 91–104.

Kornhuber, J., Quack, G., Danysz, W., Jellinger, K., Danielczyk, W., Gsell, W., Riederer, P., 1995. Therapeutic brain concentration of the NMDA receptor antagonist amantadine. Neuropharmacology 34, 713–721.

Lang, A.E., Blair, R.D.G, 1989. Anticholinergic drugs and amantadine in the treatment of Parkinson's disease. In: Calne, D.B. (Ed.), Drugs for the Treatment of Parkinson's Disease. Springer-Verlag, New York, pp. 307–323.

Meiergerd, S.M., Patterson, T.A., Schenk, J.O., 1993. D_2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies in vitro and in vivo. J. Neurochem. 61, 764–767.

Mizoguchi, K., Yokoo, H., Yoshida, M., Tanaka, T., Tanaka, M., 1994. Amantadine increases the extracellular dopamine levels in the striatum by re-uptake inhibition and by *N*-methyl-D-aspartate antagonism. Brain Res. 662, 255–258.

Nishimura, M., Sato, K., 1999. Ketamine stereoselectively inhibits rat dopamine transporter. Neurosci. Lett. 274, 131–134.

Nishimura, M., Sato, K., Okada, T., Schloss, P., Shimada, S., Tohyama, M., 1998. MK-801 blocks monoamine transporters expressed in HEK cells. FEBS Lett. 423, 376–380.

Peterson, G.L., 1977. A simplification of the protein assay method of Lowry et al. Which is more generally applicable. Anal. Biochem. 83, 346–356.

Porter, R.H.P., Greenamyre, J.T., 1995. Regional variations in the pharmacology of NMDA receptor channel blockers: implications for therapeutic potential. J. Neurochem. 64, 614–623.

Pristupa, Z.B., McConkey, F., Liu, F., Man, H.Y., Lee, F.J.S., Wang, Y.T., Niznik, H.B., 1998. Protein kinase-mediated bidirectional trafficking and functional regulation of the human dopamine transporter. Synapse 30, 79–87.

Quirion, R., Pert, C.B., 1982. Amantadine modulates phencyclidine binding site sensitivity in rat brain. Experientia 38, 955–956.

Scatton, B., Cheramy, A., Besson, M.J., Glowinski, J., 1970. Increased synthesis and release of dopamine in the striatum of the rat after amantadine treatment. Eur. J. Pharmacol. 13, 131–133.

Schwab, R.S., England, A.C., Poskanzer, D.C., Young, R.R., 1969. Amantadine in the treatment of Parkinson's disease. JAMA 208, 1168–1170.

Snell, L.D., Johnson, K.M., 1988. Comparison of the effects of MK-801 and phencyclidine on catecholamine uptake and NMDA-induced norepinephrine release. Eur. J. Pharmacol. 145, 223–226.

Sonders, M.S., Zhu, S-J., Zahniser, N.R., Kavanaugh, M.P., Amara, S.G., 1997. Multiple ionic conductances of the human dopamine transporter: the actions of dopamine and psychostimulants. J. Neurosci. 17, 960–974.

Takahashi, T., Yamashita, H., Zhang, Y-X., Nakamura, S., 1996. Inhibitory effect of MK-801 on amantadine-induced dopamine release in the rat striatum. Brain Res. Bull. 41, 363–367.

Uitti, R.J., Rajput, A.H., Ahlskog, J.E., Offord, K.P., Schroeder, D.R., Ho, M.M., Prasad, M., Rajput, A., Basran, P., 1996. Amantadine treatment is an independent predictor of improved survival in Parkinson's disease. Neurology 46, 1551–1556.

Vaughan, R.A., 2000. Regulation of dopamine transporter by phosphorylation and impact on cocaine action. In: Reith, M.E.A. (Ed.), Cerebral Signal Transduction: From First To Fourth Messengers. Humana Press, Totowa, NJ, pp. 374–399.