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Tolerance to nicotine's effects on striatal dopamine metabolism in nicotine-withdrawn mice

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Abstract

After 7-week chronic administration of nicotine to mice in their drinking water, nicotine was withdrawn for 24 h. Acute nicotine challenge (1 mg/kg s.c., 60 min) elevated the striatal concentrations of dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and decreased the concentration of 3-methoxytyramine significantly less in the mice withdrawn for 24 h from nicotine than in the control mice which had been drinking tap water under identical conditions for 7 weeks. Neither withdrawal nor the acute nicotine challenge altered the striatal dopamine concentration. No alterations were found in the density or affinity of the specific binding of [^{3}H]SCH 23390 or [^{3}H]spiperone to striatal membrane homogenates during nicotine treatment or after its withdrawal. Thus, our results show that tolerance to the acute effects of nicotine on striatal dopamine metabolism can be induced by administering nicotine to mice in the drinking water. However, neither chronic nicotine treatment nor its withdrawal seem to affect dopamine D_1 and D_2 receptors in the striatum.

Keywords: Nicotine; Tolerance; Dopamine metabolism; Striatum, mouse; Dopamine receptor

1. Introduction

Tolerance to the acute behavioural effects of nicotine (Stolerman et al., 1973, 1974; Hatchell and Collins, 1977; Falkeborn et al., 1981; Clarke and Kumar, 1983) as well as to nicotine-induced hypothermia (Hall, 1973; Mansner et al., 1974; Marks et al., 1983, 1985, 1993; Collins et al., 1988) has been shown to occur during withdrawal from chronic nicotine exposure in rats and in mice. In these studies tolerance was induced by using various routes of nicotine administration (s.c. and i.p. injections, drinking water, continuous infusions) and occurred even when nicotine was administered only 1–3 times weekly (Stolerman et al., 1974; Clarke and Kumar, 1983). In mice, the development of tolerance depends on the genotype and consequently varies between different strains (Hatchell and Collins, 1977; Marks et al., 1983, 1986). The development of tolerance does not result from induction of the nicotine-metabolizing enzymes in the liver. Instead, tolerance to nicotine seems to be pharmacodynamic in origin (i.e. adaptation of neuronal mechanisms). In the brain chronic nicotine treatment has been connected with up-regulation of the nicotinic acetylcholine receptors (Schwartz and Kellar, 1983, 1985; Marks et al., 1983, 1985, 1986, 1987; Martino-Barrows and Kellar, 1987).

Acutely administered nicotine has been shown to alter striatal dopamine metabolism (Roth et al., 1982; Haikala et al., 1986; Imperato et al., 1986; Leikola-Pelho et al., 1990). However, the occurrence of tolerance to the nicotine-induced changes in cerebral dopamine metabolism during withdrawal from chronic nicotine exposure has not been demonstrated. We have shown that nicotine can be administered chronically to mice in the drinking water at concentrations high enough to yield plasma nicotine concentrations clearly above those found in smokers and comparable to plasma concentrations induced by subcutaneous administration of 1–3 mg/kg of nicotine to mice (Pekonen et al., 1993). By using this route of administration the mice obtain nicotine mainly during their active time, as smokers usually do (Pietilä et al., 1995). Thus, to study the development of tolerance to nicotine's acute effects on striatal dopamine metabolism we administered nicotine to mice for 7 weeks in the drinking water and challenged them with acute nicotine after nicotine with-

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drawal for 24 h. For comparison, we studied whether this treatment produced tolerance to nicotine-induced hypothermia. We also estimated the binding of [^{3}H]SCH 23390 and [^{3}H]spiperone to striatal homogenates both during chronic nicotine treatment and after its withdrawal to study whether chronic nicotine affects dopamine D_1 and D_2 receptors.

NMRI mice, developed in the National Marine Research Institute (Bethesda, MD, USA), were used in our experiments. Although these mice are widely used, we did not find any previous reports concerning nicotine tolerance in these mice.

2. Materials and methods

2.1. Administration of chronic nicotine

Five-week-old male NMRI mice bred locally in the Laboratory Animal Center University of Helsinki (26–27 g) were divided randomly into nicotine-receiving and control mice. Experimental animals were maintained in accordance with the internationally accepted principles, and the experimental set-up was approved by the Committee for Animal Experiments of the Faculty of Science of the University of Helsinki.

Groups of 6–8 mice were housed in a cage and had free access to mouse chow. The lights were on from 6 a.m. to 6 p.m., and the ambient temperature was held at 22–24°C. Nicotine was administered chronically in the drinking water (the sole source of fluid) as described by Pekonen et al. (1993). The concentration of nicotine in the drinking water was gradually increased from 50 μg/ml to 500 μg/ml over 7 weeks to coax the mice to drink as steadily as possible. The control mice drank tap water during the entire treatment. After 2–3 weeks the fluid intake and the weight gain of the nicotine-treated mice started to diminish, so that after 7 weeks the mice offered the 500 μg/ml nicotine solution drank about 47% less and weighed 9% less than the corresponding control mice. Due to the decreased consumption of water the concentrations of nicotine and cotinine in plasma, and thus the amount of nicotine consumed daily, remained at about 60–65 mg/kg from the third week onward up to the end of the 7-week study (Pekonen et al., 1993; Pietilä et al., 1995). Nicotine is known to be a powerful stimulant of antidiuretic hormone release and thus reduces the output of urine (Burn et al., 1945). Since small rodents maintain their fluid balance very effectively, it appears that the mice reduced their fluid intake to compensate for the reduced fluid output. In addition, the hypodipsia produced by the higher nicotine concentrations could be partially due to the bitter taste of the solution offered. After the nicotine solutions were replaced with tap water, the nicotine-treated mice drank 15% more than the control mice during the 24-h withdrawal period, and their weights reached the weights of the control mice (Pekonen et al., 1993).

During chronic administration, the rectal temperatures were measured one day after the nicotine concentration of the solution was increased (Ellab rectal thermometer, type TE 3, Ellab Instruments, Denmark).

2.2. Challenge experiments

On the last day of nicotine administration the mice were withdrawn by replacing the nicotine solution with tap water at about 11 a.m. After 24 h of withdrawal, the mice were given acute nicotine (1 mg/kg s.c.) or 0.9% NaCl solution (saline s.c.), and 60 min later were killed by decapitation and their striata were dissected. For injections (10 ml/kg) (−)-nicotine base (Fluka, Buchs, Switzerland) was diluted with 0.9% NaCl solution, and the pH of the final solution was adjusted to 7.0–7.4 with 0.05 M HCl in 0.9% NaCl solution. The acute challenge experiment was performed at an ambient temperature of 20–22°C. The rectal temperatures were measured at 30 min and 60 min after the injection. The mice were killed by decapitation immediately following the last temperature measurement and the striata were dissected within 3 min and frozen on dry ice. The striata were weighed (mean weight 25 mg) and stored at −80°C until assayed.

2.3. Measurement of striatal dopamine and its metabolites

The striatal concentrations of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine and homovanillic acid (HVA) were measured with high-performance liquid chromatography as described by Haikala (1987). The tissue extracts were purified by Sephadex G-10 gel chromatography and the final separation of the compounds was achieved by using C_{18} reverse phase (Spherisorb ODS 5 μm) columns (25 cm, 5 mm i.d.) connected to the high performance liquid chromatography equipment. An electrochemical detector (Potentiostat LC-2A, Bioanalytical Systems) with a rotating disc working electrode was used.

After decapitation, dopamine is released and metabolised rapidly. In rats post-mortem the 3-methoxytyramine content increases quickly and therefore microwave irradiation is the preferred method of killing (Westerink and Spaan, 1982). However, in mouse striatum the steady state content of 3-methoxytyramine is several times higher and the turnover and post-mortem formation of 3-methoxytyramine are clearly slower than in rats (Haikala, 1987; Wood et al., 1988). Thus, microwave irradiation is not necessary in studies of mouse striatal 3-methoxytyramine content provided that the dissection time is constant and short enough.

2.4. Dopamine receptor binding assays

For the binding assays striata were collected as described above from control and nicotine-drinking mice

killed by decapitation on the last day of chronic treatment as well as from mice killed 24 h after withdrawal. The striata from 10 similarly treated mice were pooled and were homogenized in ice-cold buffer containing 41 mM Trizma HCl, 8 mM Trizma base, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2 \times 6H_2O$ and 1 mM $CaCl_2 \times 2H_2O$ (pH 7.1 at 37°C), using a Polytron homogenizer (setting 5 for 15 s). The homogenates were washed once by centrifugation (37 000 × *g* at 4–10°C for 20 min), resuspended in 20–25 volumes of fresh buffer and rehomogenized using the Polytron homogenizer.

Dopamine D_1 and D_2 receptor subtype assays were performed in different tubes. The saturation incubations were initiated by adding 200 μl of tissue homogenate (0.15–0.18 mg protein/ml) to triplicate tubes containing non-specific ligand and assay buffer. Non-specific ligands were 1 μM cisflupentixol for the dopamine D_1 receptor assay, and 1 μM (+)-butaclamol plus 1 μM ketanserin for the dopamine D_2 receptor assay. Ketanserin was used in a 200 times higher concentration than the radioligand concentration in dopamine D_2 receptor binding experiments to prevent the binding of [^{3}H]spiperone to 5-hydroxytryptamine type-2 receptors. In order to promote the hydrolysis of endogenous dopamine, preincubation was carried out at +37°C for 15 min. After cooling at +4°C for 15 min, five different concentrations of [^{3}H]SCH 23390 (0.75–20 nM; 100 μl) for the dopamine D_1 receptor assay and of [^{3}H]spiperone (0.5–10 nM; 100 μl) for the dopamine D_2 receptor assay were added to yield a final assay volume of 1 ml. All test tubes were incubated at +37°C for 20 min (dopamine D_2 receptor assay) or 30 min (dopamine D_1 receptor assay), and the tube contents were filtered under vacuum through Whatman GF/B glass-fibre filters by a Brandell M-24 Cell Harvester. The filters were then washed rapidly twice with 5 ml of ice-cold assay buffer. The filters were put into plastic scintillation vials, and 5 ml of 'HiSafe 3' scintillation liquid (Wallac, Finland) was added. Radioactivity trapped on the filters was counted using a Wallac 1410 liquid scintillation counter at an efficiency of 52%. The protein concentrations of homogenates were measured using a modified Lowry method (Lowry et al., 1951) in which human albumin was used as an external standard. The B_{max} and K_d values for the specific binding of [^{3}H]SCH 23390 and [^{3}H]spiperone were determined by Scatchard analyses of the saturation data (Scatchard, 1949), using a curve-fitting computer program. The correlation coefficients were $r = 0.9846 \pm 0.0025$ for the dopamine D_1 receptor assay and $r = 0.9889 \pm 0.0018$ for the dopamine D_2 receptor assay (means ± S.E.M.) for binding to a single site for each receptor.

2.5. Statistics

Results are given as means ± S.E.M. As the metabolite results consisted of several experiments, the statistical analyses were carried out by three-way analysis of variance (experiment × chronic treatment × acute challenge). As no significant interactions between the experiment and other factors were observed, the randomized block two-way analysis of variance was performed using experiments as blocks. If there were significant (chronic × acute treatment) interactions ($P < 0.05$), the analyses were continued by comparing appropriate cell means by linear contrasts. The binding results for dopamine D_1 and D_2 receptors were analyzed with one-way analysis of variance.

3. Results

3.1. Rectal temperature

During chronic treatment the rectal temperature of the nicotine-treated mice did not differ significantly from that of the water-drinking control mice (two-way analysis of variance for repeated measurements; data not shown). It is to be noted that during the chronic treatment rectal temperature was measured at 11 a.m. when the nicotine concentration was about 50 ng/ml (Pietilä et al., 1995).

In the challenge experiment the 1 mg/kg dose of nicotine, which elevates the plasma nicotine concentration to over 100 ng/ml (Pekonen et al., 1993), decreased the rectal temperature in the control mice at 30 min by −4.6 ± 0.2°C (mean ± S.E.M.) and at 60 min by −2.2 ± 0.3°C. In mice withdrawn for 24 h from 7-week chronic nicotine treatment the corresponding decrease was significantly smaller, at 30 min −2.4 ± 0.3°C ($P < 0.01$) and at 60 min −1.1 ± 0.3°C ($P < 0.05$) (two-way analysis of variance for repeated measurements followed by Tukey's post-hoc comparisons). The mean rectal temperature of control mice before nicotine administration was 38.9 ± 0.02°C ($n = 16$) and that of nicotine-withdrawn mice 38.6 ± 0.09°C ($n = 16$; $P > 0.05$).

3.2. Striatal dopamine metabolism

Neither withdrawal nor the acute nicotine challenge altered the striatal dopamine concentration (data not shown). As shown in Fig. 1 in control mice the 1 mg/kg acute dose of nicotine elevated the striatal concentrations of DOPAC and HVA by 29% and by 48%, respectively ($P < 0.01$ and $P < 0.001$), and decreased the 3-methoxytyramine concentration by 49% ($P < 0.001$) at 60 min after administration. The 24-h withdrawal from chronic nicotine did not significantly alter the levels of the striatal dopamine metabolites as compared with the levels in control mice. In mice withdrawn from chronic nicotine for 24 h, acute nicotine did not change any of the striatal dopamine metabolites studied. Thus, the striatal concentrations of DOPAC ($P < 0.01$) and HVA ($P < 0.01$) were significantly lower, and that of 3-methoxytyramine ($P < 0.05$) significantly higher than those in mice challenged with nicotine for the first time.

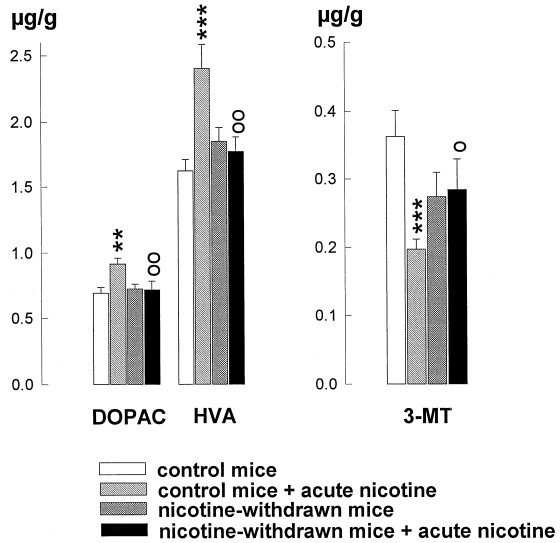

Fig. 1. The effect of an acute challenge dose of nicotine 1 mg/kg s.c. (60 min) on the striatal concentrations of dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) in control mice and in mice withdrawn for 24 h from 7-week nicotine administration in the drinking water. Given are the mean striatal concentrations of the metabolites (columns) ±S.E.M. (vertical bars); $n = 14–18$ in each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with the control mice given saline s.c. acutely; $^{\circ}P < 0.05$, $^{\circ\circ}P < 0.01$ as compared with the control mice given nicotine 1 mg/kg s.c. acutely.

3.3. Dopamine receptors

The mean K_d and B_{max} values for specific [^{3}H]SCH 23390 and [^{3}H]spiperone binding to striatal homogenates are shown in Table 1. No significant differences in the striatal dopamine D_1 or D_2 receptor density (B_{max}) or affinity (K_d) could be found between the control mice and nicotine-treated or nicotine-withdrawn mice.

Table 1
The binding of [^{3}H]SCH 23390 and [^{3}H]spiperone to striatal dopamine receptors during chronic nicotine treatment and after a 24-h withdrawal

D_1 receptors [^{3}H]SCH23390	Control	Nicotine treatment	Nicotine withdrawal
K_d	0.69 ±0.09	0.63 ±0.06	0.80 ±0.11
B_{max}	1.22 ±0.10	1.45 ±0.13	1.45 ±0.15
D_2 receptors [^{3}H]Spiperone			
K_d	0.06 ±0.01	0.06 ±0.01	0.06 ±0.01
B_{max}	0.48 ±0.03	0.49 ±0.04	0.53 ±0.06

Mean K_d (nM) and B_{max} (pmol/mg protein) ±S.E.M. of [^{3}H]SCH 23390 and [^{3}H]spiperone binding to dopamine D_1 and D_2 receptors in control mice, in mice treated with nicotine for 7 weeks (Nicotine treatment) and in mice treated for 7 weeks with nicotine followed by a 24-h withdrawal (Nicotine withdrawal). The values given are means from 5–9 Scatchard analyses; each analysis was carried out using striata from 10 mice. No significant differences were found between groups.

4. Discussion

In the present experiment, acute nicotine even at the dose of 1 mg/kg increased striatal concentrations of DOPAC and HVA and decreased that of 3-methoxytyramine in naive mice. Chronic oral treatment with nicotine for 7 weeks (and a 24-h withdrawal) abolished these effects of acute nicotine on DOPAC, HVA and 3-methoxytyramine. Thus, our results show that tolerance during withdrawal can be induced to the effects of nicotine on striatal dopamine metabolism produced by administering nicotine in the drinking water for 7 weeks. Sershen et al. (1987) treated mice with nicotine for 9 days (0.4 mg/kg, twice a day, s.c.) and found no tolerance towards the effects of acute nicotine (0.4 mg/kg, s.c.) on striatal dopamine metabolism (DOPAC, HVA). Neither did tolerance occur, in microdialysis studies, to the nicotine-induced enhancement of dopamine metabolism in the nucleus accumbens of rats treated repeatedly with nicotine (Damsma et al., 1989; Benwell and Balfour, 1992). On the contrary, in vitro repetitive stimulation with nicotine has been found to result in attenuation of nicotine-induced [^{3}H]dopamine release from rat striatal synaptosomes, which was suggested to reflect desensitisation or tachyphylaxis of nicotinic receptors (Rapier et al., 1988; Marks et al., 1993; Rowell and Hillebrand, 1994).

Previously we found that at doses of 3–10 mg/kg acute nicotine increased the striatal concentration of DOPAC but decreased that of 3-methoxytyramine in mice kept at room temperature and therefore having nicotine-induced hypothermia (Haikala et al., 1986; Leikola-Pelho et al., 1990). Elevation of room temperature abolished the nicotine-induced hypothermia as well as the decrease in 3-methoxytyramine (Haikala et al., 1986; Leikola-Pelho et al., 1990). Based on these findings, we suggested that the decrease in 3-methoxytyramine, which as an extraneuronal dopamine metabolite indicates dopamine release (Westerink and Spaan, 1982), is due to desensitisation of nicotinic acetylcholine receptors regulating striatal dopamine release. Thus, after initially activating these receptors which regulate the striatal dopamine release, nicotine desensitises them after sustained depolarization, and this effect seems to be enhanced by hypothermia. Hypothermia and cooling are known to enhance the actions of depolarizing neuromuscular blocking drugs (Zaimis and Head, 1976). DOPAC is thought to reflect the intraneuronal synthesis of dopamine (Westerink et al., 1987). Because nicotine elevates the DOPAC concentration even in hypothermic mice, the nicotinic acetylcholine receptors regulating intraneuronal dopamine synthesis probably differ from those controlling the release of dopamine. Furthermore, while the nicotine-induced increase in DOPAC content is antagonized by mecamylamine and pempidine given after the administration of nicotine, the decrease in 3-methoxytyramine content is not (Haikala and Ahtee, 1988). This finding in combination with the evidence that nicotinic antagonists

reduce the striatal 3-methoxytyramine content (Haikala and Ahtee, 1988) suggests that the nicotine-induced decrease in 3-methoxytyramine occurs when the nicotinic acetylcholine receptors are blocked by depolarisation-induced desensitisation. When these receptors are tolerant to nicotine-induced depolarisation they cannot be desensitised, and thus nicotine no longer decreases the 3-methoxytyramine concentration.

In the present study the striatal dopamine metabolite concentrations in mice treated chronically were similar to those of the control mice 24 h after withdrawal of nicotine. Previously we found that on the last day of 7-week chronic nicotine administration in the drinking water to mice the striatal dopamine, DOPAC and HVA concentrations were elevated at noon as compared with those of control mice drinking tap water (Pietilä et al., 1995). However, the striatal concentrations of DOPAC and HVA were not elevated in these mice at night, when the plasma nicotine was at its peak. Therefore, we suggested that tolerance had developed to nicotine-induced dopamine release in these mice, and indeed in the present experiment we could confirm this suggestion.

The mechanism of nicotine-induced tolerance is at present unclear. In numerous studies, chronic nicotine treatment has been shown to increase the number of nicotinic binding sites in various brain areas including the striatum (Schwartz and Kellar, 1983, 1985; Nordberg et al., 1985; Marks et al., 1985, 1986, 1987; Martino-Barrows and Kellar, 1987; Collins et al., 1988). This contradictory response of receptors to an agonist has been suggested to be a consequence of a prolonged desensitisation or even inactivation of nicotinic acetylcholine receptors (Marks et al., 1983, 1993; Schwartz and Kellar, 1985; Collins et al., 1990). However, similar changes in nicotinic receptors have been observed in mice of both tolerant and non-tolerant mouse strains after chronic nicotine treatment (Marks et al., 1991). As stated in Section 1, there are no reports concerning nicotine tolerance in NMRI mice. Therefore, we measured the rectal temperatures of mice withdrawn for 24 h from chronic nicotine drinking and, indeed, found that acute nicotine challenge induced significantly less hypothermia in chronically treated mice than in controls.

As it has been shown (Roth et al., 1982; Haikala et al., 1986; Imperato et al., 1986) that acute nicotine affects striatal dopamine metabolism, it was important to study the effects of chronic nicotine on striatal dopamine receptors. Our results show for the first time in mice that long-term nicotine administration, which significantly affects striatal dopamine metabolism, does not alter dopamine D_1 or D_2 receptor density (B_{max}) or affinity (K_d) in the striatum either during treatment or after a 24-h withdrawal period. Similarly, Kirch et al. (1992) found that continuous nicotine infusion for 21 days followed by a 7-day withdrawal period does not alter the number of dopamine D_1 or D_2 receptors in rat striatum. Thus, although nicotine is able to alter dopamine metabolism in the brain, it does not seem to affect dopamine receptors in either mice or rats. These findings agree with the fact that the enhanced responsiveness (supersensitivity) of striatal neurons to dopamine occurs only when the dopamine depletion is almost total (Zigmond et al., 1990).

In conclusion, our results indicate that nicotine can be administered to mice in the drinking water in doses that induce tolerance to the effects of nicotine on striatal dopamine metabolism during withdrawal. The 7-week treatment with nicotine and the following 24-h withdrawal failed to alter the number of striatal dopamine D_1 or D_2 receptors.

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References

Benwell, M.E.M. and D.J.K. Balfour, 1992, The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity, Br. J. Pharmacol. 105, 849.

Burn, J.H., L.H. Truelove and I. Burn, 1945, The antidiuretic action of nicotine and of smoking, Br. Med. J. 1, 403.

Clarke, P.B.S and R. Kumar, 1983, The effects of nicotine on locomotor activity in non-tolerant and tolerant rats, Br. J. Pharmacol. 78, 329.

Collins, A.C., E. Romm and J.M. Wehner, 1988, Nicotine tolerance: an analysis of the time course of its development and loss in the rat, Psychopharmacology 96, 7.

Collins, A.C., R.V. Bhat, J.R. Pauly and M.J. Marks, 1990, Modulation of nicotine receptors by chronic exposure to nicotinic agonists and antagonists, in: The Biology of Nicotine Dependence (Wiley, Chichester) p. 68.

Damsma, G., J. Day and H.C. Fibiger, 1989, Lack of tolerance to nicotine-induced dopamine release in the nucleus accumbens, Eur. J. Pharmacol. 168, 363.

Falkeborn, Y., C. Larsson and A. Nordberg, 1981, Chronic nicotine exposure in rat: a behavioural and biochemical study of tolerance, Drug Alcohol Depend. 8, 51.

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.

Haikala, H. and L. Ahtee, 1988, Antagonism of the nicotine-induced changes of the striatal dopamine metabolism in mice by mecamylamine and pempidine. Naunyn-Schmiedeberg's Arch. Pharmacol. 338, 169.

Haikala, H., T. Karmalahti and L. Ahtee, 1986, The nicotine-induced changes in striatal dopamine metabolism of mice depend on body temperature, Brain Res. 375, 313.

Hall, G.H., 1973, Effects of nicotine on thermoregulatory systems in the hypothalamus, in: The Pharmacology of Thermoregulation, eds. E. Schönbaum and P. Lomax (Karger, Basel) p. 244.

Hatchell, P.C. and A.C. Collins, 1977, Influences of genotype and sex on

behavioral tolerance to nicotine in mice, Pharmacol. Biochem. Behav. 6, 25.

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

Kirch, D.G., T.R. Taylor, I. Creese, S.-X. Xu and R.J. Wyatt, 1992, Effect of chronic nicotine treatment and withdrawal on rat striatal D_1 and D_2 dopamine receptors, J. Pharm. Pharmacol. 42, 89.

Leikola-Pelho, T., J. Heinämäki, I. Laakso and L. Ahtee, 1990, Chronic nicotine treatment changes differentially the effects of acute nicotine on the three main dopamine metabolites in mouse striatum, Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 400.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193, 265.

Mansner, R., E. Alhava and E. Klinge, 1974, Nicotine hypothermia and brain nicotine and catecholamine levels in the mouse, Med. Biol. 52, 390.

Marks, M.J., J.B. Burch and A.C. Collins, 1983, Effects of chronic nicotine infusion on tolerance development and nicotinic receptors, J. Pharmacol. Exp. Ther. 226, 817.

Marks, M.J., J.A. Stitzel and A.C. Collins, 1985, Time course study of the effects of chronic nicotine infusion on drug response and brain receptors, J. Pharmacol. Exp. Ther. 235, 619.

Marks, M.J., E. Romm, D.K. Gaffney and A.C. Collins, 1986, Nicotine-induced tolerance and receptor changes in four mouse strains, J. Pharmacol. Exp. Ther. 237, 809.

Marks, M.J., J.A. Stitzel and A.C. Collins, 1987, Influence of kinetics of nicotine administration on tolerance development and receptor levels, Pharmacol. Biochem. Behav. 27, 505.

Marks, M.J., S.M. Campbell, E. Romm and A.C. Collins, 1991, Genotype influences the development of tolerance to nicotine in the mouse, J. Pharmacol. Exp. Ther. 259, 392.

Marks, J.M., S.R. Grady and A.C. Collins, 1993, Downregulation of nicotinic receptors function after chronic nicotine infusion, J. Pharmacol. Exp. Ther. 266, 1268.

Martino-Barrows, A. and K.J. Kellar, 1987, [^{3}H]Acetylcholine and [^{3}H]nicotine label the same recognition site in rat brain, Mol. Pharmacol. 31, 169.

Nordberg, A., G. Wahlström, U. Arnelo and C. Larsson, 1985, Effect of long-term nicotine treatment on [^{3}H]nicotine binding sites in rats brain, Drug Alcohol Depend. 16, 9.

Pekonen, K., C. Karlsson, I. Laakso and L. Ahtee, 1993, Plasma nicotine and cotinine concentrations in mice after chronic oral nicotine administration and challenge doses, Eur. J. Pharm. Sci. 1, 13.

Pietilä, K., I. Laakso and L. Ahtee, 1995, Chronic oral nicotine administration affects the circadian rhythm of dopamine and 5-hydroxytryptamine metabolism in the striata of mice, Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 110.

Rapier, C., G.G. Lunt and S. Wonnacott, 1988, Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: concentration dependence and repetitive stimulation, J. Neurochem. 50, 1123.

Roth, K.A., S.L. McIntyre and J.D. Barchas, 1982, Nicotinic-catecholaminergic interactions in rat brain: evidence for cholinergic nicotinic and muscarinic interactions with hypothalamic epinephrine, J. Pharmacol. Exp. Ther. 221, 416.

Rowell, P.P. and J.A. Hillebrand, 1994, Characterization of nicotine-induced desensitization of evoked dopamine release from rat striatal synaptosomes, J. Neurochem. 63, 561.

Scatchard, G., 1949, The attractions of proteins for small molecules and ions, Ann. NY Acad. Sci. 51, 660.

Schwartz, R.D. and K.J. Kellar, 1983, Nicotinic cholinergic receptor binding sites in the brain: regulation in vivo, Science 220, 214.

Schwartz, R.D. and K.J. Kellar, 1985, In vivo regulation of [^{3}H]acetylcholine recognition sites in brain by nicotinic cholinergic drugs, J. Neurochem. 45, 427.

Sershen, H., A. Hashim and A. Lajtha, 1987, Behavioral and biochemical effects of nicotine in an MPTP-induced mouse model of Parkinson's disease, Pharmacol. Biochem. Behav. 28, 299.

Stolerman, I.P., R. Fink and M.E. Jarvik, 1973, Acute and chronic tolerance to nicotine measured by activity in rats, Psychopharmacologia 30, 329.

Stolerman, I.P., P. Bunker and M.E. Jarvik, 1974, Nicotine tolerance in rats; role of dose and dose interval, Psychopharmacologia 34, 317.

Westerink, B.H.C. and S.J. Spaan, 1982, Estimation of the turnover of 3-methoxytyramine in the rat striatum by HPLC with electrochemical detection: implications for sequence in the cerebral metabolism of dopamine, J. Neurochem. 38, 342.

Westerink, B.H.C., G. Damsma, J.B. DeVries and H. Koning, 1987, Dopamine re-uptake inhibitors show inconsistent effects on the in vivo release of dopamine as measured by intracerebral dialysis in the rat, Eur. J. Pharmacol. 135, 123.

Wood, P.L., H.S. Kim, K. Stocklin and T.S. Rao, 1988, Dynamics of the striatal 3-methoxytyramine pool in rat and mouse: species differences as assessed by steady-state measurements and intracerebral dialysis, Life Sci. 42, 2275.

Zaimis, E. and Head S., 1976, Depolarising neuromuscular blocking drugs, in: Neuromuscular Junction, ed. E. Zaimis (Springer, Berlin) p. 364.

Zigmond, M.J., E.D. Abercrombie, T.W. Berger, A.A. Grace and E.M. Stricker, 1990, Compensations after lesions of central dopaminergic neurons: some clinical and basic implications, Trends Neurosci. 13, 290.