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European Journal of Pharmacology 399 (2000) 151–160

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Muscarinic antagonists in substantia nigra influence the decarboxylation of L-dopa in striatum

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Received 26 April 2000; accepted 5 May 2000

Abstract

This study was designed to investigate whether anticholinergic drugs acting at the level of the substantia nigra can affect basal extracellular dopamine concentrations and the levodopa (L-dopa)-induced increases in dopamine levels in the striatum. Dual probe in vivo microdialysis in freely moving rats was used. One microdialysis probe was implanted in the substantia nigra and the other in the ipsilateral striatum. Muscarinic receptor antagonists were perfused into the substantia nigra and changes in neurotransmitter levels in the substantia nigra and at the axon terminals in the striatum were monitored simultaneously. Nigral perfusion of the non-selective muscarinic receptor antagonist trihexyphenidyl (1 mM) produced an increase in extracellular dopamine and γ-aminobutyric acid (GABA) levels in the substantia nigra. Perfusion with the muscarinic M_1 receptor antagonist telenzepine (0.1 μM) produced a significant decrease in nigral dopamine and GABA levels in the substantia nigra. The muscarinic M_2 receptor antagonist methoctramine (75 μM) produced an increase in dopamine levels in the substantia nigra. No significant changes in nigral extracellular GABA levels were observed. The L-dopa-induced increases in extracellular dopamine levels in the striatum were clearly attenuated under nigral perfusion of these drugs.

This in vivo study demonstrates that anticholinergic drugs perfused at the level of the substantia nigra can modulate dopamine and GABA levels and attenuate the L-dopa decarboxylation in the striatum, possibly via modulation of the nigrostriatal dopaminergic system. We add further evidence that the substantia nigra is an important site of action of antimuscarinic drugs. The attenuation of L-dopa-induced dopamine release in the striatum exerted by nigral perfusion of these antimuscarinic drugs is probably mediated via different mechanisms. This attenuation is regarded as a beneficial effect of the muscarinic antagonists as adjuncts to L-dopa in Parkinson's disease treatment. We postulate that drugs that enhance dopamine release, after L-dopa administration, in a less extreme way than L-dopa administered on its own could prevent further neurodegeneration and dyskinesias thought to result from extremely high extracellular dopamine levels following L-dopa treatment.

Keywords: Dopamine; Acetylcholine; GABA (γ-aminobutyric acid); Substantia nigra; Striatum; Antimuscarinic drug; L-Dopa

1. Introduction

The therapeutic efficacy of anticholinergic drugs in the treatment of Parkinson's disease and in the management of extrapyramidal side effects of neuroleptics, introduced the concept of a functional equilibrium between central dopamine and acetylcholine neurotransmission in the control of motor behaviour. Clinical manifestations of Parkinson's disease were ascribed to a dopamine–acetylcholine imbalance in favour of acetylcholine. The assumption of a functional interaction between dopamine–acetylcholine and the therapeutic effects of both dopaminomimetics and anticholinergic drugs in Parkinson's disease has been described primarily at the level of striatum. However, since these drugs are administered systemically in Parkinson's disease treatment, they can also act at other sites in the central nervous system such as the substantia nigra. Many studies have now shown the importance of the substantia nigra in control of movement. The substantia nigra together with the entopeduncular nucleus is the major output of the basal ganglia in the basal ganglia–thalamo-cortical motor loop (for review, see Albin et al., 1989; Alexander and Crutcher, 1990; Gerfen, 1992; Hoover and Strick, 1993; Chesselet and Delfs, 1996). Furthermore, we pointed out the importance of the substantia nigra together with the

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00353-8

striatum, as the site of action of dopamine receptor agonists, and the dopamine precursor levodopa (L-dopa) as well (Sarre et al., 1992, 1998).

Several studies indicate that acetylcholine can modulate the dopaminergic nigrostriatal pathway at the level of the substantia nigra. For instance, Blaha and Winn (1993) using in vivo chronoamperometry and intracerebral microdialysis techniques, observed that intranigral microinjections of the cholinergic agonists nicotine, carbachol or infusion of the cholinesterase inhibitor neostigmine resulted in an increase in the amperometric signal corresponding to dopamine overflow in the striatum. In the substantia nigra of the rat, the presence of cholinergic acetylcholine terminals originating from the pedunculopontine nucleus has been described to make synaptic contacts with dopamine neurons (Bolam et al., 1991). The acetylcholine terminals were found in both parts of the substantia nigra, the pars compacta and the pars reticulata (Beninato and Spencer, 1987; Gould et al., 1989). The presence of both nicotinic and muscarinic receptors in the substantia nigra supports a functional role of acetylcholine (Clarke et al., 1987; Cortez and Palacios, 1986; Nastuk and Graybiel, 1991) and a cholinergic mediation of dopamine neurons (Blaha and Winn, 1993) in this structure.

The efficacy of antimuscarinic drugs as adjuncts to L-dopa in Parkinson's disease treatment is well known. L-dopa remains the most effective treatment for Parkinson's disease. However, its clinical benefits become compromised by side effects such as dyskinesias, which are believed to result from excessive dopaminergic activity after L-dopa treatment (Gerlach, 1977; Fahn, 1989; Kostrzewa, 1995; Nutt and Holford, 1996; Sarre et al., 1996). Therefore, we postulated that drugs that affect dopamine release (after L-dopa administration) in a less extreme way could prevent further neurodegeneration and the dyskinesias thought to result from extremely high extracellular dopamine levels following L-dopa treatment (Sarre et al., 1996, 1998; Izurieta-Sànchez et al., 1998; Hastings and Zigmond, 1997).

In a previous study, we showed that intrastriatal application of the non-selective antimuscarinic drug trihexyphenidyl (1 mM) had no influence on the L-dopa-induced dopamine release in striatum. However, systemic application of the drug (1.5 mg/kg) attenuated this effect. In the line of our hypothesis, we interpreted this as a beneficial effect of trihexyphenidyl. Furthermore, this study suggested that extrastriatal mechanisms mediated this attenuation (Izurieta-Sànchez et al., 1998).

This study was designed to further investigate whether antimuscarinic drugs perfused in the substantia nigra modulate nigral extracellular dopamine and γ-aminobutyric acid (GABA) levels and can affect extracellular dopamine concentrations in striatum. The influence of this modulation on the L-dopa-induced dopamine release at the level of the striatum was also studied. We used dual probe in vivo microdialysis in freely moving rats. One probe was implanted at the level of the substantia nigra and the other at the level of the ipsilateral striatum. Muscarinic receptor antagonists were perfused in the substantia nigra and changes in neurotransmitter levels in the substantia nigra and at the terminal axons in the striatum were monitored simultaneously. Towards our aim, we tested the effect of intranigral perfusion of the non-selective muscarinic receptor antagonist trihexyphenidyl, the muscarinic M_1 receptor antagonist telenzepine and the muscarinic M_2 receptor antagonist methoctramine, on basal dopamine and GABA levels in the substantia nigra and the striatum and on the L-dopa-induced increases in dopamine levels in the striatum.

2. Materials and methods

2.1. Chemicals

The following drugs and chemicals were obtained from the sources indicated: dopamine (Sigma, St. Louis, MO, USA), L-dopa (Merck Sharp and Dohme Research Laboratories, Rahway, NJ, USA). Trihexyphenidyl, methoctramine and telenzepine from Research Biochemicals Inc., Natick, MA, USA. Disodium hydrogen phosphate dodecahydrate, disodium EDTA and orthophosphoric acid were obtained from Merck. All other reagents were obtained from Merck Belgolabo (Overijse, Belgium).

2.2. Brain microdialysis and surgery

Male albino Wistar rats (250–300 g) on a standard diet were used. All the experiments were carried out according to the National Guidelines on Animal Experiments and were approved by the Ethics Committee for Animal Experiments of the Faculty of Medicine and Pharmacy of the Vrije Universiteit Brussels. All the experiments were carried out on freely moving rats. The animals were anaesthetized with a mixture of ketamine–diazepam (50:5 mg/kg i.p.) and placed on a stereotaxic frame. A 0.1% solution of lidocaine was administered subcutaneously under the scalp before starting the surgery. The skull was exposed and intracerebral guides with an inner cannula (CMA 10, CMA Microdialysis, Stockholm, Sweden) were implanted 3.0 mm above the dialysis area in the striatum (R: +1.2; L −2.4; V: +2.8) and 2.0 mm above the substantia nigra (R–5.0; L −1.4; V +6.5) for rats smaller than 270 g (König and Klippel, 1963) and (R −5.2; L −2.0; V +7.0) for rats bigger than 270 g (Paxinos and Watson, 1982). After surgery, 4 mg/kg ketoprofen was injected intraperitoneally. The rats were allowed to recover. Then the 2 mm and 3 mm probes were placed in substantia nigra and the striatum, respectively, after removal of the inner guide. The microdialysis probe was connected to a microinfusion pump (CMA 100, CMA Microdialysis, Stockholm, Sweden) and continuously per-

fused with a modified Ringer's solution (147.5 mM Na^+, 4 mM K^+, 1.1 mM Ca^{2+}, 153.7 mM Cl^-) at a flow rate of 2 μl/min. The next day, dialysates were collected every 20 min, from substantia nigra in vials containing 10 μl of an antioxidant mixture (0.01 M HCl, 0.1% $Na_2S_2O_5$, 0.01% Na_2EDTA). After collection of the dialysate, 15 μl was taken for the analysis of GABA and the rest was used for the analysis of dopamine. From the striatum, the dialysates were collected in vials containing 10 μl of antioxidant and used for the analysis of dopamine.

2.3. Solutions for drug administration

A stock solution of 2 mM L-dopa was made in the antioxidant mixture and diluted in modified Ringer's solution to obtain a concentration of 2 μM. Stock solutions of 1 mM of methoctramine and telenzepine were made in modified Ringer's solution and diluted to the final concentration of 75 μM for methoctramine and 0.1 μM for telenzepine. Trihexyphenidyl 1 mM was prepared in modified Ringer's solution. The choice of the trihexyphenidyl concentration is based on our previous work (Izurieta-Sànchez et al., 1998), in which we performed a dose–response curve (10 μM, 100 μM, 1 mM). We chose 1 mM as this dose induced changes in dopamine levels in the striatum. For telenzepine, a dose–response curve was performed in the substantia nigra (0.01, 0.1, 1 μM). The dose chosen (0.1 μM) is the lowest selective dose, which exerts the expected decrease in dopamine levels in substantia nigra (data not shown), as previously shown in striatum with pirenzepine (Smolders et al., 1997). The dose used of methoctramine was chosen based on former studies in the laboratory (Smolders et al., 1997).

2.4. Experimental protocol

In the experiments where L-dopa was administered alone in the striatum (control experiments), basal dialysates were collected for 180 min (nine collection periods). Then, using a liquid switch (CMA 110, CMA, Stockholm, Sweden), the perfusion fluid was changed for one collection period (20 min) to a similar perfusion fluid containing 2 μM L-dopa. Then it was switched back to the modified Ringer's solution till the end of the experiment. No dialysates were collected from substantia nigra in control experiments.

In the combined experiments (muscarinic receptor antagonists in substantia nigra-L-dopa in striatum), six basal dialysates were collected from the substantia nigra (six collection periods). Then, the perfusion fluid was switched to one containing trihexyphenidyl, methoctramine, or telenzepine and continued throughout the experiment. In striatum, after 280 min (14 collection periods) perfused with Ringer's solution, L-dopa was administered for 20 min (one collection period), then it was switched back to the Ringer's solution and the dialysates were further collected during another 180 min (nine collection periods).

After each experiment, the rats were killed with an overdose of pentobarbital. Verification of the placement of the probe in substantia nigra, was performed by histology following the protocol of the study by Sarre et al. (1998).

2.5. Analysis of the dialysates

For the analysis of dopamine, a reversed-phase (C8) microbore liquid chromatography assay with single-channel electrochemical detection was used, as described before (Smolders et al., 1996). The mobile phase consisted of an aqueous solution of Na-acetate 0.1 M, citric acid 20 mM, decane sulphonic acid 2 mM, and Na_2EDTA 0.1 mM (pH 5.5). To 200 ml of buffer, 27 ml of acetonitrile was added. The limit of quantification was 2.5 fmol/20 min (0.062 nM).

For GABA, reversed-phase (C8) microbore liquid chromatography with isocratic elution and electrochemical detection was routinely used after precolumn derivatization with *o*-phthalaldehyde/*tert*-butylthiol and iodoacetamide (Smolders et al., 1995a). The limit of quantification was 6 nM.

2.6. Data presentation and statistical analysis

The data are represented graphically. The effects of the muscarinic receptor antagonists on basal dopamine and GABA output in the substantia nigra and the striatum and the effects of L-dopa on extracellular striatal dopamine in controls and under perfusion of the antimuscarinic drugs are expressed in nanomolar (nM) not corrected for the relative recovery of the microdialysis probe. The basal value is taken as the mean of six stable neurotransmitter dialysate concentrations obtained in basal conditions, before the antimuscarinic drugs were perfused through the microdialysis probe.

For statistical differences of extracellular neurotransmitter concentrations under drug treatment in comparison with basal levels, one-factor analysis of variance (ANOVA) for repeated measures and Fisher's protected least significant difference (Fisher's PLSD) post hoc tests were used. Comparisons of peak drug effects of L-dopa with and without antimuscarinic drugs were analysed with the Mann–Whitney test. The level of significance for all analyses was set at $\alpha = 0.05$.

3. Results

3.1. Basal values of dopamine in the striatum and dopamine and GABA in the substantia nigra

Basal dialysate levels (mean ± S.E.M.) for dopamine in striatum and for dopamine and GABA in substantia nigra are shown in Table 1.

Table 1
Basal dialysate concentrations of the neurotransmitters monitored in substantia nigra and striatum. Concentrations are expressed as nanomolar. Each value is the mean ± S.E.M.

	Dopamine	GABA
Striatum	1.17 ± 0.25 ($n = 26$)	
Substantia nigra	0.25 ± 0.04 ($n = 12$)	25.27 ± 2.7 ($n = 12$)

3.2. Effect of intranigral perfusion of the non-selective muscarinic receptor antagonist trihexyphenidyl (1 mM) on extracellular neurotransmitter levels in the substantia nigra and the striatum

In the substantia nigra, intranigral perfusion of trihexyphenidyl caused a significant increase in nigral dopamine release (ANOVA: $P < 0.02$). The increase was about two-fold, reaching the concentration peak in the second collection period following trihexyphenidyl administration (time point $t_{40\ min}$) and then returning to baseline values (Fig. 1). A significant and sustained increase of about five-fold was observed in nigral GABA release after trihexyphenidyl perfusion (ANOVA: $P < 0.03$) (Fig. 2a).

In the striatum, nigral perfusion of trihexyphenidyl caused a slight increase in dopamine release without reaching statistical significance (Fig. 3).

3.3. Effect of intranigral perfusion of the muscarinic M_1 receptor antagonist telenzepine (0.1 μM) on extracellular neurotransmitter levels in the substantia nigra and the striatum

Under nigral perfusion of telenzepine, a significant and sustained decrease of about two-fold was observed in nigral dopamine release (ANOVA: $P < 0.001$) (Fig. 1). A significant and sustained decrease in GABA in the substantia nigra of about two-fold was observed (ANOVA: $P < 0.05$) (Fig. 2b). No changes in dopamine release in the striatum were observed (Fig. 3).

3.4. Effect of intranigral perfusion of the muscarinic M_2 receptor antagonist methoctramine (75 μM) on extracellular neurotransmitter levels in the substantia nigra and the striatum

Intranigral perfusion of methoctramine caused a significant increase on nigral dopamine release (three-fold) ($P < 0.001$) (Fig. 1). A slight decrease of about 32% was observed in GABA release in substantia nigra without reaching significance (Fig. 2b). The slight increase in extracellular dopamine levels in the striatum was not significant (Fig. 3).

3.5. Effect of intrastriatal L-dopa (2 μM) administration on extracellular dopamine levels in the striatum

In control experiments, 2 μM L-dopa induced a significant increase in dopamine (ANOVA: $P < 0.0001$), reaching a peak concentration during the collection period following L-dopa administration and then returning to baseline values. The relative dopamine increase was about 11-fold. In absolute amounts, dopamine increased from 1.6 ± 0.22 to 19.02 ± 2.8 nM (mean ± S.E.M., $n = 11$) (Fig. 4).

3.6. Effect of intranigral perfusion of trihexyphenidyl, telenzepine and methoctramine on the L-dopa-induced increases in extracellular dopamine levels in the striatum

Nigral perfusion of trihexyphenidyl counteracted the L-dopa-induced increases in extracellular dopamine levels

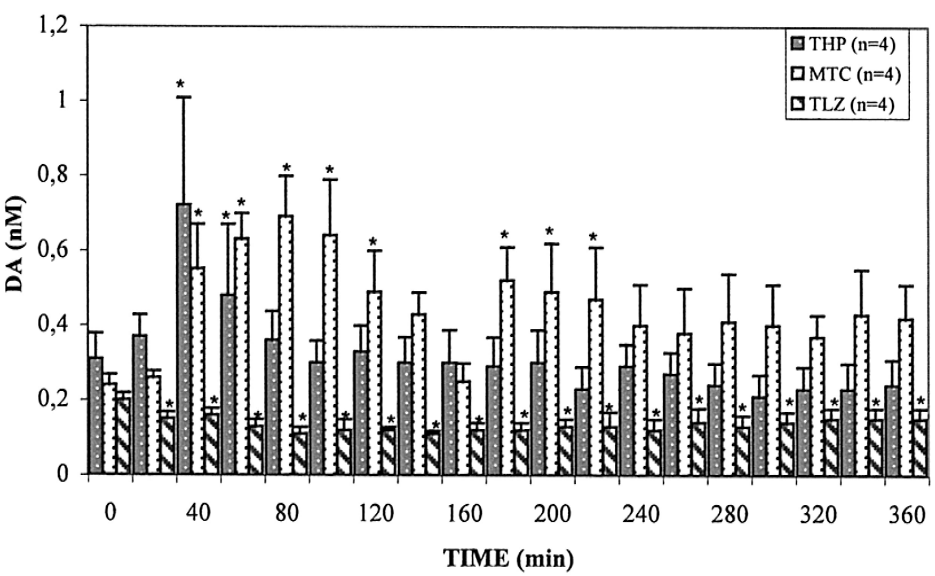

Fig. 1. Effect of nigral perfusion of trihexyphenidyl (THP) (1 mM), telenzepine (TLZ) (0.1 μM) and methoctramine (MTC) (75 μM), on nigral dopamine release. Dopamine is expressed as nanomolar (nM). Each value is the mean ± S.E.M. The 0-time point represents the mean baseline value. THP, TLZ or MTC, respectively, were administered from the 20-time point till the end of the experiment. The sign * ($P < 0.05$) represents values significantly different from the mean basal value.

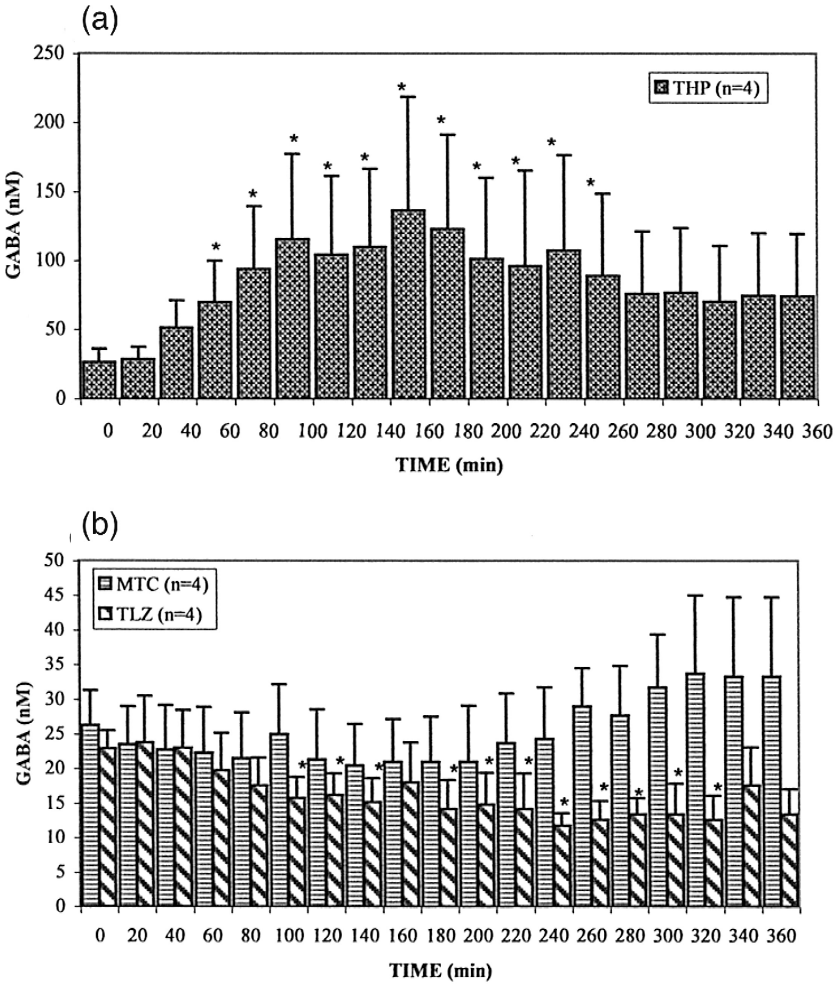

Fig. 2. Effect of nigral perfusion of THP (1 mM) (a), TLZ (0.1 μM) and MTC (75 μM) (b) on nigral GABA release. GABA is expressed as nanomolar (nM). Each value is the mean ± S.E.M. The 0-time point represents the mean baseline value. THP, TLZ or MTC were administered from the 20-time point till the end of the experiment. The sign * ($P < 0.05$) represents values significantly different from the mean basal value.

in the striatum. The peak dopamine concentration in striatum after L-dopa administration under nigral trihexyphenidyl perfusion was significantly lower than that in the control experiments ($P < 0.005$) (Fig. 4).

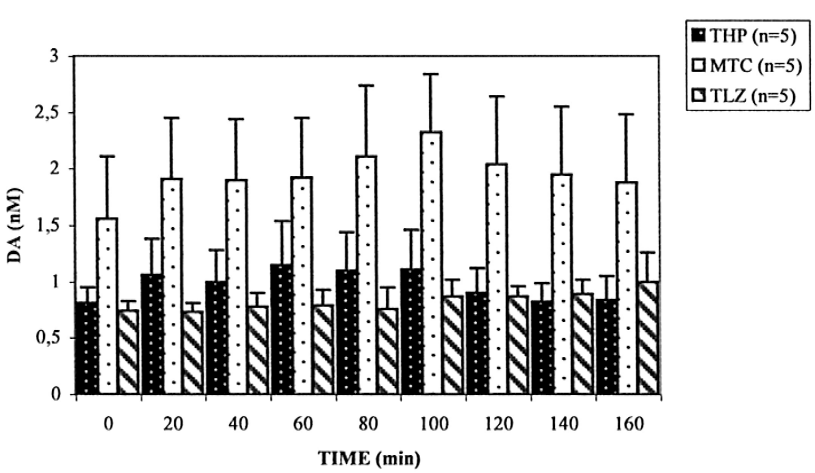

Fig. 3. Effect of nigral perfusion of trihexyphenidyl (THP) (1 mM), telenzepine (TLZ) (0.1 μM), and methoctramine (MTC) (75 μM) on the extracellular dopamine concentrations in the ipsilateral striatum. Dopamine is expressed as nanomolar (nM). Each value is the mean ± S.E.M. The 0-time point represents the mean baseline value. THP, TLZ or MTC were administered from the 20-time point onwards in the substantia nigra.

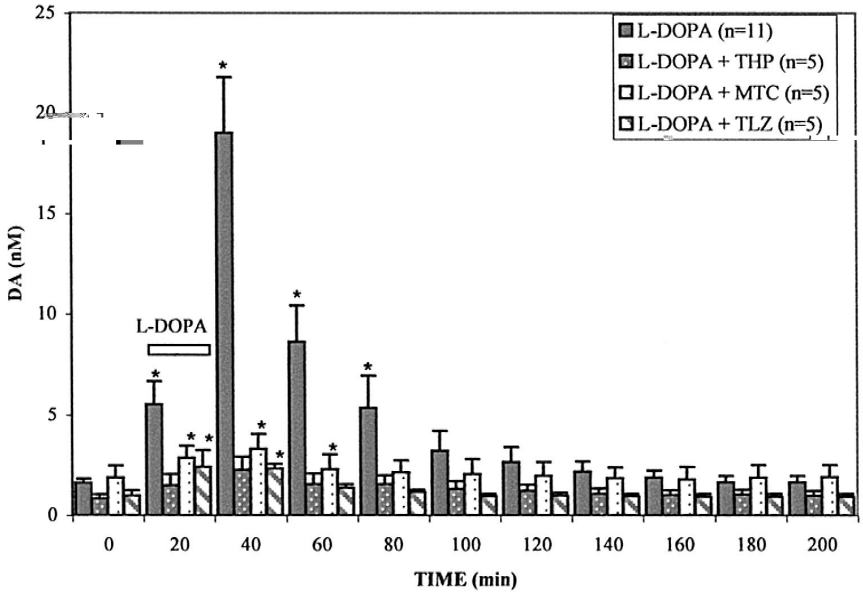

Fig. 4. Effect of nigral perfusion of trihexyphenidyl (THP) (1 mM), telenzepine (TLZ) (0.1 μM), and methoctramine (MTC) (75 μM) on the decarboxylation of L-dopa (2 μM) locally applied in the striatum. Dopamine is expressed as nanomolar (nM). Each value is the mean ± S.E.M. In the control group, the 0-time point represents the last basal value obtained before L-dopa administration. In the combined experiments, the 0-time point represents the last value obtained under perfusion of antimuscarinic drug before L-dopa administration. The antimuscarinic drugs were administered from 20-time point and continued till the end of the experiment. In both groups, L-dopa was administered at the 20-time point for 20 min indicated with the white bar. The sign * ($P < 0.05$) represents values significantly different from the mean basal value.

Under nigral perfusion of telenzepine, L-dopa induced a significant increase in extracellular dopamine levels (ANOVA: $P < 0.001$). The relative increase was about three-fold. In absolute amounts, dopamine increased from 0.74 ± 0.09 to 2.34 ± 0.22 nM (mean ± S.E.M., $n = 5$). The peak dopamine concentration in striatum after L-dopa administration under nigral perfusion of telenzepine was significantly lower than that in the control experiments ($P < 0.002$) (Fig. 4).

Under nigral perfusion of methoctramine, L-dopa induced a significant increase in extracellular dopamine levels in striatum (ANOVA: $P < 0.007$). The relative increase was about two-fold. In absolute amounts, dopamine increased from 1.56 ± 0.55 to 3.31 ± 0.75 nM min (mean ± S.E.M., $n = 5$). The peak dopamine concentration in striatum after L-dopa administration under nigral perfusion of methoctramine was significantly lower than that in the control experiments ($P < 0.003$) (Fig. 4).

4. Discussion

The modulation of nigral dopamine and GABA levels and the influence on the L-dopa-induced increases in dopamine levels in the striatum were studied, following perfusion of the selective and non-selective antimuscarinic drugs in the substantia nigra. The dual probe in vivo microdialysis was used in freely moving rats.

4.1. Effect of nigral perfusion of antimuscarinic drugs on basal neurotransmitter levels in the substantia nigra and the striatum

Muscarinic receptors in the substantia nigra have previously been classified as mixed muscarinic M_1/M_2 receptors or predominantly of the M_1 class based on their affinities for muscarinic receptor agonists and the muscarinic M_1 receptor antagonist pirenzepine, respectively (Cortez et al., 1984; Cortez and Palacios, 1986). Autoradiographic studies have also shown a high concentration of muscarinic M_3 receptors in the substantia nigra (Zubieta and Frey, 1993). The nigral component on which these receptors are localized is still unclear (Nastuk and Graybiel, 1991). However, in situ hybridization histochemistry showed that muscarinic M_5 receptors are the only muscarinic receptors expressed on dopamine neurons of substantia nigra. The unique localization of the muscarinic M_5 receptor would indicate that these receptors are involved in the regulation of the activity of the nigrostriatal pathway (Vilaró et al., 1990; Weiner et al., 1990). The exact role of this receptor subtype is still a matter of study, due to the lack of selective drugs.

In the present study, a significant increase in nigral extracellular dopamine levels was observed after continuous perfusion of trihexyphenidyl, followed by a decrease. In a former study (Izurieta-Sànchez et al., 1998), striatal perfusion of trihexyphenidyl showed a similar profile of dopamine release in the striatum. The increase in extracel-

lular dopamine levels could be explained by a blockade of the muscarinic M_2 receptor, and the subsequent decrease by the blockade of the muscarinic M_1 receptor. It is known that striatal dopamine release is stimulated by activation of muscarinic M_1 receptors and inhibited by activation of muscarinic M_2 receptors (Xu et al., 1989; De Klippel et al., 1993; Smolders et al., 1997). Thus, it is possible that in the substantia nigra the blockade of the muscarinic M_2 receptor by methoctramine resulted in an increase in extracellular dopamine levels, and the blockade of the muscarinic M_1 receptor by telenzepine resulted in a decrease of nigral extracellular dopamine levels. This was observed in our study.

The mechanism of action of anticholinergic drugs has been postulated to occur via a blockade of acetylcholine receptors. However, it has been known for a long time that these agents also act as inhibitors of the dopamine uptake in striatum (Pfeiffer and Smythies, 1970). If it is also the case in substantia nigra, this could explain the increase in extracellular dopamine levels observed after perfusion of trihexyphenidyl. The subsequent decrease could then be explained by the enhanced extracellular dopamine, which acts at dopamine D_2 auto-receptors to inhibit its own release. The reuptake inhibitor, nomifensine, also increases the extracellular dopamine levels in striatum, although the subsequent decrease in extracellular dopamine observed in this study, was not observed during nomifensine perfusion (Sarre et al., 1994). So, we could argue that the effect seen in extracellular dopamine levels under perfusion of trihexyphenidyl could be in part via reuptake inhibition together with the muscarinic M_1–M_2 receptor mediation. Another possibility is that the secondary decrease in extracellular dopamine in substantia nigra could also be the result of the inhibitory effect of GABA increase observed under trihexyphenidyl perfusion (see below).

Trihexyphenidyl induced a significant increase on nigral GABA release. On the other hand, a slight but significant decrease in GABA levels was observed under telenzepine perfusion. Methoctramine caused a slight but consistent decrease in GABA levels, however, no significance was found. In a recent study in the striatum (Smolders et al., 1997), no changes on GABA release were observed under perfusion of the muscarinic M_2 receptor antagonist methoctramine neither of the muscarinic M_1 receptor antagonist pirenzepine. However, this difference in the results could be due to a longer perfusion time of the drugs in our study compared to Smolders et al. In our experience, changes in extracellular GABA levels, occurred slowly and reached significance after at least 60 min of continuous perfusion with the antimuscarinic drugs. In an in vitro study by Kayadjanian et al. (1994), it was observed that acetylcholine and the non-selective muscarinic receptor agonist carbachol increased spontaneous [^{3}H] GABA release, and this effect was suggested to be mediated via a direct activation of the muscarinic M_2 receptor. To our knowledge, there is no evidence of the modulation of extracellular GABA levels in the substantia nigra by antimuscarinic drugs in vivo. The dopaminergic cell bodies have been shown, on morphological grounds, to receive direct synaptic input from GABAergic terminals derived from the striatum (Wassef et al., 1981) and the globus pallidus (Smith and Bolam, 1990). Further, it has been demonstrated that they receive an input from terminals that utilize acetylcholine as a transmitter, which are derived from neurons in the brainstem (see Introduction). The presence of muscarinic M_1 and M_2 receptors in the substantia nigra has been described (Cortez et al., 1984; Cortez and Palacios, 1986) but the exact localization is not clearly established (Nastuk and Graybiel, 1991; Zubieta and Frey, 1993). So, blockade of these muscarinic receptors may affect the extracellular dopamine and GABA levels in different ways and in turn modulate these neurotransmitters' release reciprocally (see below). However, these results strongly suggest that different mechanisms and/or receptors are involved in the effect of the muscarinic receptor antagonists on extracellular GABA levels. The trihexyphenidyl-induced increases in extracellular GABA levels are possibly independent of the muscarinic M_1 or M_2 receptor mediation, as a decrease was observed under perfusion with the muscarinic M_1 receptor antagonist and no significant changes with the muscarinic M_2 receptor antagonist. These data give further evidence for the presence of another muscarinic receptor (i.e. M_5, M_3) subtype in the substantia nigra that could be responsible for the effects observed in this study.

Surprisingly, no significant effects on striatal dopamine levels were observed under nigral perfusion of these muscarinic receptor antagonists. However, there was a tendency to increase dopamine levels under trihexyphenidyl and methoctramine perfusion and to decrease under telenzepine. In a similar study (Sarre et al., 1998), intranigral perfusion of L-dopa and dopamine receptor agonists and antagonists, despite the effects on nigral dopamine release, could not influence striatal dopamine release. Furthermore, Timmerman and Abercrombie (1996) showed that intranigral infusion of amphetamine also failed to influence extracellular dopamine levels in the striatum. These data confirm that there is a tight control on dopamine release in the striatum at least in intact rats (Sarre et al., 1998). Several studies suggested that cholinergic manipulation of the substantia nigra results in an increased firing rate of nigral dopamine neurons through both nicotinic and muscarinic receptors (Clarke et al., 1987; Calabresi et al., 1989; Lacey et al., 1990; Hernandez-Lopez et al., 1994; Gongora-Alfaro et al., 1996; Blaha and Winn, 1993). Our results are unable to confirm this postulate.

4.2. Effect of nigral perfusion of antimuscarinic drugs on the L-dopa induced increase of extracellular dopamine levels in striatum

The effect of L-dopa on dopamine release is well established. After striatal L-dopa administration (control experi-

ments), a significant increase in striatal dopamine release was observed. The profile of dopamine changes following L-dopa administration in striatum is similar to that observed in our former studies (Sarre et al., 1994, 1996; Izurieta-Sànchez et al., 1998).

Intranigral perfusion of trihexyphenidyl attenuated the L-dopa-induced increase of extracellular dopamine levels in striatum. The same effect was observed in our previous study (Izurieta-Sànchez et al., 1998) in which systemic (1.5 mg/kg) but not intrastriatal application (1 mM) of trihexyphenidyl attenuated the L-dopa-induced dopamine release in striatum. We suggested that the striatum possibly is not the site of action of antimuscarinic drugs and proposed the substantia nigra as possible target of these drugs. We also hypothesized that the effect observed after systemic injection of trihexyphenidyl is an indirect effect mediated via GABA. In the present study, trihexyphenidyl locally applied in substantia nigra attenuated the L-dopa-increased extracellular dopamine levels in striatum and also caused a sustained and significant increase of nigral extracellular GABA levels. By electrophysiological and immunohistochemical methods, Yung et al. (1991), identified a sub-population of presumed GABAergic interneurons in substantia nigra of guinea pigs. It has been suggested that acetylcholine can modulate nigral (Kayadjanian et al., 1994) and striatal GABA release (O'Connor et al., 1982; Limberger et al., 1986). Furthermore, striatal GABA exerts a tonic inhibition on striatal dopamine release via presynaptic $GABA_B$ receptors and postsynaptic $GABA_A$ receptors as it has been postulated by Smolders et al. (1995b). It is possible that perfusion of trihexyphenidyl also stimulated the GABAergic terminals and/or interneurons causing an increase of extracellular GABA levels, which in turn could inhibit the nigrostriatal dopaminergic neurons, leading to the attenuation of the L-dopa-induced extracellular dopamine levels in the striatum.

On the other hand, if trihexyphenidyl acts as dopamine reuptake inhibitor (Pfeiffer and Smythies, 1970) increasing extracellular dopamine at the level of the dendrites, it is well possible that this dopamine will act at dopamine D_2 autoreceptors inhibiting its own release and counteracting the effect of L-dopa on dopamine release in the striatum. Furthermore, this mechanism could act synergistically together with the muscarinic modulation of GABA explaining the attenuation of L-dopa decarboxylation observed in our study.

Nigral perfusion of the muscarinic M_2 receptor antagonist methoctramine also attenuated the L-dopa induced dopamine release in the striatum. As described above, the increase in dopamine release observed in striatum and substantia nigra under methoctramine perfusion and the further stimulation of dopamine D_2 autoreceptors could also explain this attenuation.

The L-dopa-induced dopamine release in the striatum was also attenuated by the nigral perfusion of the muscarinic M_1 receptor antagonist telenzepine. This effect could be mediated directly by the blockade of muscarinic M_1 receptors, as it is known that stimulation of this receptor causes an increase in dopamine release, confirming the tonic effect of this receptor in the regulation of dopamine efflux (Xu et al., 1989). As decreases in nigral extracellular GABA levels were observed under methoctramine and telenzepine perfusion in the substantia nigra, the mediation of GABA in the attenuation of L-dopa decarboxylation observed under perfusion of these drugs should be discarded.

It is intriguing to note that both selective and non-selective muscarinic receptor antagonists perfused in the substantia nigra, despite their opposite effects on both nigral dopamine and GABA efflux, attenuated the striatal L-dopa decarboxylation. These results are difficult to interpret. As mentioned above, the muscarinc M_5 receptor subtype is the only muscarinic receptor described to be expressed on dopamine neurons in the substantia nigra and the cloned muscarinic M_5 receptors present an intermediate affinity for the muscarinic M_1 receptor antagonist pirenzepine and also for another muscarinic M_2 receptor antagonist (Vilaró et al., 1990). So, it is possible that the antimuscarinic drugs acting at this muscarinic receptor subtype decreased the firing of dopaminergic neurons mediating the attenuation of L-dopa decarboxylation in the striatum. On the other hand, L-dopa uptake and the activity of the enzyme aromatic L-amino acid decarboxylase, responsible for the conversion of L-dopa to dopamine, may be altered under perfusion of the muscarinic receptor antagonists in the substantia nigra. However, it should be taken into account that these drugs were administered locally in the substantia nigra and L-dopa was given in the striatum. The exact mechanisms mediating the attenuation of the L-dopa decarboxylation in the striatum, by nigral perfusion of muscarinic receptor antagonists are difficult to determine. To our knowledge, there are no studies describing the muscarinic modulation of dopamine and GABA in the substantia nigra. Therefore, the interpretation of these results remains speculative. Further studies are necessary to understand the present results.

In conclusion, this study demonstrates that antimuscarinic drugs acting at the level of the substantia nigra can modulate nigral extracellular dopamine and GABA levels and attenuate the effects of L-dopa on dopamine levels in the striatum, possibly via modulation of the nigrostriatal dopaminergic system. We add further evidence that the substantia nigra is an important site of action of antimuscarinic drugs. Nigral perfusion of the non-selective muscarinic receptor antagonist trihexyphenidyl, the muscarinic M_2 receptor antagonist methoctramine and the muscarinic M_1 receptor antagonist telenzepine attenuated the L-dopa-induced dopamine release in striatum. In line with our hypothesis (see Introduction), we consider this attenuation as a beneficial effect. The observed attenuation by each drug is probably mediated via different mechanisms. Trihexyphenidyl exerted an indirect effect possibly via an

increase in nigral GABA release, methoctramine possibly via dopamine acting on dopamine D_2 auto-receptors while telenzepine decreasing dopamine levels possibly acted through a blockade of muscarinic M_1 receptors. Alternatively, it is possible that the muscarinic M_5 receptor subtype is involved in the attenuation of striatal L-dopa decarboxylation.

Acknowledgements

This work was supported by a grant from the National Fund for Scientific Research (Grant No. G. 0133.99) and the 'Geneeskundige Stichting Koningin Elisabeth'. P Izurieta-Sánchez wishes to thank also the Vrije Universiteit Brussel, for the financial support. Sophie Sarre has a post-doc position FWO-Vlaanderen.

We would like to acknowledge the excellent technical assistance of Gino De Smet, Ria Berckmans, Rose-Marie Geens and Carina De Rijck. We would also like to thank Dr. Ilse Smolders for reading this manuscript.

References

Albin, R., Young, A., Penney, B., 1989. The functional anatomy of basal ganglia disorders. Trends Neurosci. 12, 366–375.

Alexander, G., Crutcher, M., 1990. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci. 13, 266–271.

Beninato, M., Spencer, R., 1987. A cholinergic projection to the rat substantia nigra from the pedunculopontine tegmental nucleus. Brain Res. 412, 169–174.

Blaha, C., Winn, P., 1993. Modulation of dopamine efflux in the striatum following cholinergic stimulation of the substantia nigra in intact and pedunculopontine tegmental nucleus-lesioned rats. J. Neurosci. 13, 1035–1044.

Bolam, J., Francis, C., Henderson, Z., 1991. Cholinergic input to dopaminergic neurons in the substantia nigra: a double immunocytochemical study. Neuroscience 41, 483–494.

Calabresi, P., Lacey, M., North, R., 1989. Nicotinic excitation of rat ventral tegmental neurones in vitro studied by intracellular recording. Br. J. Pharmacol. 98, 135–140.

Chesselet, M., Delfs, J., 1996. Basal ganglia and movement disorders: an update. Trends Neurosci. 19, 417–422.

Clarke, P., Hommer, D., Pert, A., Skirboll, L., 1987. Innervation of substantia nigra neurons by cholinergic afferents from pedunculopontine nucleus in the rat: neuroanatomical and electrophysiological evidence. Neuroscience 23, 1011–1019.

Cortez, A., Probst, A., Palacios, J., 1984. Quantitative light microscopic localization of cholinergic muscarinic receptors in the human brain: brainstem. Neuroscience 12, 1003–1026.

Cortez, A., Palacios, J., 1986. Muscarinic cholinergic receptor subtypes in the rat brain: I. Quantitative autoradiographic studies. Brain Res. 362, 227–238.

De Klippel, N., Sarre, S., Ebinger, G., Michotte, Y., 1993. Effect of M_1 and M_2 muscarinic drugs on striatal dopamine release and metabolism: an in vivo microdialysis study comparing normal and 6-hydroxydopamine-lesioned rats. Brain Res. 630, 57–64.

Fahn, S., 1989. Adverse effects of levodopa in Parkinson's disease. In: Calne, D.B. (Ed.), Handbook of Experimental Pharmacology. Springer, Berlin, 385 pp.

Gerfen, C., 1992. The neostriatal mosaic: multiple levels of compartmental organization. Trends Neurosci. 15, 133–139.

Gerlach, J., 1977. Relationship between tardive dyskinesia, L-dopa-induced hyperkinesia and Parkinsonism. Psychopharmacology 51, 259–263.

Gould, E., Woolf, N., Butcher, L., 1989. Cholinergic projections to the substantia nigra from pedunculopontine and laterodorsal tegmental nuclei. Neuroscience 28, 611–623.

Gongora-Alfaro, J., Hernandez-Lopez, S., Martinez-Fong, D., Flores, G., Aceves, J., 1996. Circling behavior elicited by cholinergic transmission in the substantia nigra pars compacta: involvement of nicotinic and muscarinic receptors. Neuroscience 71, 729–734.

Hastings, T., Zigmond, M., 1997. Loss of dopaminergic neurons in Parkinsonism: possible role of reactive dopamine metabolites. J. Neural Transm., Suppl. 49, 103–110.

Hernandez-Lopez, S., Gongora-Alfaro, J., Martinez-Fong, D., Rosales, M., Aceves, J., 1994. Cholinergic stimulation of rostral and caudal substantia nigra pars compacta produces opposite effects on circling behaviour and striatal dopamine release measured by brain microdialysis. Neuroscience 62, 441–447.

Hoover, J., Strick, P., 1993. Multiple output channels in the basal ganglia. Science 259, 819–821.

Izurieta-Sànchez, P., Sarre, S., Ebinger, G., Michotte, Y., 1998. Effect of trihexyphenidyl, a non-selective antimuscarinic drug, on decarboxylation of L-dopa in hemi-Parkinson rats. Eur. J. Pharmacol. 353, 33–42.

Kayadjanian, N., Giovanni, H., Menetrey, A., Besson, M., 1994. Muscarinic receptor stimulation increases the spontaneous [^{3}H] GABA release in the rat substantia nigra through muscarinic receptors localized on striatonigral terminals. Neuroscience 63, 943–950.

König, J., Klippel, R., 1963. The Rat Brain. A Stereotaxic Atlas. Williams & Wilkins, Baltimore.

Kostrzewa, R., 1995. Dopamine receptor supersensitivity. Neurosci. Biobehav. Rev. 19, 1–17.

Lacey, M., Calabresi, P., North, R., 1990. Muscarine depolarizes rat substantia nigra zona compacta and ventral tegmental neurons in vitro through M_1-like receptors. J. Pharmacol. Exp. Ther. 253, 295–400.

Limberger, N., Spâth, L., Starke, K., 1986. A search for receptors modulating the release of γ-^{3}[H] aminobutyric acid in rabbit caudate nucleus slices. J. Neurochem. 46, 1109–1117.

Nastuk, M., Graybiel, A., 1991. Pharmacologically defined M_1 and M_2 muscarinic cholinergic binding sites in the cat's substantia nigra: development and maturity. Dev. Brain Res. 61, 1–10.

Nutt, J., Holford, H., 1996. The response to levodopa in Parkinson's disease: imposing pharmacological law and order. Ann. Neurol. 39, 561–573.

O'Connor, W., Tanganelli, S., Ungerstedt, U., Fuxe, K., 1982. The effects of neurotensin on GABA and acetylcholine release in the dorsal striatum of the rat: an in vivo microdialysis study. Brain Res. 573, 209–216.

Paxinos, C., Watson, C., 1982. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Pfeiffer, C., Smythies, J., 1970. In: International Review of Neurobiology vol. 13 Academic Press, New York, pp. 127–156.

Sarre, S., Herregodts, P., Deleu, D., Devrieze, A., De Klippel, N., Ebinger, G., Michotte, Y., 1992. Biotransformation of L-dopa in striatum and substantia nigra of rats with a unilateral lesion: a microdialysis study. Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 277–285.

Sarre, S., DeKlippel, N., Herregodts, P., Ebinger, G., Michotte, Y., 1994. Biotransformation of locally applied L-dopa in corpus striatum of the hemi-Parkinson's rat studied with microdialysis. Naunyn-Schmiedeberg's Arch. Pharmacol. 350, 15–21.

Sarre, S., Ebinger, G., Michotte, Y., 1996. Levodopa biotransformation in hemi-Parkinson's rats: effect of dopamine receptor agonists and antagonists. Eur. J. Pharmacol. 296, 247–260.

Sarre, S., Vandeneede, D., Ebinger, G., Michotte, Y., 1998. Biotransformation of L-dopa to dopamine in the substantia nigra of freely moving

rats: effect of dopamine receptor agonists and antagonists. J. Neurochem. 70, 1730–1739.

Smith, A., Bolam, J., 1990. The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurons. Trends Neurosci. 13, 259–265.

Smolders, I., Sarre, S., Ebinger, G., Michotte, Y., 1995a. The analysis of excitatory, inhibitory and other amino acids in rat brain microdialysates using microbore liquid chromatography. J. Neurosci. Methods 57, 47–53.

Smolders, I., De Klippel, N., Sarre, S., Ebinger, G., Michotte, Y., 1995b. Tonic GABA-ergic modulation of striatal dopamine release studied by in vivo microdialysis in the freely moving rat. Eur. J. Pharmacol. 284, 83–91.

Smolders, I., Sarre, S., Vanhaesendonck, C., Ebinger, G., Michotte, Y., 1996. Extracellular striatal dopamine and glutamate after decortication and Kainate receptor stimulation, as measured by microdialysis. J. Neurochem. 66, 2373–2380.

Smolders, I., Bogaert, L., Ebinger, G., Michotte, Y., 1997. Muscarinic modulation of striatal dopamine, glutamate and GABA release, as measured with in vivo microdialysis. J. Neurochem. 68, 1942–1948.

Timmerman, W., Abercrombie, E., 1996. Amphetamine-induced release of dendritic dopamine in substantia nigra pars reticulata: D1-mediated behavioural and electrophysiological effects. Synapse 23, 280–291.

Vilaró, T., Palacios, J., Mengod, G., 1990. Localization of M_5 muscarinic receptor mRNA in rat brain examined by in situ hybridization histochemistry. Neurosci. Lett. 114, 154–159.

Wassef, M., Berod, A., Sotelo, C., 1981. Dopaminergic dendrites in the pars reticulata of the rat substantia nigra and their striatal input. Combined immunocytochemical localization of tyrosine hydroxylase and anterograde degeneration. Neuroscience 6, 2125–2139.

Weiner, D., Levey, A., Brann, M., 1990. Expression of muscarinic acetylcholine and dopamine receptor mRNAs in the basal ganglia. Proc. Natl. Acad. Sci. U. S. A. 87, 7050–7054.

Yung, W., Häuser, M., Jack, J., 1991. Electrophysiology of dopaminergic and non-dopaminergic neurons of the guinea pig substantia nigra pars compacta in vitro. J. Physiol. 436, 643–667.

Xu, M., Mizobe, F., Yamamoto, T., Kato, T., 1989. Differential effects of M_1 and M_2 muscarinic drugs on striatal dopamine release and metabolism in freely moving rats. Brain Res. 495, 232–242.

Zubieta, J., Frey, K., 1993. Autoradiographic mapping of M_3 muscarinic receptors in the rat brain. J. Pharmacol. Exp. Ther. 264, 415–421.