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# Effect of acute nicotine administration on striatal dopamine output and metabolism in rats kept at different ambient temperatures

[1]Tiina Seppä, [1]Minna Ruotsalainen, [2]Into Laakso, [1]Raimo Tuominen & *,[1]Liisa Ahtee

[1]Division of Pharmacology and Toxicology, Department of Pharmacy, P.O. Box 56, FIN-00014 University of Helsinki, Finland and [2]Division of Pharmacognosy, Department of Pharmacy, P.O. Box 56, FIN-00014 University of Helsinki, Finland

**1** The effect of ambient temperature on the nicotine-induced (0.3, 0.5 or 0.8 mg kg$^{-1}$ s.c.) changes of the striatal concentrations of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was studied in freely-moving rats by *in vivo* microdialysis.

**2** At the ambient temperature of 30–33°C, but not at 20–23°C, nicotine doses of 0.5 ($P<0.01$) and 0.8 mg kg$^{-1}$ ($P<0.05$) significantly increased the extracellular DA concentration. The nicotine doses of 0.5 and 0.8 mg kg$^{-1}$ increased the DA metabolite levels similarly at both ambient temperatures studied ($P\leqslant0.0001$), but the dose of 0.3 mg kg$^{-1}$ only at 30–33°C (DOPAC: $P<0.05$; HVA: $P<0.01$).

**3** At 30–33°C, dihydro-$\beta$-erythroidine (DH$\beta$E 2.8 mg kg$^{-1}$ i.p.) blocked the nicotine-induced (0.5 or 0.8 mg kg$^{-1}$) increases of extracellular DA concentration but only tended to antagonize the increases of DA metabolites. Mecamylamine (5.0 mg kg$^{-1}$ i.p.) blocked the increase of DA output induced by 0.5 mg kg$^{-1}$ but not that induced by 0.8 mg kg$^{-1}$ of nicotine and fully prevented the nicotine-induced elevations of DOPAC and HVA.

**4** Elevation of ambient temperature did not affect the cerebral concentration of nicotine or the nicotine-induced elevation of serum corticosteroids. Also, the rectal temperatures of rats given nicotine at either ambient temperature did not significantly change.

**5** Our results show that the nicotine-induced output of striatal DA is enhanced at high ambient temperature. Further, our findings suggest that the nicotinic cholinoceptors mediating the effects of nicotine on striatal DA release are different from those mediating nicotine's effects on DA metabolism.

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**Keywords:** Nicotine; dopamine; ambient temperature; microdialysis; striatum; serum corticosterone

**Abbreviations:** DA, dopamine; DH$\beta$E, dihydro-$\beta$-erythroidine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; nicotinic AChRs, nicotinic acetylcholine receptors

## Introduction

Acute exposure to nicotine produces a wide range of pharmacological effects in laboratory animals, e.g. increased exploratory activity, seizures, hypothermia, analgesia and improved working memory function (see Brioni *et al*., 1997; Levin & Simon, 1998 for reviews). Existing evidence supports the view that nicotine acts on neuronal nicotinic cholinoceptors (AChRs) in the central nervous system (CNS) to modulate rather than to mediate the release of different neurotransmitters, e.g. dopamine (DA), glutamate, acetylcholine and $\gamma$-aminobutyric acid (Wonnacott, 1997). An important target site for the central actions of nicotine is the nigrostriatal dopaminergic system. Acute systemic administration of nicotine is known to increase the activity of midbrain DA neurones (Lichtensteiger *et al*., 1976; 1982; Grenhoff *et al*., 1986). Furthermore, nicotine has been shown to stimulate DA release *in vitro* in striatal slices and synaptosomal preparations (Westfall *et al*., 1983; Rapier *et al*., 1988; Dluzen & Anderson, 1998) as well as to elevate striatal 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations in rats and mice (Nose & Takemoto, 1974; Haikala *et al*., 1986; Brazell *et al*., 1990). Using *in vivo* microdialysis, the effects of acute nicotine on striatal DA were also studied in freely-moving rats, but the elevation of extracellular DA was found only under certain conditions (Imperato *et al*., 1986; Brazell *et al*., 1990; Toth *et al*., 1992; Benwell & Balfour, 1997, see also discussion). Haikala *et al*. (1986) showed that at elevated ambient temperature, smaller doses of nicotine elevated striatal DOPAC and HVA concentrations in mice and the effect lasted longer than at room temperature. Thus, altering the ambient temperature in experimental conditions could provide an important approach to study nicotinic functions.

In the present experiments, we used *in vivo* microdialysis to investigate the effect of ambient temperature on nicotine-induced striatal DA output and metabolism in freely-moving male Wistar rats. The aim was to elucidate whether ambient temperature affects functions mediated by nicotinic AChRs *in vivo*. Dihydro-$\beta$-erythroidine (DH$\beta$E), a competitive nicotinic AChR antagonist, and mecamylamine, an ion channel blocker, were used to block the effects of nicotine. The cerebral concentrations of nicotine and serum corticosterone levels were measured to study if elevated ambient temperature alters the pharmacokinetic properties of nicotine or elicits a stress response. Earlier studies have demonstrated that nicotine markedly and dose-dependently decreases the rectal temperature of mice and rats, and that this hypothermic effect can be antagonized by mecamylamine (Mansner *et al*., 1974; Horstmann, 1984) or by elevating the ambient temperature (Haikala *et al*., 1986; Leikola-Pelho *et al*., 1990). Therefore, we also measured the rectal temperatures of rats given nicotine at doses used in this study. Some of this work has been published previously in abstract form (Seppä *et al*., 1998; 1999).

*Author for correspondence; E-mail: liisa.ahtee@helsinki.fi

## Methods

### *Animals*

Male Wistar rats (8–10-week-old) weighing 250–350 g were purchased from the Laboratory Animal Center, University of Helsinki. Before surgery, the animals were housed four to a cage at an ambient temperature of 20–23°C under 12 l:12 d cycle (lights on at 0600 h). After surgery, the rats were kept singly to a cage at the ambient temperatures of either 20–23°C or 30–33°C and the experiments were performed 2 days later. The rats had free access to food and tap water. The experimental set-up was approved by the Provincial Veterinary Officer in the Provincial Government of Uusimaa, Finland.

### *Drugs and dosage regimen*

(−)-Nicotine base (Fluka AG, Buchs SG, Switzerland) was diluted with 0.9% NaCl solution (saline), and the pH of the final solution was adjusted to 7.0–7.4 with 0.05 M HCl prepared in saline. Mecamylamine hydrochloride (Merck Sharp Dohme B.V., Haarlem, Netherlands) and dihydro-$\beta$-erythroidine hydrobromide (DH$\beta$E, Research Biochemicals International, Natick, MA, U.S.A.) were dissolved in saline and administered 10 min (DH$\beta$E) or 30 min (mecamylamine) before nicotine or saline. Nicotine was administered subcutaneously (s.c.) in a volume of 1 ml kg$^{-1}$, mecamylamine and DH$\beta$E intraperitoneally (i.p.) in 2 ml kg$^{-1}$. Control animals received similar volumes of saline instead. The doses of mecamylamine and DH$\beta$E were selected on the basis of preliminary experiments in which mecamylamine at the doses of 1 or 2 mg kg$^{-1}$ and DH$\beta$E at the dose of 1.4 mg kg$^{-1}$ did not antagonize the effects of nicotine on extracellular DA, DOPAC or HVA. Furthermore in our rats, DH$\beta$E at the dose of 2.8 mg kg$^{-1}$ did not seem to antagonize nicotine's effects on respiration or nicotine-induced tremor, both of which were blocked effectively by mecamylamine at the dose of 5.0 mg kg$^{-1}$ as estimated by eye.

### *Measurement of rectal temperatures*

In a separate experiment, 15 rats were kept in groups of five at the ambient temperature of 20–23°C and another 15 rats at 30–33°C for 48 h. Thereafter, they were given saline or nicotine (0.5 or 0.8 mg kg$^{-1}$) s.c. (five rats per treatment) and the rectal temperatures were measured at 0, 30, 60, 120 and 180 min after the s.c. injections with an electric thermocouple (Ellab Instruments, Copenhagen, Denmark) inserted 5 cm into the rectum.

### *Determination of brain nicotine concentrations by GC-MS*

In these experiments, 16 rats were used. Half of the rats were housed singly at 30–33°C for 48 h before the experiment. The other eight rats were housed under similar conditions at 20–23°C. On the test day, five rats from both groups were given a single nicotine injection (0.5 mg kg$^{-1}$) between 0800 and 1100 h. For the determination of nicotine contamination from external sources, the three remaining rats of both groups were given saline s.c. Both nicotine- and saline-treated rats were killed by decapitation 60 min after the injections. The brains were removed and placed on a brain mould (RBM-4000C, ASI Instruments, U.S.A.), and coronal slices were cut between 2.70 and −4.70 mm from bregma. The slices were immediately weighed and homogenized in 0.05 M HCl in 3.0 ml g$^{-1}$ of brain tissue.

Nicotine concentrations in the homogenates were measured by modifying the methods of Leikola-Pelho *et al*. (1990) and Deutch *et al*. (1992). In brief, 100 ng of quinoline in water was added as an internal standard (I.S.) in 1.0 ml of homogenate. The samples were alkalinized with 1.0 ml of 5 M NaOH and extracted with *n*-butyl chloride (2.5 ml). The *n*-butyl chloride extract was acidified with 1.0 ml 1 M HCl and the organic solvent was removed. The remaining aqueous phase was treated with 200 $\mu$l of 5 M NaOH and extracted with chloroform (400 $\mu$l). After centrifugation, most of the aqueous layer was discarded and the remaining water was removed by freezing the samples overnight at −20°C. Subsequently, the unfrozen organic layer was transferred to a 2 ml septum cap vial and the chloroform was evaporated under nitrogen gas. Of the remaining solution (20 $\mu$l), 2–3 $\mu$l were injected into the gas chromatograph-mass spectrometer (GC-MS) system. Analyses were performed on a Hewlett-Packard 5970 quadropole MS coupled to a Hewlett-Packard 5890 GC using a NB-54 fused silica column (15 m). Fragment ions of m/z 84 (nicotine), 129 (quinoline, I.S.) and 98 (cotinine) were used for single ion monitoring. Sensitivity of this assay was 10 ng ml$^{-1}$ and the correlation coefficient of the standard samples (mouse brain tissue) between 25 and 300 ng ml$^{-1}$ was 0.989. Coefficient of variation (reproducibility) of the method was 4.8% ($n=3$) for nicotine at the concentration of 100 ng ml$^{-1}$ homogenate.

### In vivo *microdialysis*

Rats were implanted with self-made modified I-shaped microdialysis probes (Ruotsalainen & Ahtee, 1996) under stereotaxic guidance during general halothane and local lidocaine (5.0 mg ml$^{-1}$) anaesthesia. The exposed length of dialysis membrane of the probes was 4 mm. Coordinates for probe implantation were calculated relative to bregma and dura (A+1.0, L+2.7, D−6.0) according to the atlas of Paxinos & Watson (1986). Microdialysis experiments were performed approximately 42–46 h after the surgery.

On the experimental day, the implanted probes were connected to a microperfusion pump. The pump was set to a perfusion speed of 2 $\mu$l min$^{-1}$ and the rats were allowed to equilibrate for at least 1 h. Following this interval, dialysate samples were collected every 20 min. Concentrations of DA, DOPAC and HVA were quantified by HPLC with electrochemical detection as described earlier (Ruotsalainen & Ahtee, 1996). The perfusion medium was a modified Ringer solution containing in mM: NaCl 147.0, $CaCl_2$ 1.2, KCl 2.7, $MgCl_2$ 1.0 and ascorbic acid 0.04. Nicotine or saline were administered after a stable outflow of DA and its metabolites was established as shown by at least three consecutive constant samples. After the experiments, the rats were decapitated and their brains were examined by eye for verification of the correct placement of the dialysis probe. Only results derived from rats with correctly positioned probes were included in the statistical analysis.

### *Determination of serum corticosterone levels*

To elucidate the role of stress in nicotine-induced DA release at the elevated ambient temperature, 24 rats underwent a similar surgical operation as described above to implant the microdialysis probes. The rats were kept at either 20–23°C or 30–33°C and given nicotine (0.5 mg kg$^{-1}$) or saline s.c. 60 min before decapitation (six rats in all groups). The injections were

given between 0930–1000 h. The timing was identical to earlier microdialysis experiments in order to avoid corticosterone fluctuations due to the circadian rhythm. Trunk blood was collected into iced tubes, immersed in ice for 30 min, and the serum was separated by centrifugation. The samples were stored frozen at −80°C until assayed. Serum corticosterone concentrations were measured in duplicates using the Immuchem™ double antibody $^{125}$I radioimmunoassay obtained from ICN Biomedicals Incorporation (Costa Mesa, CA, U.S.A.).

### *Data analysis*

Data are given as means ± s.e.mean. Changes in rectal temperatures were compared with two-way analysis of variance (ANOVA) for repeated measures. Student's *t*-test was used for the comparison of the cerebral nicotine concentrations at 20–23°C vs 30–33°C. The statistical analysis of microdialysis data was performed using two-way ANOVA for repeated measures. When appropriate, multiple comparisons were conducted using the contrast analysis with Bonferroni levels. Data from dose-response and antagonist experiments were tested separately. The average of three stable samples before drug administration was considered as the control level (baseline) and was defined as 100%. The absolute basal levels of DA and its metabolites were compared using *F*-test for equal variances followed by *t*-test assuming equal variances. Serum corticosterone levels were compared using Kruskall-Wallis nonparametric ANOVA followed by Mann-Whitney *U*-test. A probability value $P<0.05$ was regarded as significant.

## Results

### *Rectal temperatures*

The mean rectal temperature of rats kept at 20–23°C measured before drug administration was 37.6 ± 0.07°C ($n=15$) and that of rats kept at 30–33°C 37.7 ± 0.11°C ($n=15$), respectively. The mean rectal temperatures (30–180 min; $n=5$ in all groups) for rats kept at 20–23°C or 30–33°C and given saline were 38.1 and 37.8°C, respectively. The corresponding values in nicotine-treated rats were 38.0°C (0.5 mg kg$^{-1}$) and 37.9°C (0.8 mg kg$^{-1}$) at 20–23°C and 38°C (0.5 and 0.8 mg kg$^{-1}$) at 30–33°C. Two-way ANOVA revealed a significant pretreatment (temperature) × treatment (nicotine) interaction ($P<0.05$) although neither temperature nor nicotine treatment alone altered significantly the rectal temperatures of rats during the 3-h testing period. This could be traced back to the finding that in rats kept at 20–23°C the handling effect produced by subcutaneous saline injection tended to elevate the rectal temperatures more than in rats kept at 30–33°C.

### *Cerebral nicotine concentrations*

At 1 h after giving 0.5 mg kg$^{-1}$ of nicotine s.c. the cerebral nicotine concentration in rats kept at 20–23°C ($n=5$) was 185.5 ± 15.7 ng g$^{-1}$ and that in rats kept at 30–33°C ($n=5$) 148.4 ± 16.6 ng g$^{-1}$, respectively. These values did not differ significantly (Student's *t*-test). Traces of cotinine were also found but could not be properly quantified.

### *Extracellular levels of DA and its metabolites (dose-response experiments)*

The absolute basal values of DA in the dialysates calculated from three consecutive samples prior to drug administration were 14.80 ± 0.53 and 13.35 ± 0.36 fmol min$^{-1}$ for rats kept at 20–23°C ($n=25$) and 30–33°C ($n=24$), respectively; the corresponding values of DOPAC were 0.75 ± 0.02 and 0.72 ± 0.01 pmol min$^{-1}$, and those of HVA 0.61 ± 0.02 and 0.65 ± 0.01 pmol min$^{-1}$, respectively. Thus, the elevation of ambient temperature had no effect on the basal extracellular striatal concentrations of DA or its metabolites (Student's *t*-test). Two-way ANOVA revealed that nicotine treatment (dose × time interaction) significantly altered the extracellular levels of DA ($F=2.04$, $P<0.001$), DOPAC ($F=4.34$, $P\leqslant0.0001$) and HVA ($F=5.24$, $P\leqslant0.0001$). Multiple comparisons showed that the smallest dose of nicotine studied

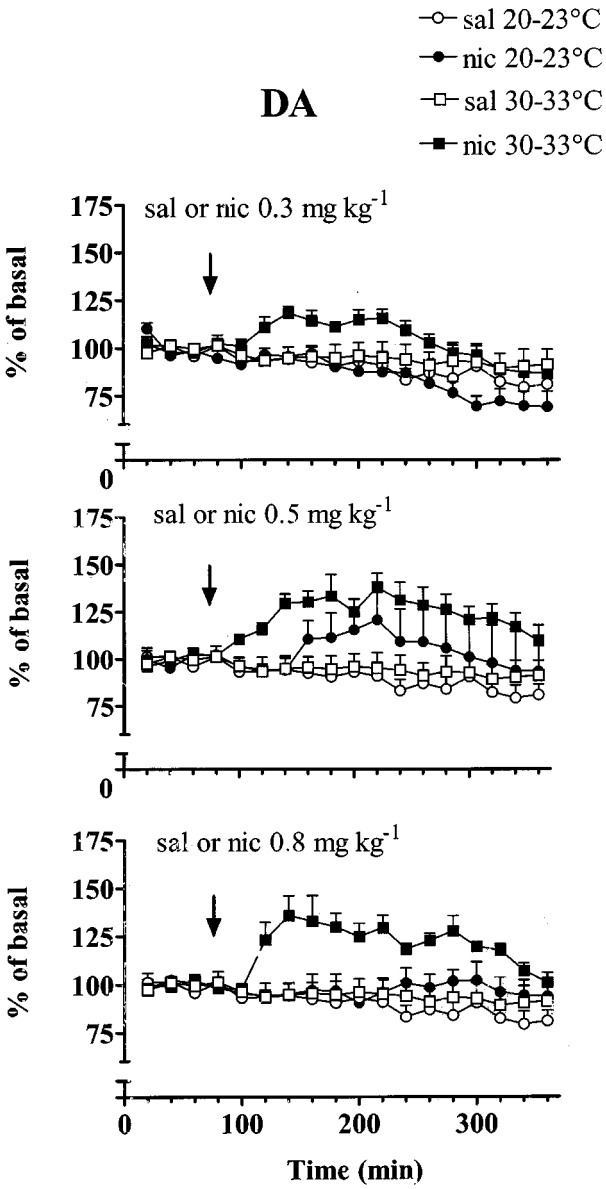


**Figure 1** The influence of different ambient temperatures on the effects of nicotine (0.3, 0.5 or 0.8 mg kg$^{-1}$ s.c.) on the extracellular concentrations of dopamine (DA) in the striatum. The rats were kept at 20–23°C or 30–33°C for 2 days before and during the experiment. Saline or nicotine were administered at the time indicated by arrows. The results (means ± s.e.mean) are expressed as percentages of the three consecutive samples collected before the injection of nicotine or saline (6–7 rats in all groups). Contrast analysis after analysis of variance for repeated measurements, 100–360 min, revealed the following statistically significant interactions: Saline 30–33°C vs nicotine 0.5 mg kg$^{-1}$ 30–33°C ($P<0.05$), saline 30–33°C vs nicotine 0.8 mg kg$^{-1}$ 30–33°C ($P<0.05$).

(0.3 mg kg$^{-1}$) did not significantly alter the extracellular DA concentration at either ambient temperature (Figure 1). Furthermore, larger nicotine doses (0.5 or 0.8 mg kg$^{-1}$) did not have significant effects on DA output at 20–23°C, but at the elevated temperature nicotine at these doses elevated the extracellular DA concentration significantly (Figure 1). The maximal concentrations, 138% after 0.5 mg kg$^{-1}$ and 136% of the control level after 0.8 mg kg$^{-1}$ of nicotine, respectively, were about similar.

The nicotine dose 0.3 mg kg$^{-1}$ elevated the extracellular concentrations of DOPAC and HVA significantly only at 30–33°C (Figures 2 and 3). However, the larger nicotine doses (0.5 or 0.8 mg kg$^{-1}$) elevated the extracellular DOPAC and HVA concentrations similarly and significantly at both temperatures

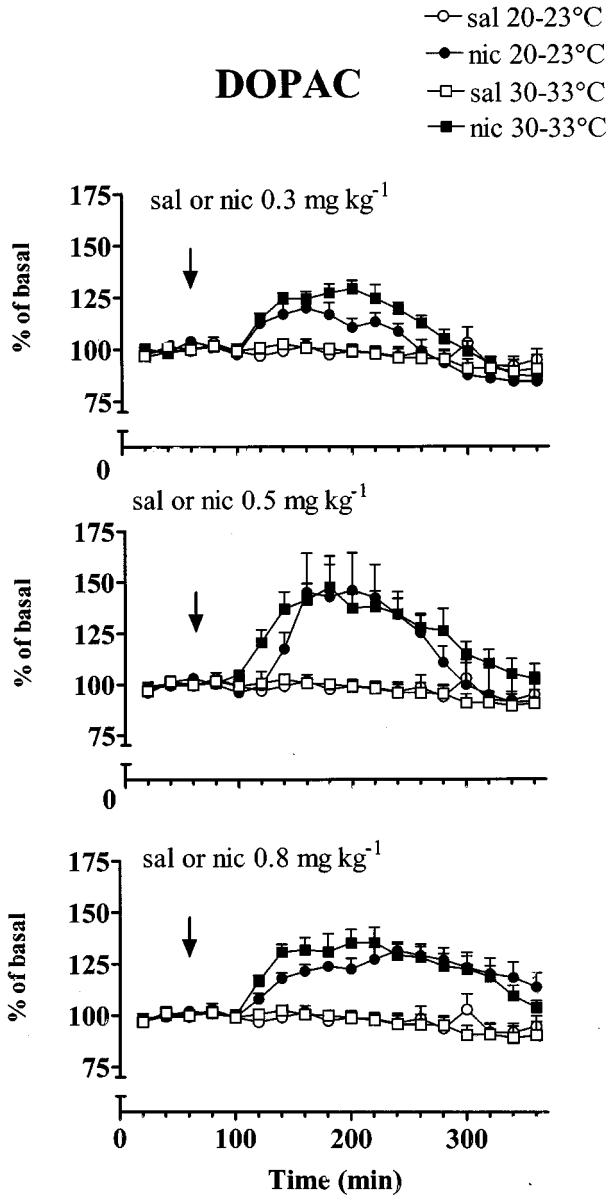


**Figure 2** The influence of different ambient temperatures on the effects of nicotine (0.3, 0.5 or 0.8 mg kg$^{-1}$ s.c.) on the extracellular concentrations of DOPAC in the striatum. The rats were kept at 20–23°C or 30–33°C for 2 days before and during the experiment. Saline or nicotine were administered at the time indicated by arrows. The results (means±s.e.mean) are expressed as percentages of the three consecutive samples collected before the injection of nicotine or saline (6–7 rats in all groups). Contrast analysis after analysis of variance for repeated measurements, 100–360 min, revealed the following statistically significant interactions: Saline 30–33°C vs nicotine 0.3 mg kg$^{-1}$ 30–33°C ($P<0.05$); saline 20–23°C vs nicotine 0.5 mg kg$^{-1}$ 20–23°C ($P<0.01$); saline 30–33°C vs nicotine 0.5 mg kg$^{-1}$ 30–33°C ($P<0.001$); saline 20–23°C vs nicotine 0.8 mg kg$^{-1}$ 20–23°C ($P<0.001$); saline 30–33°C vs nicotine 0.8 mg kg$^{-1}$ 30–33°C ($P<0.0001$).

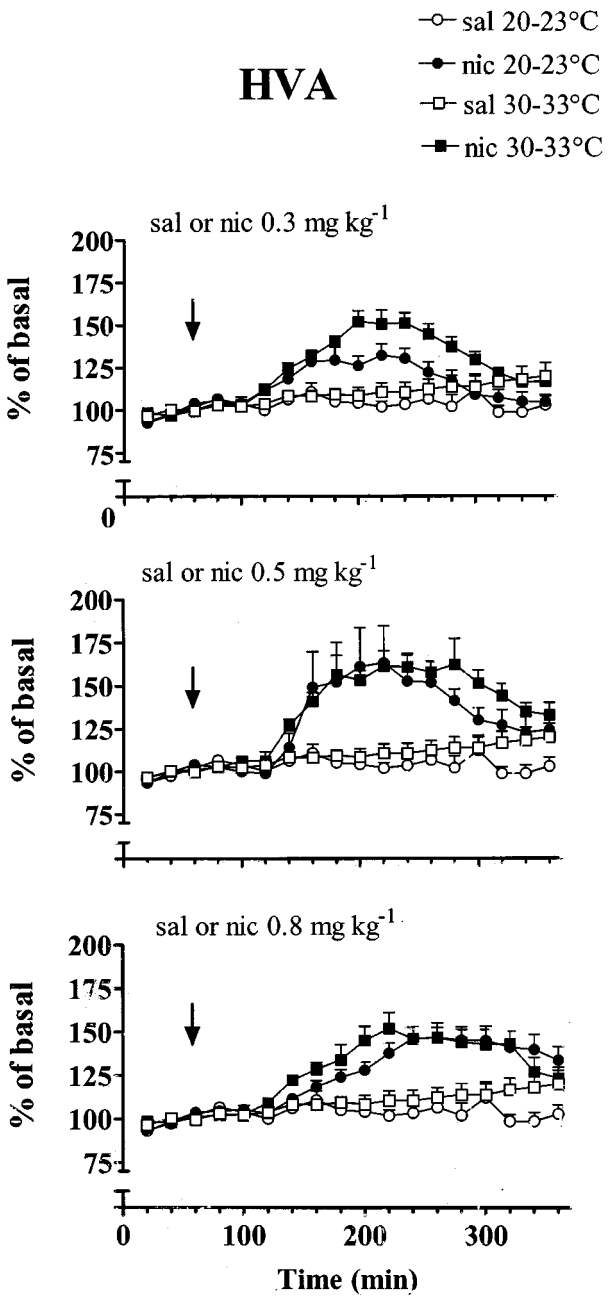


**Figure 3** The influence of different ambient temperatures on the effects of nicotine (0.3, 0.5 or 0.8 mg kg$^{-1}$ s.c.) on the extracellular concentrations of HVA in the striatum. The rats were kept at 20–23°C or 30–33°C for 2 days before and during the experiment. Saline or nicotine were administered at the time indicated by arrows. The results (means±s.e.mean) are expressed as percentages of the three consecutive samples collected before the injection of nicotine or saline (6–7 rats in all groups). Contrast analysis after analysis of variance for repeated measurements, 100–360 min, revealed the following statistically significant interactions: Saline 30–33°C vs nicotine 0.3 mg kg$^{-1}$ 30–33°C ($P<0.01$); saline 20–23°C vs nicotine 0.5 mg kg$^{-1}$ 20–23°C ($P<0.0001$); saline 30–33°C vs nicotine 0.5 mg kg$^{-1}$ 30–33°C ($P<0.0001$); saline 20–23°C vs nicotine 0.8 mg kg$^{-1}$ 20–23°C ($P<0.001$); saline 30–33°C vs nicotine 0.8 mg kg$^{-1}$ 30–33°C ($P<0.001$).

studied. Thus, the responses of DA metabolites to nicotine were only marginally enhanced by elevating the ambient temperature above 20–23°C. As shown in Figures 2 and 3, nicotine 0.5 mg kg$^{-1}$ elevated striatal DOPAC maximally up to 146% and HVA up to 163% at 20–23°C and at 30–33°C, the maximal elevations were 147% and 162% for DOPAC and HVA, respectively. The dose of 0.8 mg kg$^{-1}$ of nicotine elevated DOPAC up to 131% and HVA to 147% at 20–23°C respectively, and up to 135 and 152% at 30–33°C. Thus, the responses of DA metabolites to nicotine were not enhanced by elevating the dose of nicotine above 0.5 mg kg$^{-1}$.

*Effects of nicotinic AChR antagonists on the nicotine-induced changes of striatal DA output and metabolism at 30–33°C*

To study if the nicotine-induced elevations of striatal extracellular DA, DOPAC and HVA concentrations at the ambient temperature of 30–33°C are mediated by nicotinic AChRs we estimated the effect of nicotine after blocking the nicotinic AChRs either with the competitive antagonist DH$\beta$E or with the ion channel blocker mecamylamine. Pretreatment with DH$\beta$E (2.8 mg kg$^{-1}$) 10 min prior to nicotine completely prevented the

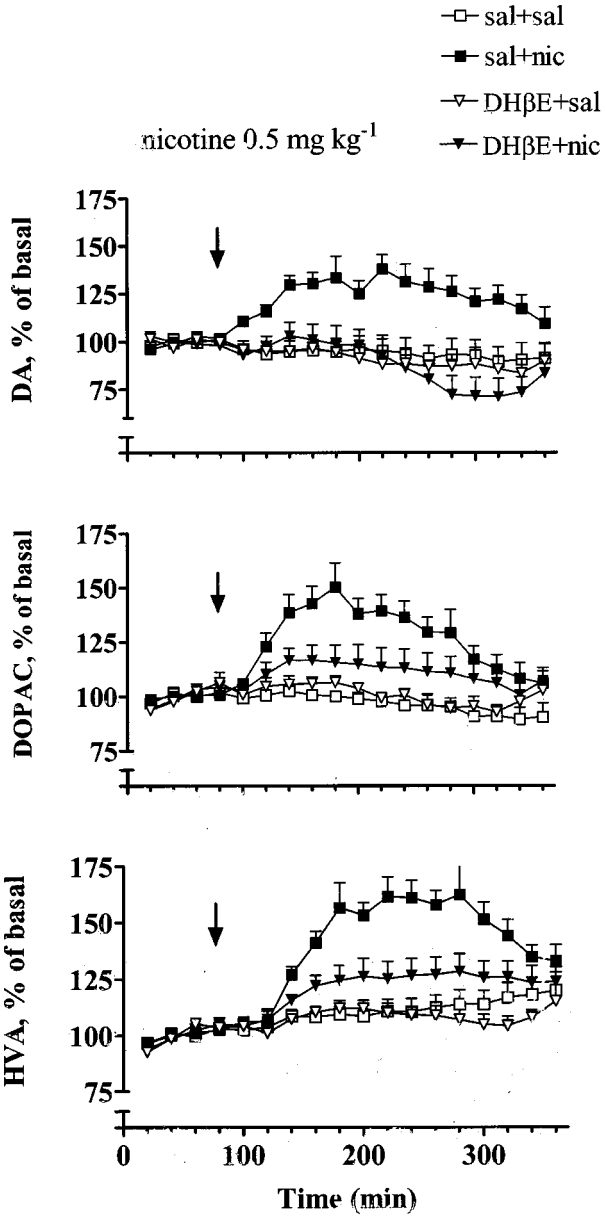


**Figure 4** The effect of dihydro-$\beta$-erythroidine (DH$\beta$E) on the nicotine-induced increases of the extracellular concentrations of DA, DOPAC and HVA in the striatum of rats kept at 30–33°C. The rats were given either saline or DH$\beta$E 2.8 mg kg$^{-1}$ i.p. 10 min prior to nicotine 0.5 mg kg$^{-1}$ or saline. Saline or nicotine was given s.c. at the time indicated by arrows. The results (means±s.e.mean) are expressed as percentages of the three consecutive samples collected before the injection of nicotine or saline (5–7 rats in all groups). DH$\beta$E significantly ($P<0.05$; two-way ANOVA; 100–360 min) antagonized the effect of nicotine on extracellular DA, and tended to antagonize those on DOPAC ($P<0.07$) and HVA ($P<0.08$).

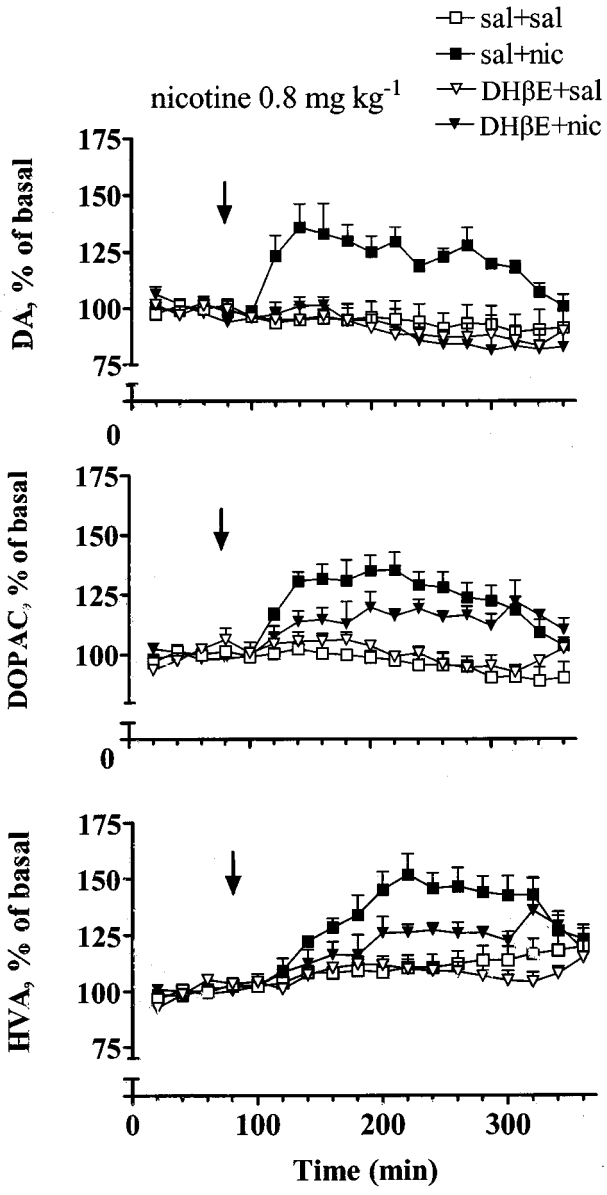


**Figure 5** The effect of dihydro-$\beta$-erythroidine (DH$\beta$E) on the nicotine-induced increases of the extracellular concentrations of DA, DOPAC and HVA in the striatum of rats kept at 30–33°C. The rats were given either saline or DH$\beta$E 2.8 mg kg$^{-1}$ i.p. 10 min prior to nicotine 0.8 mg kg$^{-1}$ or saline. Saline or nicotine was given s.c. at the time indicated by arrows. The results (means±s.e.mean) are expressed as percentages of the three consecutive samples collected before the injection of nicotine or saline (5–7 rats in all groups). DH$\beta$E significantly ($P<0.05$; two-way ANOVA; 100–360 min) antagonized the effect of nicotine on extracellular DA, and tended to antagonize that on DOPAC ($P<0.08$) but not that on HVA ($P=0.33$).

increases of extracellular DA concentration induced by 0.5 mg $kg^{-1}$ or 0.8 mg $kg^{-1}$ of nicotine (Figures 4 and 5). However, DH$\beta$E did not fully prevent the nicotine-induced increases of DA metabolite concentrations. DH$\beta$E strongly tended to inhibit the increase of extracellular DOPAC and HVA concentrations induced by the dose of 0.5 mg $kg^{-1}$ of nicotine (Figure 4). Similarly DH$\beta$E tended to inhibit the effects of 0.8 mg $kg^{-1}$ dose of nicotine (Figure 5) on extracellular DOPAC and also on HVA.

Mecamylamine (5.0 mg $kg^{-1}$) 30 min prior to nicotine antagonized the elevation of extracellular DA by 0.5 mg $kg^{-1}$ of nicotine (Figure 6) but not that induced by 0.8 mg $kg^{-1}$ of nicotine (Figure 7). However, mecamylamine completely prevented the increases of extracellular DOPAC concentration induced either by 0.5 or 0.8 mg $kg^{-1}$ of nicotine. Similarly, the elevations of extracellular HVA concentration by 0.5 and 0.8 mg $kg^{-1}$ of nicotine were totally antagonized by mecamylamine.

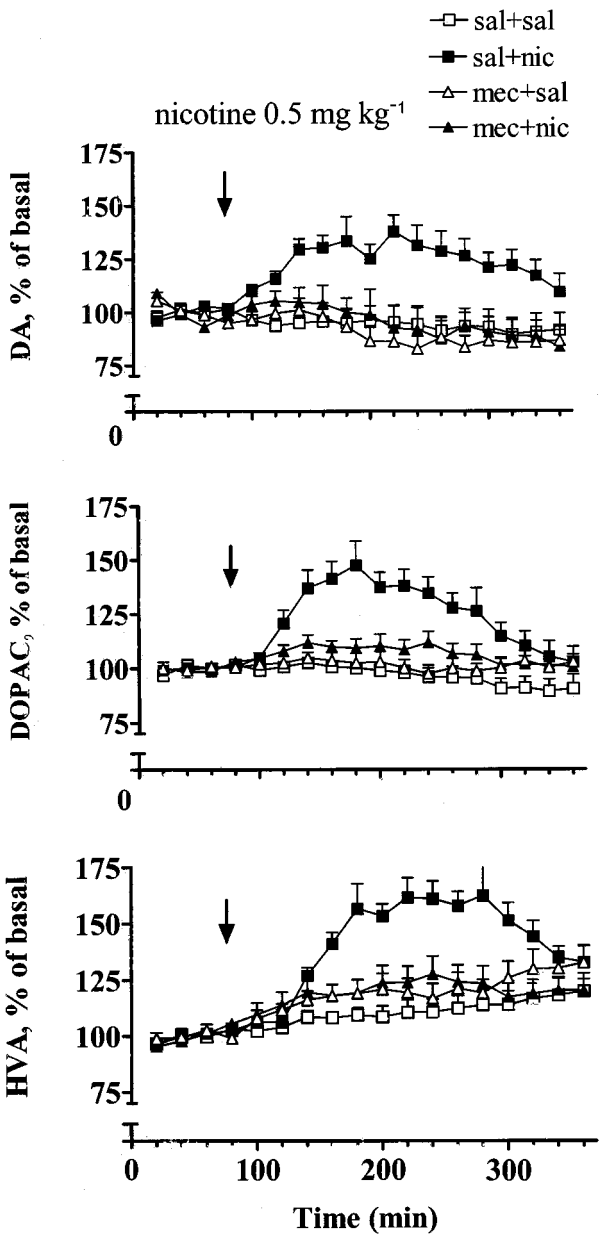


**Figure 6** The effect of mecamylamine on the nicotine-induced increases of the extracellular concentrations of DA, DOPAC and HVA in the striatum of rats kept at 30–33°C. The rats were given either saline or mecamylamine 5.0 mg $kg^{-1}$ i.p. 30 min prior to nicotine 0.5 mg $kg^{-1}$ or saline. Saline or nicotine was given s.c. at the time indicated by arrows. The results (means ± s.e.mean) are expressed as percentages of the three consecutive samples collected before the injection of nicotine or saline (6–7 rats in all groups). Mecamylamine significantly (two-way ANOVA; 100–360 min) antagonized the effects of nicotine on extracellular DA ($P=0.052$), on DOPAC ($P<0.01$) and on HVA ($P<0.01$).

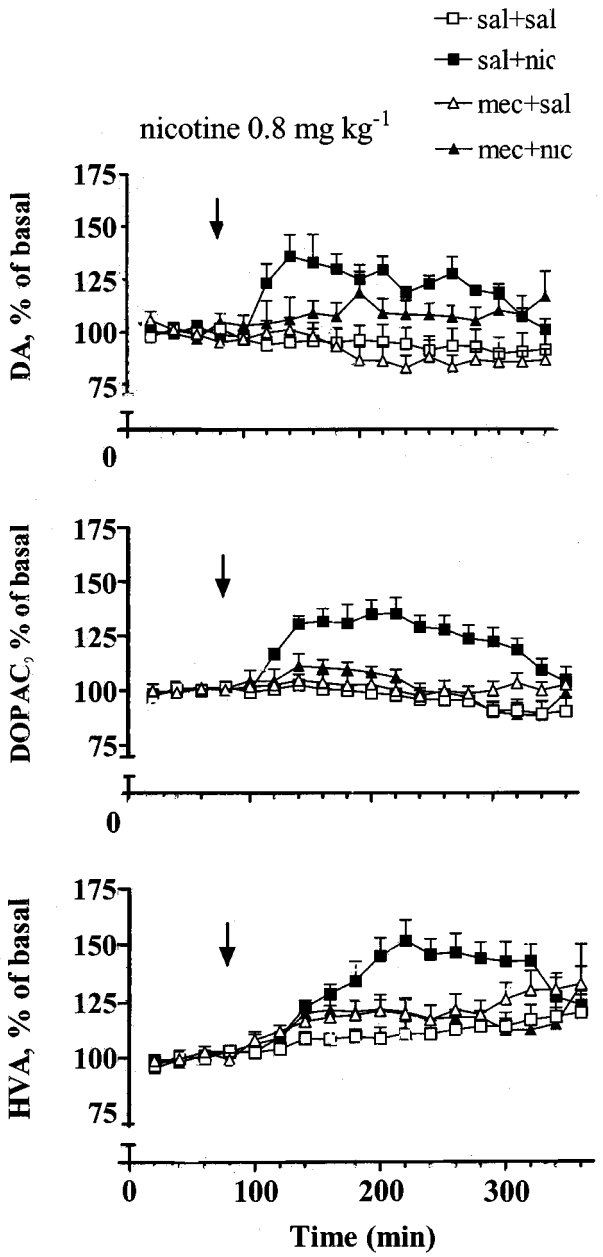


**Figure 7** The effect of mecamylamine on the nicotine-induced increases of the extracellular concentrations of DA, DOPAC and HVA in the striatum of rats kept at 30–33°C. The rats were given either saline or mecamylamine 5.0 mg $kg^{-1}$ i.p. 30 min prior to nicotine 0.8 mg $kg^{-1}$ or saline. Saline or nicotine was given s.c. at the time indicated by arrows. The results (means ± s.e.mean) are expressed as percentages of the three consecutive samples collected before the injection of nicotine or saline (6–7 rats in all groups). Mecamylamime significantly (two-way ANOVA; 100–360 min) antagonized the effects of nicotine on DOPAC ($P<0.001$) and on HVA ($P<0.05$), but not on DA.

*Serum corticosterone levels*

At 1 h after giving 0.5 mg $kg^{-1}$ of nicotine s.c. the serum corticosterone concentration in rats kept at 20–23°C was $554.5 \pm 84.0$ ng $ml^{-1}$ and that in rats kept at 30–33°C $521.8 \pm 169.6$ ng $ml^{-1}$. The corresponding values in rats given saline were $84.9 \pm 10.7$ and $67.3 \pm 26.0$ ng $ml^{-1}$, respectively (six rats in all groups). Statistical analysis of the data revealed that nicotine significantly and similarly increased the serum corticosterone concentration at both ambient temperatures studied ($P<0.01$; Mann-Whitney *U*-test). Furthermore, the serum corticosterone levels in saline-treated rats were similar at both ambient temperatures ($P>0.33$).

## Discussion

In the present experiments we found that nicotine (0.5 or 0.8 mg $kg^{-1}$) significantly enhanced striatal DA output in rats kept at an ambient temperature of 30–33°C but not in rats kept at 20–23°C. Furthermore, at the dose 0.3 mg $kg^{-1}$ nicotine significantly elevated extracellular striatal DOPAC and HVA concentrations in rats kept at 30–33°C but not in those kept at 20–23°C. The effects of the doses 0.5 or 0.8 mg $kg^{-1}$ on DOPAC and HVA levels were similar and significant at both ambient temperatures studied. Nicotinic AChR antagonists mecamylamine and DH$\beta$E blocked or reduced the increases of extracellular DA or its metabolites suggesting that nicotine's effects on striatal DA release and metabolism are mediated by nicotinic AChRs. The efficacies of DH$\beta$E and mecamylamine in inhibiting nicotine-induced increases of extracellular concentrations of DA and metabolites differed so that DH$\beta$E preferentially blocked the elevation of DA but mecamylamine that of its metabolites.

These data are in agreement with earlier reports from our laboratory showing that nicotine's effects on striatal DA metabolism of mice are enhanced at elevated ambient temperature (Haikala *et al*., 1986; Leikola-Pelho *et al*., 1990). However, in mice the nicotine-induced changes in DA release and metabolism are closely related to changes in body temperature, whereas in rats the elevation of ambient temperature profoundly affected the striatal DA release and metabolism without altering the body temperature. In mice kept at elevated ambient temperature to antagonize the hypothermic effect of nicotine, nicotine's stimulating effects on striatal DA metabolism are larger and found at smaller nicotine doses than in mice kept at room temperature (Haikala *et al*., 1986; Leikola-Pelho *et al*., 1990). Indeed, in mice, repeated nicotine doses which induced profound hypothermia even decreased striatal DA release as judged from effects on DA metabolites (Haikala, 1986). Acute nicotine has been reported to induce hypothermia also in rats (Hall, 1973; Horstmann, 1984). However, in the present experiments the rectal temperatures of rats were not decreased by any of the nicotine doses tested at either temperature studied. Furthermore, the baseline rectal temperatures of rats were not affected by elevating the ambient temperature as was also reported recently by Vuckovic *et al*. (1998). Interestingly, Gong *et al*. (1999) recently reported that in rats sleeping at 31.5°C the expression of Fos protein in certain hypothalamic areas was increased as compared with rats sleeping at 22°C and suggested that sleep achieved at elevated ambient temperature is associated with enhanced activation of specific preoptic area/basal forebrain neurons. Thus, it is likely that elevation of ambient temperature affects the functioning of the body without altering the body temperature which is most probably compensated for by thermoregulatory mechanisms. It is reasonable to conclude that pharmacological or physiological interventions affect such altered activity differently from their effects under standard room temperature.

Using *in vivo* microdialysis, the DA-releasing effect of systemically-administered nicotine in the striatum has proven somewhat difficult to demonstrate, since either no change or an increase in striatal DA release has been reported. Some investigators (Imperato *et al*., 1986; Damsma *et al*., 1988) have used perfusion media that contain higher than physiological concentrations of $Ca^{2+}$ and consequently report significant enhancement of striatal DA release by nicotine. Benwell & Balfour (1997) used nomifensine, a DA uptake inhibitor, and reported similar results. In contrast, when the $Ca^{2+}$ concentration of the perfusion medium was kept within the physiological range in the absence of an uptake blocker, striatal extracellular DA concentration was not altered by intravenous administration of nicotine (Brazell *et al*., 1990). This finding is in agreement with the results obtained in the present experiments in rats kept at 20–23°C, in which nicotine did not significantly elevate striatal extracellular DA. Several mechanisms could be responsible for the enhancement of nicotine-induced striatal DA output at the elevated ambient temperature. These include alterations in conformational state of the receptor, in pharmacokinetics of nicotine or changes in physiological functions. The basal striatal concentrations of DA or its metabolites were not altered by elevating the temperature indicating that the change of ambient temperature alone did not alter striatal DA release or metabolism in our rats. The present data argue also against a pharmacokinetic explanation, i.e. enhanced penetration of nicotine into the brain at the elevated temperature, because cerebral nicotine concentrations were not increased at 30–33°C as compared with 20–23°C.

Exposure to stressful stimuli or administration of glucocorticoids are known to increase DA release *in vivo* (Piazza *et al*., 1996). A baseline range of serum corticosterone concentration of 50–300 ng $ml^{-1}$ can be expected in mice and rats depending on sample collection methods (Shimizu *et al*., 1983). Thus, the serum corticosterone concentrations below 100 ng $ml^{-1}$ indicate that the rats used in this study showed no marks of neuroendocrine stress by implantation of microdialysis probes and by exposure to elevated ambient temperature. Takahashi *et al*. (1998) showed that in the striatum, combined chronic nicotine treatment and stress significantly increased DA release. There is also evidence that glucocorticoids modulate the effects of nicotine (Johnson *et al*., 1995) and that steroid hormones act as allosteric modulators at nicotinic AChRs (Ke & Lukas, 1996). However, acute nicotine treatment elevated the serum corticosterone concentrations to a similar degree at both ambient temperatures studied, but increased DA release only at 30–33°C. Thus, the nicotine-induced DA release is not correlated with elevated corticosterone levels, and it is unlikely that the differences in nicotine's effects on striatal DA induced by altering the ambient temperature are stress-related.

Nicotine's effects are characterized by the fact that after initially stimulating its receptors it subsequently desensitizes them; the same basic mechanism of action is shared by the neuromuscular blocking drugs which induce a depolarization block. The concept of receptor desensitization is commonly linked to situations where drugs are given chronically or repeatedly. However, Rowell & Duggan (1998) have shown that already a relatively short exposure, $\geqslant 5$ min, to relatively small concentrations of nicotine produced long-lasting nicotinic AChR inactivation at rat striatal synaptosomes, and,

with others, provided evidence that the subunit composition of nicotinic AChRs determines the degree to which the receptors are desensitized (Rowell & Duggan, 1998; Fenster *et al*., 1997). Furthermore, in an *in vivo* study, James *et al*. (1994) demonstrated that nicotine can acutely desensitize brain nicotinic AChRs at doses similar to those used in the present study. Interestingly, there are studies to suggest that cooling prolongs the action of depolarizing neuromuscular blocking drugs and this could alter the functioning of nicotinic AChRs (Zaimis & Head, 1976). Thus, it is tempting to speculate that the temperature-dependent differences in nicotine's effects observed in the present study are due to a related phenomenon. For example, it could be that a larger proportion of the nicotinic AChRs regulating striatal dopaminergic neurons has undergone conformational change leading to desensitization at 20–23°C than at the elevated temperature of 30–33°C.

In a previous study, in which the effects of nicotine on the striatal DA system were investigated in mice kept at different ambient temperatures, Leikola-Pelho *et al*. (1990) suggested that the nicotinic AChRs involved in the regulation of striatal DA metabolism and DA release differed from each other. Dopaminergic neurons innervating the striatum are thought to have nicotinic AChRs in somatodendritic regions as well as on nigral axons presynaptically and preterminally, a distinction based on tetrodotoxin sensitivity (Marshall *et al*., 1996; Wonnacott, 1997). According to a recent study, at least two different subtypes of nicotinic AChRs control striatal DA release (Kaiser *et al*., 1998). The enhancement of DA metabolism seems to be linked to the activation of nicotinic AChRs in the somatodendritic region, because locally applied nicotine failed to increase the extracellular concentrations of DOPAC and HVA in the striatum and nucleus accumbens (Toth *et al*., 1992; Marshall *et al*., 1997). Also, the data presented here that the mechanisms controlling striatal DA release seem to be more readily affected by ambient temperature than those controlling the extracellular levels of DA metabolites, indicate the multiplicity of nicotinic AChRs involved in the control of striatal DA system.

The findings that mecamylamine preferentially blocked the nicotine-induced elevation of extracellular DOPAC and HVA concentrations, whereas DH$\beta$E significantly blocked the nicotine-induced elevation of DA concentration but not that of DOPAC or HVA, are in agreement with the idea that the effects of nicotine on striatal DA release are mediated by nicotinic AChR subtypes different from those mediating nicotine's effects on DA metabolite levels. Mecamylamine and DH$\beta$E bind to different structures on nicotinic AChRs (Brioni *et al*., 1997). DH$\beta$E is a competitive antagonist at the acetylcholine binding site whereas mecamylamine is thought to block the ion channel. Interestingly, in our rats, DH$\beta$E unlike mecamylamine did not seem to antagonize nicotine's effects on respiration or nicotine-induced tremor. These observations agree with the finding that synaptic ganglionic-like $\alpha3\beta4$ nicotinic AChRs are relatively insensitive to blockade by DH$\beta$E (Harvey *et al*., 1996). In contrast to DH$\beta$E, mecamylamine blocked totally the nicotine-induced elevations of DOPAC and HVA concentrations but surprisingly, not that of DA after 0.8 mg kg$^{-1}$ of nicotine. Thus, our studies with nicotinic AChR antagonists strengthen the notion that different nicotinic AChRs mediate nicotine's effects on DA release and metabolism.

In summary, we conclude that the functional state of nicotinic AChRs *in vivo* can be modulated by altering the ambient temperature. This modulation may underlie processes such as changes in the conformational state/desensitization properties of these receptors. Furthermore, our findings suggest that the nicotinic cholinoceptors mediating the effects of nicotine on striatal DA release are different from those mediating nicotine's effects on DA metabolism. The temperature-dependency of nicotine-DA interactions in the striatum as well as our findings that more than one kind of nicotinic AChR might mediate nicotine's effects on striatal DA are of importance considering attempts to develop nicotine analogues to treat neurodegenerative disorders, for example Parkinson's disease (Holladay *et al*., 1997). Already in the 1920s, nicotine was found to alleviate the immobility associated with postencephalitic parkinsonism (Moll, 1926). Further, in epidemiological studies, a negative association between parkinsonism and cigarette smoking has been consistently found (Kessler, 1973; Baron, 1986).

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