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ORIGINAL ARTICLE

Sanna Janhunen · Paula Mielikäinen · Päivi Paldánius · Raimo K. Tuominen · Liisa Ahtee · Seppo Kaakkola

The effect of nicotine in combination with various dopaminergic drugs on nigrostriatal dopamine in rats

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Abstract It is well established that nicotine activates brain dopaminergic systems and in addition has neuroprotective actions. Thus, nicotinic acetylcholine receptor (nAChR) agonists might be beneficial in the treatment of Parkinson's disease, and it is important to study the interactions of nicotine with drugs affecting the nigrostriatal dopaminergic pathway. We used brain microdialysis to study the effects of nicotine on extracellular levels of dopamine (DA) and its metabolites in the rat dorsal striatum in combination with drugs inhibiting either DA uptake (nomifensine), catechol-O-methyltransferase (COMT; tolcapone), monoamine oxidase B (MAO-B; selegiline) or DA receptors (haloperidol). Nicotine (0.5 mg/kg, s.c.) modestly increased DA output, and this effect was antagonised by mecamylamine but not by hexamethonium. Nomifensine (3 mg/kg, i.p.) substantially further enhanced the nicotine-induced increase in DA output and nomifensine+nicotine also evoked a strong mecamylamine-sensitive ipsilateral rotational behaviour in 6-hydroxydopamine lesioned rats. Tolcapone (10 mg/kg, i.p.) did not alter DA output, but markedly decreased homovanillic acid (HVA) and increased 3,4-dihydroxyphenylacetic acid (DOPAC). Selegiline pretreatment (5×1 mg/kg, i.p.) significantly increased extracellular DA and decreased DOPAC and HVA. Haloperidol (0.1 mg/kg, s.c.) slightly increased DA output and more clearly DOPAC and HVA. Tolcapone, selegiline or haloperidol did not enhance the nicotine-induced DA output. These results indicate that the activation of nigrostriatal nAChRs induces a significant DA release in the striatum, which is potentiated by DA uptake inhibition but not by COMT, MAO-B or presynaptic DA receptor inhibition. Our findings therefore agree with the notion that the termination of the effect of DA in the synapse mainly occurs via neuronal reuptake. Thus, selective nAChR agonists, possibly in combination with a DA uptake inhibitor, might improve dopaminergic transmission in Parkinson's disease.

Keywords Nicotine · Nomifensine · Tolcapone · Selegiline · Haloperidol · Extracellular dopamine · Rotational behaviour

S. Janhunen · P. Mielikäinen · P. Paldánius · R. K. Tuominen · L. Ahtee (✉)
Division of Pharmacology and Toxicology,
Faculty of Pharmacy, University of Helsinki,
P.O. Box 56, 00014 Helsinki, Finland
e-mail: Liisa.Ahtee@helsinki.fi
Tel.: +358-9-19159468
Fax: +358-9-19159471

S. Kaakkola
Department of Neurology, University of Helsinki,
00014 Helsinki, Finland

Introduction

Degeneration of nigrostriatal dopaminergic neurons and consequent decrease of striatal dopamine (DA) is the major neuropathological change in Parkinson's disease. The aetiology for the degeneration, however, is still largely unknown. Epidemiological studies have shown that the occurrence of Parkinson's disease is significantly less common among smokers and even former smokers than among those who have never smoked (Allam et al. 2004; Baron 1986; Gorell et al. 1999; Morens et al. 1995). As nicotine is the main pharmacologically active substance in cigarette smoke, this finding has stimulated many researchers to elucidate whether nicotine and nicotinic acetylcholine receptors (nAChRs) have any role in the aetiopathology of Parkinson's disease and in its treatment (for a review see for example Quik 2004).

A number of studies have demonstrated that nicotine stimulates the release of DA in striatum, as studied in vitro using striatal slices (Arqueros et al. 1978; Giorguieff-Chesselet et al. 1979; Teng et al. 1997; Westfall 1974) or synaptosomes (Rapier et al. 1990; Rowell 1995; Sakurai et al. 1982; Whiteaker et al. 1995), and in vivo using microdialysis (Damsma et al. 1988; Di Chiara and Imperato 1988; Imperato et al. 1986; Marshall et al. 1997; Toth et al. 1992). The nAChR subtypes localised on dopaminergic and glutamatergic nerves are thought to mediate this effect (Kaiser and Wonnacott 2000; Wonnacott 1997; Wonnacott

et al. 2000). However, nAChRs also exist on dopaminergic cell bodies and dendrites in the substantia nigra (Clarke and Pert 1985; Court and Clementi 1995; Klink et al. 2001; Sorenson et al. 1998) and a cholinergic pathway with nAChRs from pedunculopontine nuclei to the substantia nigra has been described (Clarke et al. 1987; Futami et al. 1995).

It has been suggested that even nicotine itself may have an antiparkinsonian effect (Balfour and Fagerström 1996; Domino et al. 1999). In addition, several potent nAChR agonists have been recently developed and many of them are presently under evaluation as antiparkinsonian drugs (Mihailescu and Drucker-Colin 2000; Vernier et al. 1998). Given these findings, and the fact that parkinsonian patients use a number of drugs enhancing the brain dopaminergic transmission, we wanted to further study the involvement of nicotinic mechanisms in the striatal dopaminergic transmission and potential suitability of nicotine or related nAChR agonists as antiparkinsonian drugs. Thus, to study how nicotine alters the endogenous dopaminergic transmission we compared the effects of nicotine (0.5 mg/kg) in combination with various drugs affecting synaptic DA by performing a series of microdialysis experiments in awake and freely moving rats. A DA uptake inhibitor, nomifensine (3 mg/kg), a catechol-O-methyltransferase (COMT) inhibitor, tolcapone (10 mg/kg), a monoamine oxidase B (MAO-B) inhibitor, selegiline (5× 1 mg/kg, a MAO-B selective dose), and a DA receptor blocker, haloperidol (0.1 mg/kg), were used at doses that have been reported to alter the endogenous dopaminergic transmission significantly although not maximally (Carboni et al. 1989; Imperato et al. 1986; Kaakkola and Wurtman 1992; O'Connor et al. 1995; Schiffer et al. 2003). Furthermore, additional studies using the well-known parkinsonian animal model, rotational behaviour, were carried out in order to explore whether changes in the striatal dopaminergic transmission reverberate to the rotational behaviour of rats.

Preliminary results have been previously published in abstract form (Janhunen et al. 2001; Kaakkola et al. 2000).

Materials and methods

Animals Male Wistar rats weighing 240–380 g were used in both microdialysis and rotational behaviour experiments. The rats were housed under a 12-h light-dark cycle at a constant room temperature (24±2°C) with food (Altromin 1314 standard-diet; Chr. Petersen A/S, Ringstedt, Denmark) and tap water freely available. All animal procedures were conducted in accordance with the Council Directive 86/609/EEC and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, and they were approved by the Institutional Animal Care and Use Committee, and the chief veterinarian of the County Administrative Board.

Surgeries For insertion of guide cannulae in microdialysis experiments the rats were anaesthetised with halothane (3.5% for induction and 2% for maintenance) and mounted in a stereotaxis apparatus (Stoelting, IL, USA) with the incisor bar set at −3.3 mm. The skull was exposed, and a burr hole was drilled to insert a guide cannula (MD-2250; Bioanalytical Systems, West Lafayette, IN, USA). The coordinates for the caudate-putamen were as follows: AP+1.0, L+2.7, V-2.0 (Paxinos and Watson 1986). The surgery was conducted similarly in all rats despite variability in their weight. The guide cannula was held in place using dental acrylic cement and three stainless screws attached to the skull. After the surgery, the rats were treated with tramadol (1.0 mg/kg s.c.) for postoperative analgesia, placed individually in test cages and allowed to recover for 6–8 days.

The rats used for unilateral destruction of nigrostriatal dopaminergic neurones were separate from those used for the microdialysis study. The rats were anaesthetised with halothane (3.5% for induction and 2% for maintenance) and mounted in the stereotaxis apparatus with the incisor bar set at −3.3 mm. Thirty minutes prior to 6-OHDA injection the rats were administered desmethylimipramine (15 mg/kg i.p.) to prevent the uptake of 6-OHDA into the noradrenergic nerve terminals. A burr hole was drilled above the medial forebrain bundle (MFB). Unilateral lesions of the right nigrostriatal pathway were made by injecting 6-hydroxydopamine (6-OHDA, 8 μg/4 μl, 1.0 μl/min for 4 min) into the MFB with the 10 μl Hamilton syringe. The coordinates were as follows: AP -4.2, L -1.4, V -8.2 (Paxinos and Watson 1986). The surgery was conducted similarly in all rats despite variability in their weight. Upon completion, the injection needle was kept in place for an additional minute to minimise backflow of the solution. The rats were allowed to recover from the surgery for 1–2 days in individual cages and thereafter housed in groups as before the surgery.

Microdialysis On the day before a microdialysis experiment, a dummy probe (MD-2204, 4-mm-long dialysis surface; Bioanalytical Systems) was inserted into the guide cannula; the tip of the probe extending 6.0 mm below the dura. In the morning of the experimental day, the dummy probe was replaced by an identical new probe. The probe was infused with artificial cerebrospinal fluid (pH 7.4) containing 147 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCL, 1.0 mM $MgCl_2$, and 0.04 mM ascorbic acid at a rate of 2 μl/min using a microperfusion pump (CMA Microdialysis AB, Solna, Sweden). Following an approximately 2-h equilibration period, the dialysate samples were then collected every 15 min (30 μl) and they were immediately analysed by high-performance liquid chromatography (HPLC) with electrochemical detection. During the microdialysis experiments, the rats were allowed to move freely in their cages at a room temperature of 21±1°C.

At the end of the experiments the rats were anaesthetised with CO_2, killed by decapitation and the brains were removed. Frozen coronal sections were prepared and stained with thionine blue for verification of the probe location. Only data from animals with proper probe placements in the caudate-putamen were used.

Rotational behaviour After a 14-day recovery period, rotational behaviour of the rats was measured in black circular plastic bowls (37 cm in diameter and 15 cm high) with a 40-cm high transparent plexiglas cylinder surrounding the bowls. Each rat was attached to a rotation sensor with a spring tether connected to the plastic collar around the neck of the rat. The rotation sensor detected full (360°) clockwise (in this case, ipsilateral) and counter clockwise (contralateral) turns. Each rat was taken from the cage, placed individually into the test bowl and allowed to habituate for 45 min. Thereafter, the rotation counts were recorded in 15-min intervals for 3 h. At the end of rotation experiments, the 6-OHDA lesions were verified by measuring DA concentrations in depleted and intact dorsal striata.

HPLC analysis Dialysate levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were immediately analysed by HPLC with electrochemical detection. The HPLC system consisted of a Coulochem II detector (ESA, MA, USA) equipped with a 5014B microdialysis cell, a Pharmacia LKB model 2248 HPLC pump (Pharmacia LKB, Uppsala, Sweden), and a SSI model LP-21 pulse damper (Scientific Systems, State College, PA, USA). The column (Spherisorb ODS2, 3 μm, 4.6×100 mm; Waters, Milford, MA, USA) was kept at 40°C with a column heater (Croco-Cil, Saint-Foy-la-Grande, France). The mobile phase consisted of 0.1 M NaH_2PO_4, pH 4.0 (adjusted with 0.1 mM citric acid), 0.85–0.95 mM octane sulphonic acid, 15% (v/v) methanol and 1.2 mM EDTA. The flow rate of the mobile phase was 1.0 ml/min. A CMA/200 autoinjector (CMA Microdialysis AB) was utilised for injecting 20 μl of the dialysate sample into the HPLC system. DA was reduced with an amperometric detector (set at −150 mV) whereas DOPAC, HVA and 5-HIAA were oxidised with a coulometric detector (set at +350 mV). Samples were quantified by comparing peak heights with those of standards.

To verify the 6-OHDA lesions, both depleted and intact dorsal striata of the 6-OHDA-lesioned rats were dissected, the striatal samples were immediately frozen on dry ice and stored at −80°C until assay. Samples were homogenised and purified as described earlier (Haikala 1987). The samples were assayed for the concentration of DA by using an HPLC system with a C-18 reverse-phase column (Spherisorb S5 ODS2, 4.6×250 mm; Waters) and electrochemical detection (+200 mV, ESA Coulochem 5014A; ESA, Bedford, CA, USA). Only rats with DA depletion of more than 95% in the lesioned right dorsal striatum compared with the intact left dorsal striatum were included in the final data analysis. The mean DA depletion of rats included in the results was 99.6% (range 95.1–100%, n=45).

Drugs and their administration Haloperidol (Orion-Pharma, Espoo, Finland), hexamethonium chloride (Sigma, St. Louis, MO, USA), mecamylamine hydrochloride (Merck, Whitehouse Station, NJ, USA), (-)-nicotine base (Fluka, Buchs, Switzerland), selegiline (a gift from Orion-Pharma) and tramadol hydrochloride (Orion-Pharma) were dissolved in saline. The pH of nicotine solution was adjusted to 7.0–7.4 with 0.05 M HCl and that of haloperidol solution to 7.2 with 1 M NaOH. Nomifensine maleate (RBI, Natick, MA, USA) and desmethylimipramine (Sigma) were dissolved in sterile water. Tolcapone (a gift from Orion-Pharma) was suspended in phosphate buffer (pH 7.4) containing a drop of Tween 80. 6-OHDA (Sigma) was dissolved in saline containing 0.02% ascorbic acid. Haloperidol, nicotine, selegiline and tramadol were injected subcutaneously (s.c.) while the other drugs were given intraperitoneally (i.p.). Doses refer to free base. All reagents were of analytical grade. In all microdialysis experiments except for those studying selegiline, all rats were injected twice in 30-min intervals after collecting baseline samples; with saline (0.9% NaCl solution) only (control), with saline and an active drug or with two active drugs, as indicated in the figures. Selegiline (1 mg/kg, s.c.) was given repeatedly on 5 consecutive days and the microdialysis experiments were conducted on the 5th day, when the selegiline injection was given 2 h before starting collecting the baseline samples and thus 3 h before the nicotine injection. In rotational behaviour experiments the rats were treated twice or thrice in 15-min intervals, as indicated in the Fig. 6.

Data analysis Dialysate values reported were not corrected for probe in vitro recovery, which was approximately 20% for dopamine and 10–15% for its metabolites and 5-HIAA. The average concentration of three or four stable samples (<20% variation) before the first drug injection was defined as the baseline level (=100%). Data are expressed as means±SEM of the percentage change from the baseline values. The statistical analysis of the results was performed by two-way analysis of variance (treatment×time) for repeated measures (time). Post hoc comparisons were performed by using Newman-Keuls test in all cases when the main effect (treatment) was positive (P<0.05; Statistica version 5.1; StatSoft, Tulsa, OK, USA). Almost constantly when a positive main effect was found the interaction term also showed statistical significance. In the selegiline experiments, the baseline dialysate levels of DA, DOPAC, HVA and 5-HIAA were analysed using the Student's t test. The data for total ipsilateral rotations were analysed using one-way analysis of variance followed by the Newman-Keuls test.

Results

Basal levels of dopamine, DOPAC, HVA and 5-HIAA in dialysate samples

In control rats (n=18), baseline dialysate levels of DA, DOPAC, HVA and 5-HIAA per sample (mean±SEM) were 54.1±5.4 fmol, 18.0±1.4 pmol, 12.9±1.4 pmol and 3.3±0.2 pmol respectively. The baseline levels in all drug-treated rats before treatment were comparable with those

of the control rats and there were no statistically significant differences in the baseline values between various treatment groups in separate experiments.

Effects of nicotine, mecamylamine and hexamethonium

Nicotine (0.5 mg/kg s.c.) produced a moderate but significant increase in the extracellular DA level up to 140% of the baseline level, $F(3,22)=3.9$, Newman-Keuls test $P<0.05$ vs. saline control group (Fig. 1). Nicotine also significantly increased the dialysate levels of DOPAC up to 125% of the baseline level, $F(3,22)=5.7$, $P<0.05$ vs. saline control group, and HVA up to 137% of the baseline level, $F(3,22)=8.1$, $P<0.01$. The maximum increase for HVA occurred about 15 to 30 min later than that for DOPAC (Fig. 1). The HVA level remained elevated at the end of the collecting period (270 min), whereas the DA and DOPAC levels had almost restored to their baseline levels during the collecting period.

Neither a brain penetrating nAChR antagonist, mecamylamine (5 mg/kg i.p.), nor a peripheral nAChR antagonist, hexamethonium (5 mg/kg i.p.), changed the striatal

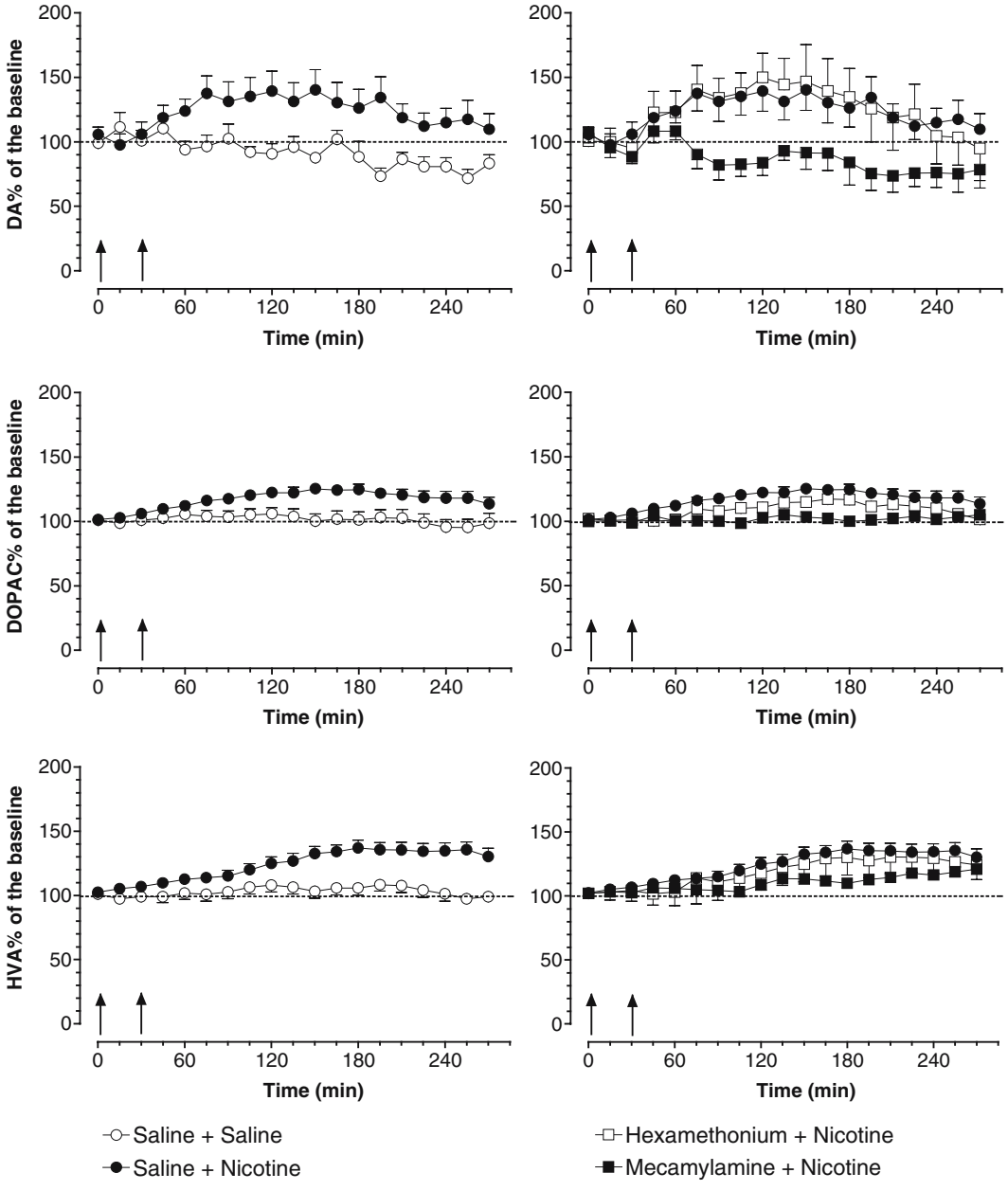

Fig. 1 The effects of nicotine (0.5 mg/kg s.c.) alone and after a pretreatment with mecamylamine (5 mg/kg i.p.) or hexamethonium (5 mg/kg i.p.) on the striatal extracellular levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Rats were given mecamylamine, hexamethonium or saline (*first arrow*) after collecting the baseline samples and 30 min later nicotine or saline (*second arrow*). The data, starting from the last baseline sample, are expressed as percentage changes of three to four consecutive baseline samples collected before injections (means±SEM, $n=6–8$)

extracellular levels of DA, DOPAC or HVA (data not shown). Pretreatment with mecamylamine significantly prevented the nicotine-induced increases in the extracellular levels of DA, $F(3,22)=3.9$, Newman-Keuls test $P< 0.05$ vs. nicotine group (Fig. 1) and DOPAC, $F(3,22)=5.7$, $P< 0.01$. Mecamylamine also significantly prevented the nicotine-induced increase in the HVA level, $F(3,22)=8.1$, $P< 0.05$, but its effect started to gradually disappear 210 min after the nicotine administration (Fig. 1). In contrast to mecamylamine, hexamethonium did not alter the nicotine-induced increases in the striatal levels of DA and its metabolites (Fig. 1). Nicotine, mecamylamine or hexamethonium did not significantly change the extracellular 5-HIAA level (data not shown).

Effect of nomifensine with nicotine

The administration of the DA uptake inhibitor, nomifensine (3 mg/kg i.p.), significantly increased the striatal DA level, $F(3,23)=7.9$, Newman-Keuls test $P<0.05$ vs. saline control group (Fig. 2), but failed to significantly alter the levels of DOPAC, HVA or 5-HIAA (Fig. 2; saline+saline group omitted from the figure due to clarity). The peak increase in the DA level after nomifensine was about 200% of the baseline level.

The combination of nomifensine (3 mg/kg) and nicotine (0.5 mg/kg) induced a substantial elevation in the extracellular DA level up to 370% of the baseline level (Fig. 2). The increase was significant when compared with the saline control group, $F(3,23)=7.9$, Newman-Keuls test $P< 0.001$, with the nomifensine group, $P<0.05$, and with the nicotine group, $P<0.05$. After the combination of nomifensine and nicotine the extracellular levels of DOPAC and HVA were at the same level as after nomifensine alone, but lower than after nicotine alone, DOPAC $F(3,23)=6.2$, $P<0.01$, HVA $P=0.065$ (Fig. 2).

Effect of tolcapone with nicotine

The administration of the catechol-O-methyltransferase inhibitor, tolcapone (10 mg/kg i.p.), did not alter the extracellular DA level when compared with the saline control group. Tolcapone significantly increased the striatal DOPAC level up to 142% of the baseline level, $F(3,24)= 12.2$, Newman-Keuls test $P<0.01$ vs. saline control group, and significantly decreased the HVA level down to 13% of the baseline level, $F(3,24)=48.2$, $P<0.001$ (Fig. 3, saline+saline group omitted from the figure due to clarity).

Pretreatment with tolcapone (10 mg/kg) had no significant effect on the 0.5 mg/kg nicotine-induced increase in the DA level, although it slightly tended to retard the elevation of DA (Fig. 3). The combination of tolcapone and nicotine considerably elevated the extracellular level of DOPAC (up to 177% of the baseline level). This increase was significantly higher than that induced by either tolcapone, $F(3,24)=12.2$, Newman-Keuls test $P<0.05$, or nicotine alone, $P<0.01$. The striatal HVA level after the combined treatment of tolcapone and nicotine remained almost as low as after the tolcapone treatment alone, $P>0.10$.

Tolcapone alone or in combination with nicotine had no effect on the extracellular level of 5-HIAA (data not shown).

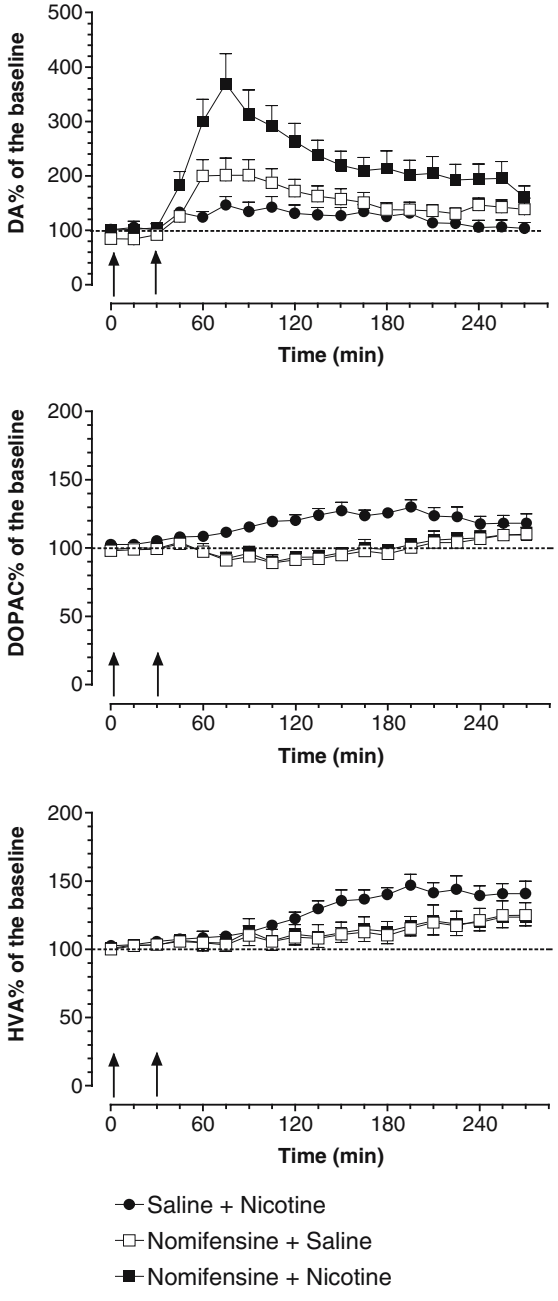

Fig. 2 The effects of nomifensine (3 mg/kg i.p.) on the changes induced by nicotine (0.5 mg/kg s.c.) or saline in the striatal extracellular levels of DA, DOPAC and HVA. Rats were given nomifensine or saline (*first arrow*) after collecting the baseline samples and 30 min later nicotine or saline (*second arrow*). It is to be noted that the scale of the ordinates differs between the graphs showing DA and its metabolites. The data, starting from the last baseline sample, are expressed as percentage changes of three to four consecutive baseline samples collected before injections (means ±SEM, n=6–8)

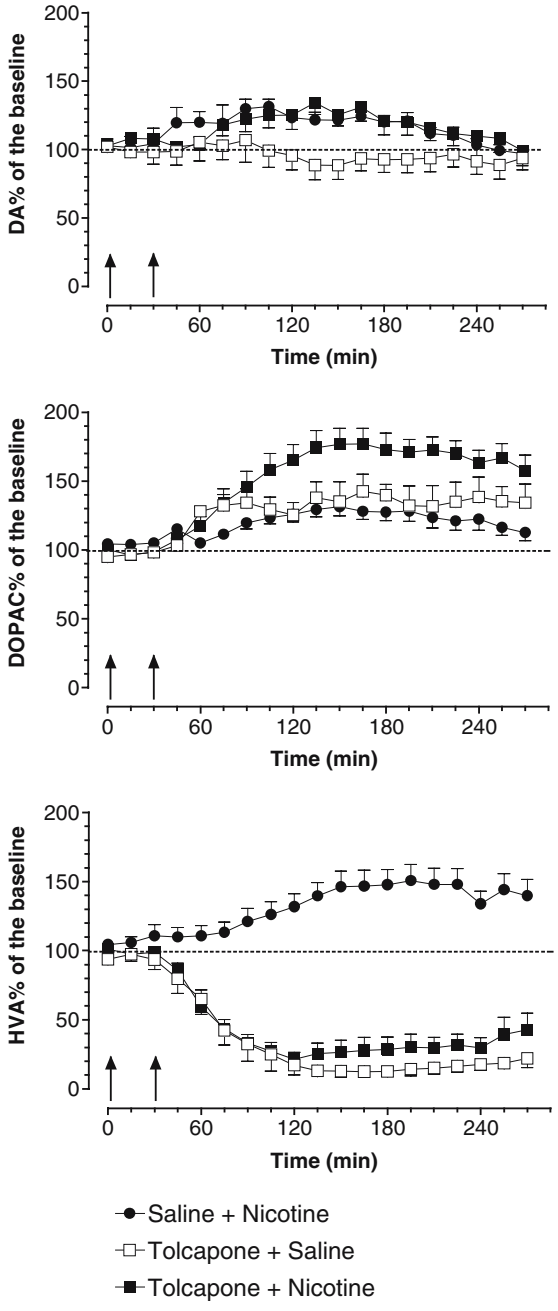

Fig. 3 The effects of tolcapone (10 mg/kg i.p.) on the changes induced by nicotine (0.5 mg/kg s.c.) or saline in the striatal extracellular levels of DA, DOPAC and HVA. Rats were given tolcapone or saline (*first arrow*) after collecting the baseline samples and 30 min later nicotine or saline (*second arrow*). The data, starting from the last baseline sample, are expressed as percentage changes of three to four consecutive baseline samples collected before injections (means±SEM, n=6–8)

Effect of selegiline with nicotine

When given repeatedly on 4 consecutive days and on the experimental day 2 h before collecting the dialysate samples, the monoamine oxidase B inhibitor, selegiline (1 mg/kg s.c.), significantly elevated the extracellular DA level in the baseline samples more than 5-fold compared with the samples of the rats correspondingly treated with saline (selegiline: 394±98 fmol, n=10, saline: 72±23 fmol, n=6, means±SEM per 20 μl, Student's t test P<0.05). Selegiline treatment also significantly decreased the level of DOPAC in the baseline samples (selegiline: 7.9±0.9 pmol, saline: 21.2±3.8 pmol, P<0.05) and tended to decrease that of HVA (selegiline: 8.2±0.9 pmol, saline: 14.1±3.2 pmol, P>0.05). Selegiline treatment did not alter the level of 5-HIAA in the baseline samples (selegiline: 3.6±0.3 pmol, saline: 3.5±0.6 pmol).

The selegiline-elevated DA level was decreasing during the collecting period (i.e. 2–6 h after the last selegiline administration), and at the end of the experiment it had decreased by 50% from the elevated level seen in the baseline samples. Initially during the collecting period the selegiline-decreased levels of DA metabolites continued to decrease, but the DOPAC level returned at the end of the experiment towards the levels of the baseline samples. The HVA level further decreased more than the DOPAC level and remained low at the end of experiment (Fig. 4, saline+saline/nicotine groups omitted from the figure due to clarity).

Nicotine (0.5 mg/kg), given acutely 3 h after the fifth selegiline (1 mg/kg) injection, modestly although not significantly elevated the levels of DA and its metabolites, particularly that of HVA, that were decreasing during the collecting period (Fig. 4; P>0.10).

Effect of haloperidol with nicotine

The DA receptor blocker, haloperidol (0.1 mg/kg s.c.), induced a moderate increase in the extracellular DA level up to 180% of the baseline level (Fig. 5). Due to high variability between haloperidol-treated rats the increase failed to reach significance in pair-wise comparison with the control group (P=0.054). The administration of haloperidol clearly elevated the levels of DA metabolites; both the DOPAC and HVA levels were significantly increased compared with the saline control group, DOPAC: $F(3,18)$=39.0, Newman-Keuls test P<0.001; HVA: $F(3,18)$=27.6, P<0.001 (Fig. 5). The maximal increase in the DOPAC level occurred 120 min after the administration of haloperidol, after which the DOPAC level remained almost unchanged for the rest of the collecting period. The HVA concentration was maximally increased about an hour later, at which level it remained until the end of the collecting period (270 min).

The combination of haloperidol (0.1 mg/kg) and nicotine (0.5 mg/kg) did not lead to any further significant increase in the levels of DA, DOPAC or HVA compared with that induced by haloperidol alone (Fig. 5). No significant treatment effects were observed in the 5-HIAA levels after nicotine, haloperidol or their combinations (data not shown).

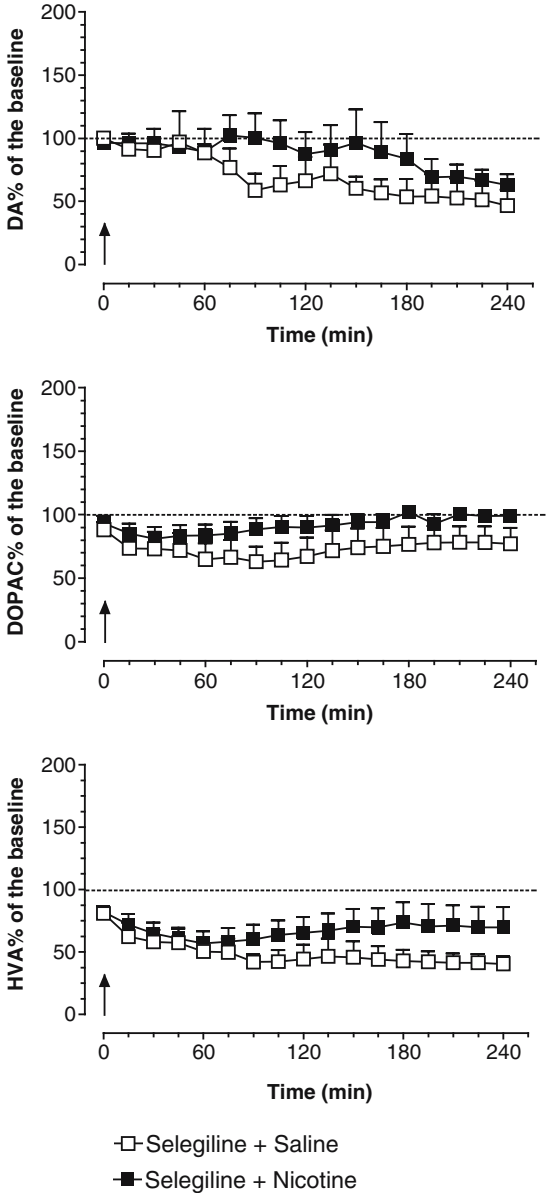

Fig. 4 The effects of nicotine (0.5 mg/kg s.c.) or saline on the extracellular levels of DA, DOPAC and HVA in the rats previously repeatedly treated with selegiline (5×1 mg/kg s.c.). Rats were given five repeated selegiline injections on 4 consecutive days and on the experimental day 2 h before starting to collect the baseline samples. Nicotine or saline (*arrow*) was given after collecting four consecutive baseline samples. The data, starting from the last baseline sample, are expressed as percentage changes of four consecutive baseline samples collected before nicotine or saline injections (means±SEM, *n*=4–6)

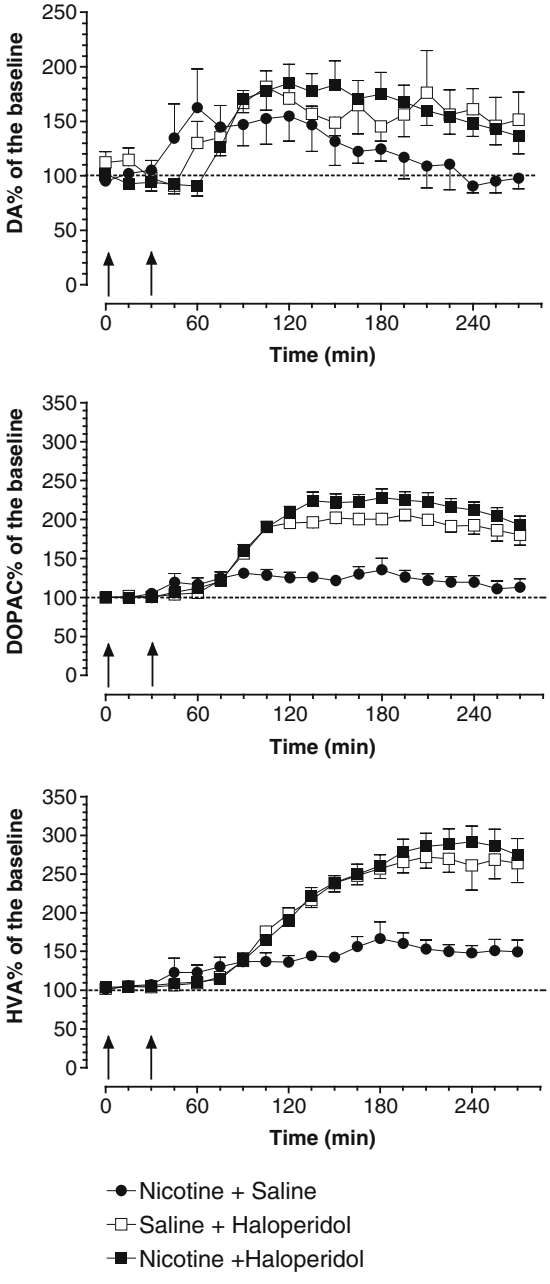

Fig. 5 The effects of haloperidol (0.1 mg/kg s.c.), nicotine (0.5 mg/kg s.c.) and their combination on the striatal extracellular levels of DA, DOPAC and HVA. Rats were given nicotine or saline (*first arrow*) after collecting the baseline samples and 30 min later haloperidol or saline (*second arrow*). It is to be noted that the scale of the ordinates differs between the graphs showing DA and its metabolites. The data, starting from the last baseline sample, are expressed as percentage changes of the consecutive baseline samples collected before injections (means±SEM, *n*=4–6)

Rotational behaviour

Nicotine (0.5 mg/kg s.c.) alone induced only a very modest ipsilateral rotation (Fig. 6). A slightly more intense rotation was induced by nomifensine (3 mg/kg i.p.). When these two drugs were combined, a strong ipsilateral rotational behaviour was observed. The net rotation differed significantly from that induced by either nicotine, $F(2,18)=5.5$, Newman-Keuls test $P<0.05$, or nomifensine alone, $F(2,18)=12.2$, $P<0.05$. Mecamylamine (5 mg/kg i.p.) did not induce any clear ipsi- or contralateral rotation. However, the pretreatment with mecamylamine significantly ($P<0.05$) antagonised the rotation induced by the combination of nomifensine and nicotine (Fig. 6).

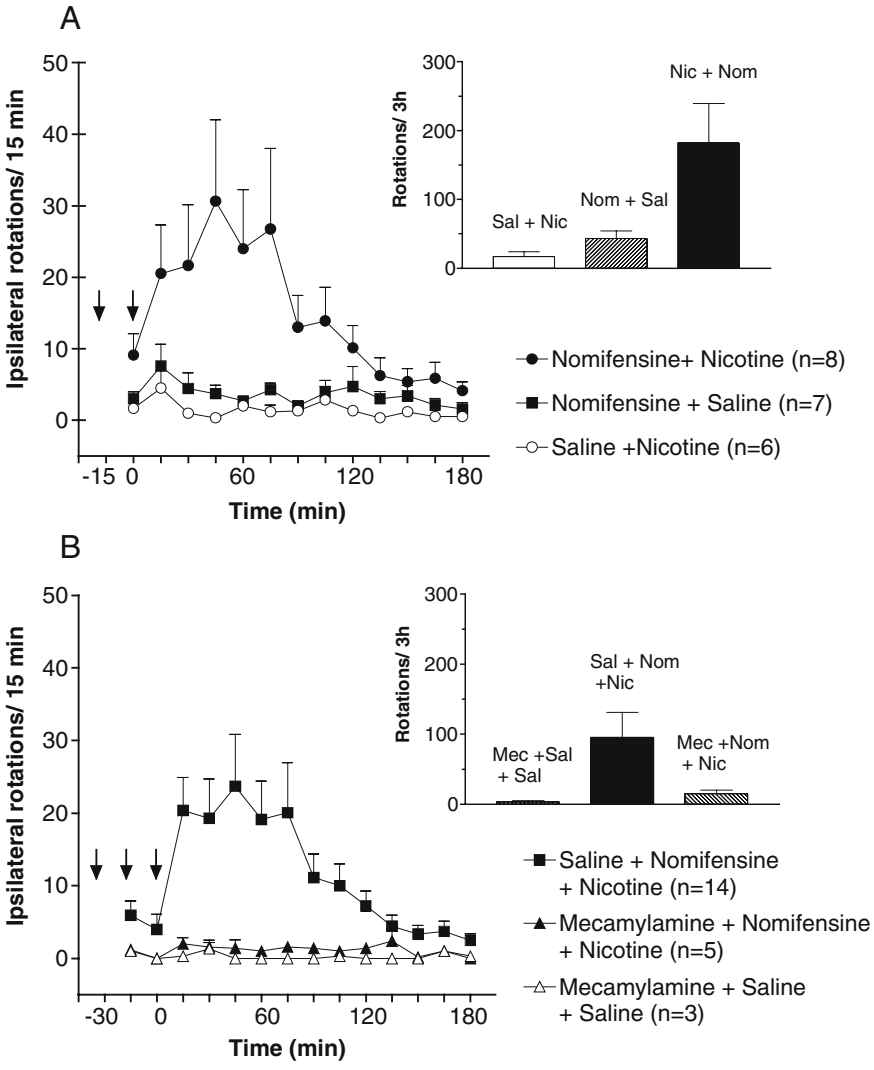

Fig. 6 The effects of mecamylamine, nicotine, nomifensine and their combination on ipsilateral rotational behaviour in rats with unilateral nigrostriatal 6-OHDA lesions. **a** Rats were given nomifensine (*Nom*, 3 mg/kg i.p.) or saline (*Sal*; *first arrow*) and 15 min later nicotine (*Nic*, 0.5 mg/kg s.c.) or saline (*second arrow*) after which the ipsilateral rotations were measured in 15-min intervals for 3 h. **b** Rats were given mecamylamine (*Mec*, 5 mg/kg i.p.) or saline (*first arrow*), 15 min later nomifensine (3 mg/kg i.p.) or saline (*second arrow*) and another 15 min later nicotine (0.5 mg/kg s.c.) or saline (*third arrow*), after which the ipsilateral rotations were measured in 15-min intervals for 3 h. The inserts present 3-h cumulative ipsilateral rotations. Data are expressed as means±SEM, *n*=3–14

Discussion

To investigate nicotine's effects on endogenous dopaminergic transmission in the nigrostriatal pathway, striatal extracellular levels of DA and its metabolites were estimated after administration of nicotine alone and in combination with drugs inhibiting either DA uptake, COMT, MAO-B or presynaptic DA receptors. Such a comparison has not been previously carried out in one and the same study, and indeed the effects of COMT inhibition on nicotine's effects on the synaptic DA have not been previously studied. The drugs were studied at doses that alter the endogenous dopaminergic transmission significantly, although not maximally (Carboni et al. 1989; Imperato et al. 1986; Kaakkola and Wurtman 1992; O'Connor et al. 1995; Schiffer et al. 2003). Inhibition of COMT by tolcapone, MAO-B by selegiline or presynaptic DA receptors by haloperidol did not further enhance the nicotine-elevated DA output. However, the DA uptake inhibitor nomifensine in combination with nicotine induced a considerable increase in the extracellular DA level in the dorsal striatum. The interaction was also observed at a behavioural level, as the combination caused a clear ipsilateral rotational behaviour in rats with unilateral destruction of nigrostriatal dopaminergic nerve tract.

Systemic administration of a small dose of nomifensine increased the extracellular level of DA, while the level of DOPAC did not increase. This is consistent with the mechanism of action of nomifensine, an inhibitor of DA reuptake (Hoffmann 1982) and also with previous results (Butcher et al. 1991; Church et al. 1987; Kaakkola and Wurtman 1992; Nakachi et al. 1995). The increases in the striatal extracellular DA levels induced by nomifensine alone or by the combination of nomifensine and nicotine were reflected in the ipsilateral rotational behaviour in the 6-OHDA-lesioned rats. Both the neurochemical and the behavioural interaction were inhibited by mecamylamine, indicating the involvement nAChRs in the release of DA in the dorsal striatum. Previously, it has been shown that intra-accumbal administration of nomifensine in combination with a systemic administration of nicotine resulted in a significant increase in the DA concentration in the dialysate in the nucleus accumbens (Benwell and Balfour 1992). A similar synergistic interaction between nicotine

and other DA uptake inhibitors (cocaine, methylphenidate) has been recently reported by Gerasimov et al. (2000). They found that nicotine and a relatively high dose of cocaine or methylphenidate produced a synergistic elevation in the extracellular DA level in the nucleus accumbens.

Acute systemic nicotine increased the DA output in the dorsal striatum as earlier reported after systemic administration (Damsma et al. 1988; Di Chiara and Imperato 1988; Ferger and Kuschinsky 1997; Imperato et al. 1986) and also after intrastriatal application of nicotine (Marshall et al. 1997; Toth et al. 1992). The effect appears to be mediated by brain nAChRs, as the brain penetrating nAChR blocker mecamylamine, but not the peripheral nAChR blocker hexamethonium, antagonised the increase. The effect was, however, quite modest, which may be due to rather low ambient temperature or it may simply be due to the brain area studied. We have shown earlier that nicotine's effect on the DA output in the dorsal striatum is dependent on ambient temperature and is more pronounced at elevated ambient temperatures (Seppä et al. 2000). The experiments presented here were performed at room temperature (21±1°C). We, as well as others, have also shown that the effect of nicotine on the DA output is more pronounced in the nucleus accumbens than in the dorsal striatum (Brazell et al. 1990; Imperato et al. 1986; Seppä and Ahtee 2000).

Catechol-O-methyltransferase inhibitors, such as entacapone and tolcapone, have been recently introduced into therapy for Parkinson's disease (Kaakkola 2000). Their mechanism of action in clinical use is based on the inhibition of peripheral metabolism of levodopa and subsequently they improve the bioavailability of levodopa and prolong its elimination half-life. In addition to a peripheral action, tolcapone penetrates the blood–brain barrier and inhibits the O-methylation of brain DA (Zürcher et al. 1993). Thus, theoretically it might also have a central antiparkinsonian effect. Microdialysis studies have, however, demonstrated that systemic administration of tolcapone does not elevate the extracellular level of DA, although the DA metabolism is altered (Huotari et al. 1999; Kaakkola and Wurtman 1992). We also found similar effects of tolcapone in this study.

A MAO-B inhibitor, selegiline, is also used to improve efficacy of levodopa in the treatment of parkinsonian patients (Yahr et al. 1983). In our study, repeated administration of selegiline increased the basal extracellular concentration of DA and decreased the striatal concentrations of DA metabolites, especially DOPAC, but did not alter the 5-HT metabolite, 5-HIAA. This suggests that selegiline inhibited mainly MAO-B at this dose and treatment regimen. These findings agree with earlier studies in which selegiline at low doses had modest effects on the extracellular DA acutely (Butcher et al. 1990; Kaakkola and Wurtman 1992; Wu et al. 2000), but increased it when given repeatedly (Lamensdorf et al. 1996, 1999).

In our study, nicotine did not have clear additive or synergistic effects with either tolcapone or with selegiline, as their combinations did not induce any further increase in the extracellular DA level. The probable explanation is that the role of DA uptake in the termination of the effect of DA in the synapse is of the most importance and the role of COMT or MAO-B is minimal in normal metabolic conditions in the rat striatum (Huotari et al. 2002; Raevskii et al. 2002). Thus, the slight increase in the extracellular DA induced by nicotine is probably controlled more by DA transporter than by either COMT or MAO-B. As a result, the effects of nicotine in combination with drugs other than DA uptake inhibitor were not sufficient to cause marked leakage of DA from the synaptic cleft into the extracellular fluid, the compartment actually sampled by the microdialysis probe. The situation may change when levodopa is combined, as then tolcapone and selegiline potentiate the increase in the extracellular DA level in the striatum (Huotari et al. 1999; Kaakkola and Wurtman 1993). Whether nicotine or nicotine derivatives in combination with levodopa and tolcapone/selegiline have beneficial effects on parkinsonian symptoms remains to be elucidated in future studies.

A low dose of haloperidol slightly increased the extracellular level of DA in the dorsal striatum. There was, however, a considerable variability between animals. This has been observed also by other researchers (Drew et al. 1990; O'Connor et al. 1995). It is likely that the elevation in the DA output seen after haloperidol is mediated by autoreceptors localised on dopaminergic terminals (Westerink and de Vries 1989). Nicotine did not alter the effects of haloperidol on the DA output and metabolism. Probably the reuptake mechanism is able to terminate any enhancement of the output of DA induced by combined haloperidol and nicotine. The enhanced DA output from dopaminergic terminals induced by nicotine might also, at least partly, antagonise the haloperidol-induced blockade of presynaptic DA receptors.

We have earlier shown that intranigral administration of nicotine induces contralateral rotation in rats (Kaakkola 1980). Nicotine can also enhance levodopa-induced contraversive rotation in hemiparkinsonian monkeys (Domino et al. 1999). These experimental findings indicate that nicotine or nicotine derivatives may have a therapeutic application in Parkinson's disease, as also suggested by others (Quik and Kulak 2002; Rusted et al. 2000). There are several case reports stating that nicotine may improve clinical symptoms of parkinsonian patients (Fagerström et al. 1994; Kelton et al. 2000; Marshall and Schnieden 1966; Villafane et al. 2001). However, in several studies chronic nicotine has had no significant effect on parkinsonian disability (Clemens et al. 1995; Ebersbach et al. 1999; Vieregge et al. 2001; Zdonczyk et al. 1988). The negative results may be related to desensitisation or inactivation of nAChR subtypes, or tolerance to the effects of nicotine in the striatal dopaminergic system (Grady et al. 1994; Marks et al. 1993; Pietilä et al. 1995, 1996). To our knowledge there are no clinical reports on the use of DA uptake inhibitors alone or in combination with nicotine in Parkinson's disease, and this may be due to the abuse liability of these compounds.

One solution to avoid desensitisation or inactivation might be the use of more selective nAChR agonists. In

animal models of Parkinson's disease several such agonists have shown promising effects (Menzaghi et al. 1997; Sacaan et al. 1996; Schneider et al. 1998). In addition, as there are many types of nAChRs in the brain, the development of subtype selective nAChR agonists would be one way to further improve parkinsonian therapy, particularly in the early stage of the disease. Several nAChR subtypes are involved in the DA release in the nigrostriatal system. On the dopaminergic terminals at least α3, α4, α5, α6, β2 and β3 subunits have been described and suggested to be involved in the DA release (Kulak et al. 2002; Quik et al. 2002; Salminen et al. 2004; Wonnacott et al. 2000; Zhou et al. 2001; Zoli et al. 2002). In addition, α2, α4, α5, α6, α7, β2 and β3 subunits appear to exist on dopaminergic cell bodies and dendrites in the substantia nigra (Arroyo-Jimenez et al. 1999; Azam et al. 2002; Klink et al. 2001; Quik et al. 2000; Sorenson et al. 1998). nAChR ligands directed to α6* nAChRs might be particularly relevant for Parkinson's disease (Quik and Kulak 2002; Quik et al. 2003). On the other hand, α7* nAChRs may be a relevant target as well, since their activation increases not only the release of DA in the striatum (Kaiser and Wonnacott 2000), but also the levels of tyrosine hydroxylase mRNA in the substantia nigra (Serova and Sabban 2002). These effects may be indirectly mediated through α7* nAChR activation on glutamatergic nerve terminals leading to an increase in glutamate release and activation of dopaminergic cells (Kaiser and Wonnacott 2000).

In conclusion, our studies indicate that nicotine is able to increase the striatal extracellular levels of DA, the effect that is potentiated by DA uptake inhibitor nomifensine. The potentiation is also observed at a behavioural level. No such potentiation was found when nicotine was combined with drugs affecting DA metabolism (tolcapone, selegiline) or inhibiting DA receptors (haloperidol). Thus, our findings indicate that striatal DA released by nicotine is rapidly inactivated by reuptake of DA into nerve terminals, and, as discussed above, the role of COMT or MAO-B is minimal in normal metabolic conditions in the rat striatum. Subtype selective nicotine derivatives, possibly combined with a DA uptake inhibitor, may have a therapeutic application in the treatment of early Parkinson's disease.

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References

Allam MF, Campbell MJ, Hofman A, Del Castillo AS, Fernandez-Crehuet Navajas R (2004) Smoking and Parkinson's disease: systematic review of prospective studies. Mov Disord 19:614–621

Arqueros L, Naquira D, Zunino E (1978) Nicotine-induced release of catecholamines from rat hippocampus and striatum. Biochem Pharmacol 27:2667–2674

Arroyo-Jimenez MM, Bourgeois JP, Marubio LM, Le Sourd AM, Ottersen OP, Rinvik E, Fairen A, Changeux JP (1999) Ultrastructural localization of the alpha4-subunit of the neuronal acetylcholine nicotinic receptor in the rat substantia nigra. J Neurosci 19:6475–6487

Azam L, Winzer-Serhan UH, Chen Y, Leslie FM (2002) Expression of neuronal nicotinic acetylcholine receptor subunit mRNAs within midbrain dopamine neurons. J Comp Neurol 444:260–274

Balfour DJ, Fagerström KO (1996) Pharmacology of nicotine and its therapeutic use in smoking cessation and neurodegenerative disorders. Pharmacol Ther 72:51–81

Baron JA (1986) Cigarette smoking and Parkinson's disease. Neurology 36:1490–1496

Benwell ME, Balfour DJ (1992) The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. Br J Pharmacol 105:849–856

Brazell MP, Mitchell SN, Joseph MH, Gray JA (1990) Acute administration of nicotine increases the in vivo extracellular levels of dopamine, 3,4-dihydroxyphenylacetic acid and ascorbic acid preferentially in the nucleus accumbens of the rat: comparison with caudate-putamen. Neuropharmacology 29:1177–1185

Butcher SP, Fairbrother IS, Kelly JS, Arbuthnott GW (1990) Effects of selective monoamine oxidase inhibitors on the in vivo release and metabolism of dopamine in the rat striatum. J Neurochem 55:981–988

Butcher SP, Liptrot J, Aburthnott GW (1991) Characterisation of methylphenidate and nomifensine induced dopamine release in rat striatum using in vivo brain microdialysis. Neurosci Lett 122:245–248

Carboni E, Imperato A, Perezzani L, Di Chiara G (1989) Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28:653–661

Church WH, Justice JB Jr, Byrd LD (1987) Extracellular dopamine in rat striatum following uptake inhibition by cocaine, nomifensine and benztropine. Eur J Pharmacol 139:345–348

Clarke PB, Pert A (1985) Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res 348:355–358

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

Clemens P, Baron JA, Coffey D, Reeves A (1995) The short-term effect of nicotine chewing gum in patients with Parkinson's disease. Psychopharmacology 117:253–256

Court J, Clementi F (1995) Distribution of nicotinic subtypes in human brain. Alzheimer Dis Assoc Disord 9 [Suppl 2]:6–14

Damsma G, Westerink BH, de Vries JB, Horn AS (1988) The effect of systemically applied cholinergic drugs on the striatal release of dopamine and its metabolites, as determined by automated brain dialysis in conscious rats. Neurosci Lett 89:349–354

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85:5274–5278

Domino EF, Ni L, Zhang H (1999) Nicotine alone and in combination with L-DOPA methyl ester or the D(2) agonist N-0923 in MPTP-induced chronic hemiparkinsonian monkeys. Exp Neurol 158:414–421

Drew KL, O'Connor WT, Kehr J, Ungerstedt U (1990) Regional specific effects of clozapine and haloperidol on GABA and dopamine release in rat basal ganglia. Eur J Pharmacol 187:385–397

Ebersbach G, Stock M, Muller J, Wenning G, Wissel J, Poewe W (1999) Worsening of motor performance in patients with Parkinson's disease following transdermal nicotine administration. Mov Disord 14:1011–1013

Fagerström KO, Pomerleau O, Giordani B, Stelson F (1994) Nicotine may relieve symptoms of Parkinson's disease. Psychopharmacology 116:117–119

Ferger B, Kuschinsky K (1997) Biochemical studies support the assumption that dopamine plays a minor role in the EEG effects of nicotine. Psychopharmacology 129:192–196

Futami T, Takakusaki K, Kitai ST (1995) Glutamatergic and cholinergic inputs from the pedunculopontine tegmental nucleus to dopamine neurons in the substantia nigra pars compacta. Neurosci Res 21:331–342

Gerasimov MR, Franceschi M, Volkow ND, Rice O, Schiffer WK, Dewey SL (2000) Synergistic interactions between nicotine and cocaine or methylphenidate depend on the dose of dopamine transporter inhibitor. Synapse 38:432–437

Giorguieff-Chesselet MF, Kemel ML, Wandscheer D, Glowinski J (1979) Regulation of dopamine release by presynaptic nicotinic receptors in rat striatal slices: effect of nicotine in a low concentration. Life Sci 25:1257–1262

Gorell JM, Rybicki BA, Johnson CC, Peterson EL (1999) Smoking and Parkinson's disease: a dose-response relationship. Neurology 52:115–119

Grady SR, Marks MJ, Collins AC (1994) Desensitization of nicotine-stimulated [3H]dopamine release from mouse striatal synaptosomes. J Neurochem 62:1390–1398

Haikala H (1987) Use of a novel type of rotating disc electrode and a flow cell with laminar flow pattern for the electrochemical detection of biogenic monoamines and their metabolites after Sephadex gel chromatographic purification and high-performance liquid chromatographic isolation from rat brain. J Neurochem 49:1033–1041

Hoffmann I (1982) Pharmacology of nomifensine. Int Pharmacopsychiatry 17 [Suppl]:4–20

Huotari M, Gainetdinov R, Mannisto PT (1999) Microdialysis studies on the action of tolcapone on pharmacologically-elevated extracellular dopamine levels in conscious rats. Pharmacol Toxicol 85:233–238

Huotari M, Santha M, Lucas LR, Karayiorgou M, Gogos JA, Mannisto PT (2002) Effect of dopamine uptake inhibition on brain catecholamine levels and locomotion in catechol-O-methyltransferase-disrupted mice. J Pharmacol Exp Ther 303:1309–1316

Imperato A, Mulas A, Di Chiara G (1986) Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. Eur J Pharmacol 132:337–338

Janhunen S, Kaakkola S, Tuominen RK, Ahtee L (2001) Nicotine induces rotation in combination with nomifensine in unilaterally 6-hydroxydopamine lesioned rats. Parkinsonism Relat Disord 7 [Suppl 1]:559

Kaakkola S (1980) Contralateral circling behaviour induced by intranigral injection of morphine and enkephalin analogue FK 33-824 in rats. Acta Pharmacol Toxicol 47:385–393

Kaakkola S (2000) Clinical pharmacology, therapeutic use and potential of COMT inhibitors in Parkinson's disease. Drugs 59:1233–1250

Kaakkola S, Wurtman RJ (1992) Effects of COMT inhibitors on striatal dopamine metabolism: a microdialysis study. Brain Res 587:241–249

Kaakkola S, Wurtman RJ (1993) Effects of catechol-O-methyltransferase inhibitors and L-3,4-dihydroxyphenylalanine with or without carbidopa on extracellular dopamine in rat striatum. J Neurochem 60:137–144

Kaakkola SK, Tuominen RK, Mielikäinen P, Paldanius P, Ahtee L (2000) Effects of nomifensine and tolcapone on nicotine-induced dopamine release in rat striatum. Soc Neurosci Abstr 26:900

Kaiser S, Wonnacott S (2000) Alpha-bungarotoxin-sensitive nicotinic receptors indirectly modulate [3H]dopamine release in rat striatal slices via glutamate release. Mol Pharmacol 58:312–318

Kelton MC, Kahn HJ, Conrath CL, Newhouse PA (2000) The effects of nicotine on Parkinson's disease. Brain Cogn 43:274–282

Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP (2001) Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. J Neurosci 21:1452–1463

Kulak JM, Musachio JL, McIntosh JM, Quik M (2002) Declines in different β2* nicotinic receptor populations in monkey striatum after nigrostriatal damage. J Pharmacol Exp Ther 303:633–639

Lamensdorf I, Youdim MB, Finberg JP (1996) Effect of long-term treatment with selective monoamine oxidase A and B inhibitors on dopamine release from rat striatum in vivo. J Neurochem 67:1532–1539

Lamensdorf I, Porat S, Simantov R, Finberg JP (1999) Effect of low-dose treatment with selegiline on dopamine transporter (DAT) expression and amphetamine-induced dopamine release in vivo. Br J Pharmacol 126:997–1002

Marks MJ, Grady SR, Collins AC (1993) Downregulation of nicotinic receptor function after chronic nicotine infusion. J Pharmacol Exp Ther 266:1268–1276

Marshall DL, Redfern PH, Wonnacott S (1997) Presynaptic nicotinic modulation of dopamine release in the three ascending pathways studied by in vivo microdialysis: comparison of naive and chronic nicotine-treated rats. J Neurochem 68:1511–1519

Marshall J, Schnieden H (1966) Effect of adrenaline, noradrenaline, atropine, and nicotine on some types of human tremor. J Neurol Neurosurg Psychiatry 29:214–218

Menzaghi F, Whelan KT, Risbrough VB, Rao TS, Lloyd GK (1997) Interactions between a novel cholinergic ion channel agonist, SIB-1765F and L-DOPA in the reserpine model of Parkinson's disease in rats. J Pharmacol Exp Ther 280:393–401

Mihailescu S, Drucker-Colin R (2000) Nicotine, brain nicotinic receptors, and neuropsychiatric disorders. Arch Med Res 31: 131–144

Morens DM, Grandinetti A, Reed D, White LR, Ross GW (1995) Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? Neurology 45:1041–1051

Nakachi N, Kiuchi Y, Inagaki M, Inazu M, Yamazaki Y, Oguchi K (1995) Effects of various dopamine uptake inhibitors on striatal extracellular dopamine levels and behaviours in rats. Eur J Pharmacol 281:195–203

O'Connor WT, Drew KL, Ungerstedt U (1995) Differential cholinergic regulation of dopamine release in the dorsal and ventral neostriatum of the rat: an in vivo microdialysis study. J Neurosci 15:8353–8361

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, vol 2. Academic, New York

Pietilä K, Laakso I, Ahtee L (1995) Chronic oral nicotine administration affects the circadian rhythm of dopamine and 5-hydroxytryptamine metabolism in the striata of mice. Naunyn-Schmiedebergs Arch Pharmacol 353:110–115

Pietilä K, Salminen O, Leikola-Pelho T, Ahtee L (1996) Tolerance to nicotine's effects on striatal dopamine metabolism in nicotine-withdrawn mice. Eur J Pharmacol 318:17–22

Quik M (2004) Smoking, nicotine and Parkinson's disease. Trends Neurosci 27:561–568

Quik M, Kulak JA (2002) Nicotine and nicotinic receptors; relevance to Parkinson's disease. Neurotoxicology 23:581–594

Quik M, Polonskaya Y, Gillespie A, Lloyd GK, Langston JW (2000) Differential alterations in nicotinic receptor alpha6 and beta3 subunit messenger RNAs in monkey substantia nigra after nigrostriatal degeneration. Neuroscience 100:63–72

Quik M, Polonskaya Y, McIntosh JM, Kulak JM (2002) Differential nicotinic receptor expression in monkey basal ganglia: effects of nigrostriatal damage. Neuroscience 112:619–630

Quik M, Sum JD, Whiteaker P, McCallum SE, Marks MJ, Musachio J, McIntosh JM, Collins AC, Grady SR (2003) Differential declines in striatal nicotinic receptor subtype function after nigrostriatal damage in mice. Mol Pharmacol 63:1169–1179

Raevskii KS, Gainetdinov RR, Budygin EA, Mannisto P, Wightman M (2002) Dopaminergic transmission in the rat striatum in vivo in conditions of pharmacological modulation. Neurosci Behav Physiol 32:183–188

Rapier C, Lunt GG, Wonnacott S (1990) Nicotinic modulation of [3H]dopamine release from striatal synaptosomes: pharmacological characterisation. J Neurochem 54:937–945

Rowell PP (1995) Nanomolar concentrations of nicotine increase the release of [3H]dopamine from rat striatal synaptosomes. Neurosci Lett 189:171–175

Rusted JM, Newhouse PA, Levin ED (2000) Nicotinic treatment for degenerative neuropsychiatric disorders such as Alzheimer's disease and Parkinson's disease. Behav Brain Res 113:121–129

Sacaan AI, Menzaghi F, Dunlop JL, Correa LD, Whelan KT, Lloyd GK (1996) Epibatidine: a nicotinic acetylcholine receptor agonist releases monoaminergic neurotransmitters: in vitro and in vivo evidence in rats. J Pharmacol Exp Ther 276:509–515

Sakurai Y, Takano Y, Kohjimoto Y, Honda K, Kamiya HO (1982) Enhancement of [3H]dopamine release and its [3H]metabolites in rat striatum by nicotinic drugs. Brain Res 242:99–106

Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, Grady SR (2004) Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. Mol Pharmacol 65:1526–1535

Schiffer WK, Azmoodeh M, Gerasimov M, Volkow ND, Fowler JS, Dewey SL (2003) Selegiline potentiates cocaine-induced increases in rodent nucleus accumbens dopamine. Synapse 48: 35–38

Schneider JS, Pope-Coleman A, Van Velson M, Menzaghi F, Lloyd GK (1998) Effects of SIB-1508Y, a novel neuronal nicotinic acetylcholine receptor agonist, on motor behavior in parkinsonian monkeys. Mov Disord 13:637–642

Seppä T, Ahtee L (2000) Comparison of the effects of epibatidine and nicotine on the output of dopamine in the dorsal and ventral striatum of freely-moving rats. Naunyn-Schmiedebergs Arch Pharmacol 362:444–447

Seppä T, Ruotsalainen M, Laakso I, Tuominen R, Ahtee L (2000) Effect of acute nicotine administration on striatal dopamine output and metabolism in rats kept at different ambient temperatures. Br J Pharmacol 130:1147–1155

Serova L, Sabban EL (2002) Involvement of alpha 7 nicotinic acetylcholine receptors in gene expression of dopamine biosynthetic enzymes in rat brain. J Pharmacol Exp Ther 303:896–903

Sorenson EM, Shiroyama T, Kitai ST (1998) Postsynaptic nicotinic receptors on dopaminergic neurons in the substantia nigra pars compacta of the rat. Neuroscience 87:659–673

Teng L, Crooks PA, Buxton ST, Dwoskin LP (1997) Nicotinic-receptor mediation of S(-)nornicotine-evoked -3H-overflow from rat striatal slices preloaded with -3H-dopamine. J Pharmacol Exp Ther 283:778–787

Toth E, Sershen H, Hashim A, Vizi ES, Lajtha A (1992) Effect of nicotine on extracellular levels of neurotransmitters assessed by microdialysis in various brain regions: role of glutamic acid. Neurochem Res 17:265–271

Vernier JM, Holsenback H, Cosford ND, Whitten JP, Menzaghi F, Reid R, Rao TS, Sacaan AI, Lloyd GK, Suto CM, Chavez-Noriega LE, Washburn MS, Urrutia A, McDonald IA (1998) Conformationally restricted analogues of nicotine and anabasine. Bioorg Med Chem Lett 8:2173–2178

Vieregge A, Sieberer M, Jacobs H, Hagenah JM, Vieregge P (2001) Transdermal nicotine in PD: a randomized, double-blind, placebo-controlled study. Neurology 57:1032–1035

Villafane G, Degos J-D, Lagrue G, Cesaro P (2001) Long-term nicotine treatment in Parkinson's disease: report of a case. Parkinsonism Relat Disord 7 [Suppl 1]:S73

Westerink BH, de Vries JB (1989) On the mechanism of neuroleptic induced increase in striatal dopamine release: brain dialysis provides direct evidence for mediation by autoreceptors localized on nerve terminals. Neurosci Lett 99:197–202

Westfall TC (1974) Effect of nicotine and other drugs on the release of 3H-norepinephrine and 3H-dopamine from rat brain slices. Neuropharmacology 13:693–700

Whiteaker P, Garcha HS, Wonnacott S, Stolerman IP (1995) Locomotor activation and dopamine release produced by nicotine and isoarecolone in rats. Br J Pharmacol 116:2097–2105

Wonnacott S (1997) Presynaptic nicotinic ACh receptors. Trends Neurosci 20:92–98

Wonnacott S, Kaiser S, Mogg A, Soliakov L, Jones IW (2000) Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. Eur J Pharmacol 393:51–58

Wu WR, Zhu ZT, Zhu XZ (2000) Differential effects of L-deprenyl on MPP+- and MPTP-induced dopamine overflow in microdialysates of striatum and nucleus accumbens. Life Sci 67:241–250

Yahr MD, Mendoza MR, Moros D, Bergmann KJ (1983) Treatment of Parkinson's disease in early and late phases. Use of pharmacological agents with special reference to deprenyl (selegiline). Acta Neurol Scand Suppl 95:95–102

Zdonczyk D, Royse V, Koller WC (1988) Nicotine and tremor. Clin Neuropharmacol 11:282–286

Zhou FM, Liang Y, Dani JA (2001) Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. Nat Neurosci 4:1224–1229

Zoli M, Moretti M, Zanardi A, McIntosh JM, Clementi F, Gotti C (2002) Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. J Neurosci 22:8785–8789

Zürcher G, Dingemanse J, Da Prada M (1993) Potent COMT inhibition by Ro 40-7592 in the periphery and in the brain. Preclinical and clinical findings. Adv Neurol 60:641–647