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Effect of prolonged nicotine infusion on response of rat catecholamine biosynthetic enzymes to restraint and cold stress

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Abstract

There is a paradoxical relationship between nicotine and stress. To help elucidate their relationship on catecholamine biosynthesis, rats were infused with nicotine for 7–14 days before exposure to cold or restraint stress. Nicotine (5 mg/kg/day, 14 days) did not alter basal plasma corticosterone or its elevation with 24 h cold stress, but prevented corticosterone elevation following 2 h restraint stress. In adrenal medulla (AM), response of dopamine β-hydroxylase (DBH), but not tyrosine hydroxylase (TH) mRNA, to both stressors was attenuated in nicotine-infused rats. In locus coeruleus (LC), restraint stress elevated TH and DBH mRNA in saline-, but not in nicotine-infused rats. Cold stress triggered a similar response of TH and DBH mRNAs in LC with and without nicotine infusion. With shorter nicotine infusion (8 mg/kg/day, 7 days), TH mRNA in AM was not induced by restraint stress on one (1×) or two (2×) consecutive days nor was DBH mRNA in AM or LC by 2×. The findings demonstrate that constant release of nicotine can modulate, or even prevent, some stress responses at the level of the HPA axis and gene expression of catecholamine biosynthetic enzymes in LC and AM.

Keywords: Corticosterone; mRNA; Adrenal medulla; Locus coeruleus; Tyrosine hydroxylase; Dopamine β-hydroxylase; Nicotine; Restraint; Cold stress

1. Introduction

There is a paradoxical relationship between nicotine and stress. On one hand cigarette smoking is reported to be calming, and on the other hand it can trigger many of the same physiological responses seen with stress. The reported calming effect of smoking is more evident during stressful situations (Schachter et al., 1977; Rose et al., 1983). Chronic smokers are more resistant to stress when allowed to smoke than when prevented from smoking. Nicotine is likely responsible for this effect since this calming effect was not observed in people smoking low- or non-nicotine containing cigarettes (Silverstein, 1982). Moreover, stress is an important factor contributing to relapse among smokers attempting to quit (Shiffman, 1982; Abrams et al., 1987).

Despite this calming effect, nicotine was shown to activate several stress reactive systems. Administration of nicotine leads to activation of the hypothalamic–pituitary–adrenal (HPA) axis resulting in the higher plasma levels of the stress hormones, adrenocorticotropin hormone (ACTH) and cortisol (Balfour, 1980; Seyler et al., 1984). Nicotine also stimulates release of catecholamines (CA) from the adrenal medulla, sympathetic nerve endings and brain CA neurons (Haass and Kubler, 1997). Administration of nicotine, like stress, increases heart rate, systolic and diastolic blood pressures and enhances electroencephalogram (EEG) measured potentials in humans and experimental animals (USDHHS, 1998). These cardiovascular effects are largely attributed to the large increases in circulating epinephrine and norepinephrine (NE) in humans and animals (Cryer et al., 1976; Haass and Kubler, 1997). Nicotine stimulation of the ascending NE neurons from the nucleus of the solitary tract was shown to be essential for induction and release of ACTH (Matta et al., 1993). Nicotine also acts centrally to stimulate norepinephrine release from locus coeruleus neurons which is considered a crucial site in CNS stress response (Mitchell, 1993; Dani and De Biasi, 2001).

Nicotine also promotes catecholamine biosynthesis by activating tyrosine hydroxylase (TH), the first and major rate-limiting enzyme (Fossom et al., 1991; Smith et al., 1991; Hiremagalur and Sabban, 1995). In addition, nicotine not only

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increases gene expression of TH, but also other subsequent catecholamine biosynthetic enzymes [dopamine β hydroxylase (DBH) and phenylethanolamine *N*-methyltransferase (PNMT)] in the periphery and in many catecholaminergic regions of the CNS (Fossom et al., 1991; Hofle et al., 1991; Mitchell et al., 1993; Hiremagalur and Sabban, 1995; Serova et al., 1999).

Recent studies show a relationship between genetic polymorphisms in the TH and DBH genes and smoking, indicating that these genes may be involved in nicotine addiction. (McKinney et al., 2000; Anney et al., 2004).

Several hypotheses were advanced to explain the nicotine and stress paradox. It was suggested that the basis could be psychological and not necessarily due to biological explanations. For example it has been suggested that abstinence from nicotine smoking results in increased stress in smokers, and smoking only appears to reduce stress by contrast (Schachter et al., 1977; Schachter, 1978). It was considered that smoking might provide an alternative source of attention thus they are less aware of negative somatic experiences (Silverstein, 1982). However, a considerable amount of recent research points to a biological basis for the nicotine and stress paradox. Chronic (11 days) nicotine administration by osmotic minipumps at high concentrations (12 mg/kg/day nicotine dihydrochloride, expressed as free base) prevented the stress triggered increase in acoustic startle amplitude and pre-pulse amplitude (Acri, 1994). Repeated nicotine injections led to a reduction in footshock stress induced dopamine utilization in the prefrontal cortex and immobility responses (George et al., 1998). Previous studies from our laboratory revealed that prolonged infusion of rats with nicotine attenuated several of the responses to immobilization stress, including activation of gene expression for catecholamine biosynthetic enzymes, TH and DBH (Serova et al., 1999). The attenuation of gene expression for catecholamine biosynthetic enzymes in response to immobilization stress in rats with nicotine infusion was tissue specific (Serova et al., 1999). In adrenal medulla, the immobilization induced elevation of TH mRNA levels was significantly less in nicotine-infused rats compared to saline-infused rats. The response of DBH mRNA to immobilization stress was abolished in both adrenal medulla and locus coeruleus, the site of cell bodies for the major noradrenergic system in the brain. However, it is unknown how nicotine infusion would affect different types of stress and the optimal conditions required to modulate the stress responses in different CA locations.

In this study we examine the effects of infusion of nicotine on the response of TH and DBH gene expression to cold stress and also to restraint, a milder form of immobilization stress, more likely to be encountered by humans. Prior to exposure to stress, rats were pretreated with nicotine by two different treatment paradigms. In the first paradigm, animals were infused 5 mg/kg/day of nicotine for 14 days and then subjected to either 2 h restraint stress or to 24 h cold stress. In the second paradigm, nicotine was infused at 8 mg/kg/day for 7 days prior to exposure to single or twice repeated restraint stress. Levels of TH and DBH mRNA in the adrenal medulla and locus coeruleus as well as plasma nicotine and corticosterone concentrations were compared to control saline treated animals. The findings will help to understand the interactions between nicotine and stress, and how individuals addicted to nicotine respond to stress, and can be helpful in preventing relapse.

2. Materials and methods

2.1. Animal manipulations

All animal experiments were approved by the Institutional Animal Care and Use Committee and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Male Sprague–Dawley rats (250–300 g) from Taconic Farms (Germantown, NY) were used for this study. They were housed four per cage in a barrier area to minimize stress on a 12-h light/dark cycle at 23±2 °C with free access to food and water.

Three experimental procedures in which animals pretreated with nicotine were exposed to stress were used in this study and are shown in Table 1. Nicotine-di-D-tartrate (Sigma, St. Louis, MO) dissolved in saline was administrated by continual infusion with an osmotic pump (model 2002; Alzet, Palo Alto, CA). Control groups received an equal volume of saline. Alzet pumps were implanted subcutaneously in the nape of the neck under pentobarbital (50 mg/kg) anesthesia to deliver 5 mg/kg/day nicotine (calculated as free base) for 14 days, 8 mg/kg/day nicotine for 7 days or an equal volume of saline. Animals were then divided into groups of 8 animals each which were either subjected to the restraint or cold stress, or unstressed.

2.1.1. Restraint stress

Rats were placed in a small metal cylinder (diameter: 6 cm) for 2 h once (1×) or 2 h daily on two consecutive days (2×) and euthanized 3 h after stress, a time found to be maximal for elevation of TH mRNA in adrenal medulla with immobilization stress (Nankova et al., 1994).

2.1.2. Cold stress

Rats were kept at 4 °C, two rats per metal cage without bedding for 24 h and euthanized immediately afterwards, based on the time course previously observed for cold triggered changes in TH mRNA levels in the rat adrenal medulla (Baruchin et al., 1990).

Rats were euthanized by decapitation. The adrenals were removed, and the left and right adrenal medulla dissected and frozen separately from each animal in liquid nitrogen. The brain was dissected using a tissue slicer with digital micrometer. The LC was punched from frontal sections, 9.2–10.4 mm from Bregma, and frozen in liquid nitrogen. Blood was collected into EDTA-containing tubes on ice, plasma separated and kept at −70 °C.

Table 1
Summary of experimental procedures

	Nicotine pretreatment	Stress
Experiment 1	5 mg/kg/day, 14 days	Restraint 1× or cold 24 h
Experiment 2	5 mg/kg/day, 14 days	Cold 24 h
Experiment 3	8 mg/kg/day, 7 days	Restraint 1×, 2× or cold 3 h

2.2. Determination of plasma corticosterone and nicotine levels

Plasma corticosterone levels were determined by ^{125}I radioimmunoassay (Biomedical, Inc., Costa Mesa, CA) according to the manufacturer's instruction. Samples were diluted with steroid diluent (1:50–200). The sensitivity of assay was approximately 25 ng/ml. The Intra-assay and Inter-assay coefficients of variation were 4.4% and 6.5%, respectively.

Plasma nicotine levels were measured by ^{3}H radioimmunoassay (Kit purchased from Dr. Van Vunakis, Brandeis University, MA) according to the supplier's instruction (Langone and VanVunakis, 1982; Langone et al., 1993). Plasma was diluted (1:2–1:50) with 0.14 M NaCl, 0.01 M Tris–HCl, pH 7.4, containing 0.1% gelatin. The standard curve was determined by using nicotine concentrations that ranged from 0.5 to 50 ng/ml. Plasma nicotine competed with a known amount of ^{3}H-labelled nicotine for the binding sites on a limited number of specific antibody molecules. After equilibration, the antibody-bound nicotine was separated and precipitated from unbound free nicotine by adding anti-rabbit globulin. The radioactivity in the precipitate was compared to the values from the standard curve run in parallel. The inter-assay coefficient of variation was 6–10%.

2.3. Isolation of RNA and determination of mRNA levels

2.3.1. Adrenal medulla (AM)

The relative levels of adrenomedullary TH and DBH mRNA levels were determined by Northern blot analyses as previously described (Nankova et al., 1994; Serova et al., 1999). Briefly, the adrenal medulla from each separate animal was homogenized in RNA-STAT 60 (Tel-Test, Friendswoods, TX). The total RNA from individual samples was then isolated and fractionated on 1.2% agarose gels. The RNA was subsequently transferred to Gene-Screen Plus membranes (New England Nuclear, Boston, MA) and hybridizations were performed with TH cDNA, DBH cDNA, and genomic probe for 18S rRNA. The probes were labeled with [^{32}P]α-dCTP (6000 Ci/mmol; Perkin Elmer, Boston, MA) by using the random primer method (Megaprime; Amersham, Arlington Heights, IL). Hybridization was performed at 42 °C in a prehybridization solution and 10^6 dpm of ^{32}P-labeled probes. The hybridizations with the cDNA probes and the washing of the filters were done as described previously (Nankova et al., 1994; Kvetnansky et al., 1996). Following hybridization and exposure to X-ray film (Kodak, Rochester, NY) the blots were stripped and then rehybridized with subsequent probes. Autoradiograms with signals within the linear range were scanned using Bio-Rad GS-800 Calibrated Densitometer and normalized to levels of 18S rRNA.

2.3.2. Locus coeruleus (LC)

The levels of TH and DBH mRNAs in LC were analyzed by real-time RT-PCR. Total RNA was isolated from tissues using RNA-STAT 60 (Tel-Test, Friendswoods, TX) and purified with RNAqueous-Micro RNA isolation kit (Ambion, Austin, TX). The A260/A280 was >1.85. The amount of total RNA from each sample was quantified using Ribo-Green fluorescent dye (Molecular Probes, Eugene, OR). Total RNA (100–300 ng) was reverse transcribed (AMV, Roche, Indianapolis, IN) in 5 μl using AMV reverse transcriptase (Sigma, St. Louis, MO). 5 μl of RT mixture contained 1 × RT buffer (Sigma, St. Louis, MO), 1 mM dNTP mix, 5 units of RNAse inhibitor (Roche, Indianapolis, IN), 1 μM specific reverse primer and 5 unit of AMV reverse transcriptase (Sigma, St. Louis, MO). The following specific primers were used for the RT reaction:

TH gene 5′-TCAGGCTCCTCTGACAG-3′
DBH gene 5′-GCACAGTAATCACCTTCC-3′.

RT was carried out at 42 °C for 1 h followed by 10 min 85 °C. Then the reactions were diluted to 20 μl with autoclaved H_2O.

Quantitative real-time PCR was performed using Light Cycler System with SYBR Green detection (Roche Applied Science). The following primers were used for real-time PCR:

THfor 5′-GTGAACCAATTCCCCATG-3′
THrev 5′-CAGTACACCGTGGAGAG-3′
DBHfor 5′-CACCACATCATCATGTATGAGG-3′
DBHrev 5′-CCTGTCTGTGCAGTAGCCAG-3′.

The primers were designed so that sense and antisense primers matched sequences in different exons. In this case contamination with the genomic DNA would result in amplification of the longer product which never was observed. For the Light Cycler reaction a master mix of the following reaction components (Roche, Indianapolis, IN) was prepared (per 1 reaction; 20 μl): 12.8 μl PCR grade H_2O for TH (12.4 μl for DBH); 1.2 μl 25 mM $MgCl_2$ (1.6 μl for DBH); 2 μl 5 μM primer mix; 2 μl LightCycler FastStart DNA Master SYBR Green. 18 μl of the master mix were distributed to each capillary and 2 μl of the diluted samples were added.

The following LightCycler protocols were used: For TH-denaturation program (95 °C for 7 min), a four-segment amplification and quantification program repeated 37 times (95 °C for 3 s; 59 °C for 3 s; 72 °C for 18 s; 85 °C for 0 s for a single fluorescence measurement), melting curve program (70 °C to 99 °C) and finally a cooling program down to 40 °C. For DBH-denaturation program (95 °C for 7 min), a four-segment amplification and quantification program repeated 37 times (95 °C for 4 s; 54 °C for 3 s; 72 °C for 18 s; 85 °C for 0 s for a single fluorescence measurement), melting curve program (70 °C to 99 °C) and a cooling program down to 40 °C. The threshold cycle was determined using Fit Points method to provide optimal standard curve values (0.98 to 1.0). For quantification assays standard curve was used, produced by amplification of several 10 fold dilutions ($\geq$4) of linearized plasmids contained TH or DBH cDNA. The results of real-time PCR were normalized to the amount of total RNA in the PCR reaction.

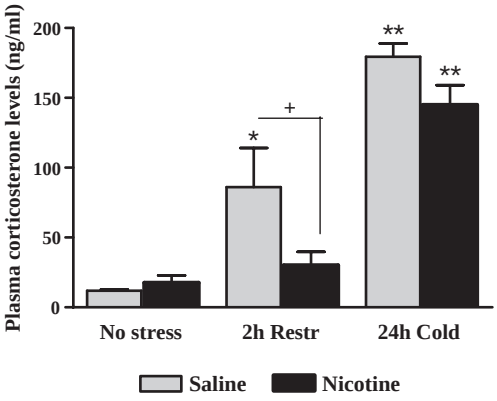

Fig. 1. Effect of nicotine infusion (5 mg/kg/day) on plasma corticosterone levels in response to a single 2 h restraint stress (Restr) or 24 h cold stress. Data are expressed as mean ± S.E.M, relative to the mean of the unstressed saline-infused animals taken as 1. *$p < 0.05$ and **$p < 0.001$ relative to respective control (No stress); +$p < 0.05$ relative to saline-infused rats exposed to restraint stress.

2.4. Statistical analyses

The data were analyzed with GraphPad (San Diego, CA). One-way ANOVA, followed by Fisher's least-significant difference test for comparison of the means (for more than two experimental groups) or Student's *t*-test (for two experimental groups) were used to evaluate the data. Data are expressed as mean ± S.E.M. A level of $p < 0.05$ was accepted as statistically significant.

3. Results

To study whether nicotine infusion can modulate the responses to restraint or cold stress, rats were implanted with osmotic pumps to deliver nicotine at 5 mg/kg/day. Control animals received an equal volume of saline by osmotic pumps. After 14 days of the infusion of nicotine or saline, the rats were then exposed to either restraint stress for 2 h and euthanized 3 h afterward or to cold stress for 24 h and euthanized immediately. These durations of the stress were chosen based on previous experiments to elicit optimal changes in rat TH and DBH mRNA levels (Kvetnansky and Sabban, 1993; Nankova et al., 1994; Kvetnansky et al., 2003).

The plasma corticosterone levels at the time of euthanasia are shown in Fig. 1. Similar to our previous finding (Serova et al., 1999), the prolonged nicotine infusion did not significantly alter basal plasma corticosterone levels. Both types of stress triggered significant elevations in plasma corticosterone in the saline infused rats. However in the nicotine treated rats, corticosterone was not significantly elevated following the restraint stress. In contrast cold stress triggered a significant elevation in corticosterone in both controls and nicotine infused rats, although the rise tended to be somewhat smaller in the nicotine treated animals.

The effects of nicotine on stress triggered changes in TH and DBH mRNA levels were examined in the adrenal medulla and locus coeruleus.

The results in the adrenal medulla are shown in Fig. 2. Basal levels were similar in nicotine- or saline-infused rats. Exposure of the rats to restraint stress or to cold stress led to a significant increase in TH mRNA levels to 2.5–3.0 fold basal levels, in both saline-infused controls and in the nicotine treated rats. However, while restraint or cold stress significantly elevated DBH mRNA levels about 50% higher than basal levels in saline-treated rats, these stressors did not trigger any change in DBH in the nicotine infused rats. Thus, infusion of nicotine prevented the stress-triggered elevation of DBH, but not TH, mRNA in the adrenal medulla.

In the locus coeruleus (Figs. 3 and 4), there was a tendency towards elevated basal TH and DBH mRNA levels in the rats infused with nicotine compared to saline. Exposure of saline-infused rats to 2 h of restraint stress led to a significant rise in both TH and DBH mRNA levels to over double control values (Fig. 3). There was no significant difference between unstressed and restrained rats given nicotine infusion in the levels of TH and DBH mRNA.

Next, we examined the effect of cold stress on TH and DBH mRNA levels in the LC of saline and nicotine infused rats. Following 24 h of cold, both groups of the animals

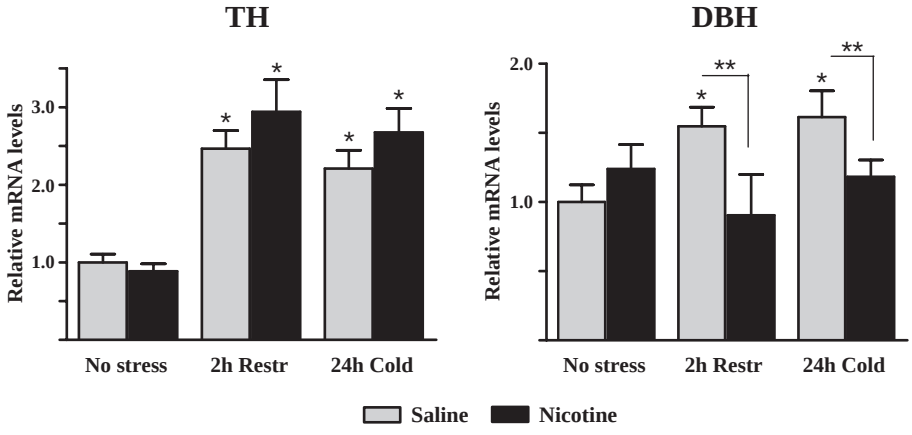

Fig. 2. Effect of 5 mg/kg/day nicotine infusion on stress-elicited changes in TH and DBH mRNA levels in rat adrenal medulla. Rats were infused with saline or nicotine (5 mg/kg/day) for 14 days and then subjected to no stress, 2 h restraint (Restr), or 24 h cold stress. Data are expressed as mean ± S.E.M, relative to the mean of the unstressed saline-infused animals taken as 1. *$p < 0.05$ relative to respective controls (No stress); **$p < 0.05$ relative to saline-infused rats exposed to stress.

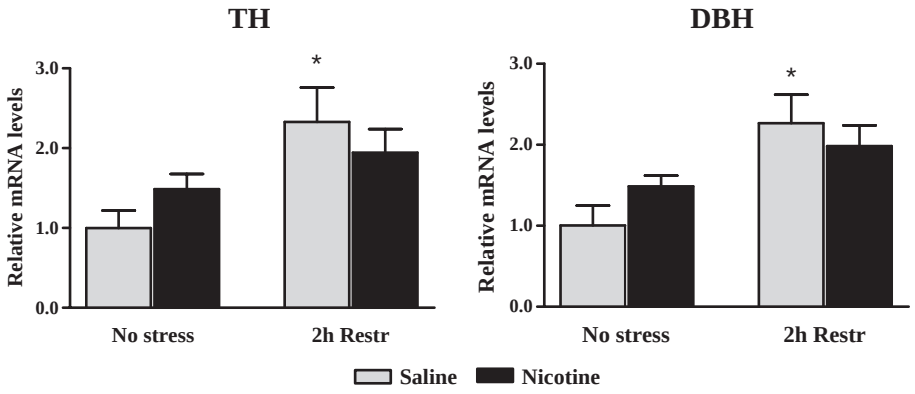

Fig. 3. Effect of 5 mg/kg/day nicotine infusion on restraint-elicited changes in TH and DBH mRNA levels in locus coeruleus. Rats were infused with saline or nicotine (5 mg/kg/day) for 14 days and subjected to 2 h restraint stress. Data are expressed as mean ± S.E.M, relative to the mean of the unstressed saline-infused animals taken as 1. *$p < 0.05$ relative to respective control (No stress).

(saline and nicotine infused) displayed a dichotomous response to cold stress. Therefore this experiment was repeated again and the combined results are shown for TH and DBH in Fig. 4. In some of the cold-exposed animals (at least 1/3 of the cold treated rats) TH mRNA levels in the LC were much lower than in animals not exposed to cold. However the rest of the animals displayed TH mRNA levels higher (although not significantly) than controls. A similar dichotomous response to cold stress was also observed for DBH mRNA levels in the LC. Changes in TH and DBH were similar in the same animals—the animals in which cold triggered a decline in TH mRNA also showed a decline in DBH, and similarly animals with an elevation of TH mRNA to cold, also had an increase in DBH.

In these experiments, the 14 day infusion of 5 mg/kg nicotine elevated DBH mRNA levels in the LC without exposure to stress, which was not observed in our earlier study (Serova et al., 1999) where the rats were infused with 5 mg/kg nicotine for 12 days. Since 14 days is close to the maximal for the pumps and since it was found that 7 days of nicotine infusion is sufficient for rats to display features of nicotine addiction (Malin, 2001), therefore we examined whether 7 days of nicotine infusion would alter the response to restraint stress in the adrenal medulla and the LC. In this experiment we used a higher concentration of nicotine (8 mg/kg/day) since it is reported to provide nicotine concentrations similar to those attained with self administration (LeSage et al., 2003). We examined the effect of this mode of nicotine infusion on the response to a single 2 h restraint as in our previous experiments, as well as an additional 2 h restraint repeated on the next day.

To assess the nicotine delivery with infusion by mini-pumps, we measured nicotine concentrations in the plasma of the nicotine infused rats (see Table 2). Plasma nicotine levels were about 24 ng/ml following infusion with 5 mg/kg for 14 days. Similar results were obtained in the two separate experiments. In saline infused rats plasma nicotine levels were less than 8 ng/ml. After 7 days infusion of 8 mg/kg nicotine, plasma nicotine levels were about 141 ng/ml or about 6 fold higher than those in the previous experiments, even though the concentration of nicotine in the osmotic pumps was only 60% greater.

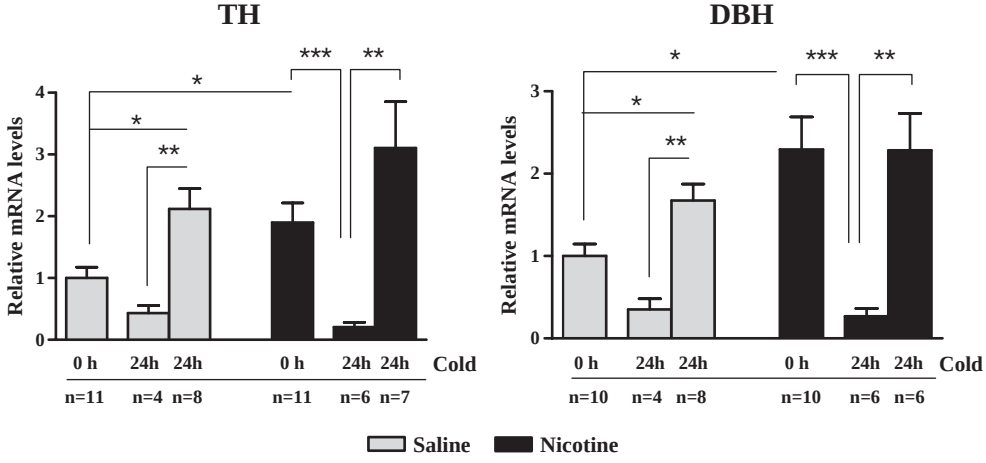

Fig. 4. Effect of 5 mg/kg/day nicotine infusion on cold stress-elicited changes in TH and DBH mRNA levels in locus coeruleus. Rats were infused with saline or nicotine (5 mg/kg/day) for 14 days and subjected to 24 h cold stress. Data are expressed as mean ± S.E.M, relative to the mean of the unstressed saline-infused animals taken as 1. *$p < 0.05$ vs. saline-infused no stress control; **$p < 0.05$ low response group vs. high response group; ***$p < 0.05$ vs. nicotine-infused no stress group.

Table 2
Plasma nicotine concentration in rats with saline or nicotine infusion

Treatment		Plasma nicotine concentration (ng/ml)
Saline, 7 days		≤7.89±2.7
Nicotine 5 mg/kg/day, 14 days	Exp 1	22.0±6.2*
	Exp 2	23.7±3.6*
Nicotine 8 mg/kg/day, 7 days	Exp 3	141.1±30.6*,+

Data are expressed as mean±S.E.M.
*$p<0.05$ relative to saline.
+$p<0.05$ relative to nicotine 5 mg/kg/day.

This second paradigm of nicotine administration did not alter basal TH or DBH mRNA levels in the adrenal medulla or the locus coeruleus.

In the adrenal medulla (Fig. 5): Exposure to a single 2 h restraint stress (1× Restr) led to a significant rise in TH mRNA in the saline infused controls, but not in the nicotine infused rats. No significant elevation was observed with the second daily restraint (2× Restr) in either the saline or nicotine infused group. These results indicate that the infusion of nicotine (8 mg/kg/day, 7 days) attenuated the elevation of TH mRNA in the adrenal medulla by the single restraint stress. DBH mRNA levels were 2–3 fold higher in nicotine as well as in saline infused rats exposed to a single 2 h restraint stress. However, DBH mRNA levels after 2× Restr were significantly lower than after first episode of restraint in nicotine treated animals, and were similar to basal levels.

In the locus coeruleus (Fig. 6): The restraint stress triggered changes in levels of TH mRNA were similar with saline and nicotine infusion; with the large variation none of the levels were significantly different from the unstressed controls in nicotine infused rats. In contrast DBH mRNA in the locus coeruleus of saline infused rats was significantly elevated by either a single 2 h restraint or two daily 2 h restraint stress. In response to single restraint stress DBH mRNA levels were similarly elevated in both groups of rats. However, the response to the second daily restraint was not observed in the nicotine infused rats. The nicotine treatment actually prevented the elevation of DBH to twice repeated restraint stress.

4. Discussion

The results of this study demonstrate the complexity of the interactions between nicotine and stress. While prolonged infusion of nicotine can attenuate some of the responses to stress, it varied with the type and repetition of stress as well as the specifics of the nicotine infusion such as duration, dose, and the tissue examined. Thus, pretreatment with 5 mg/kg/day nicotine for 14 days prevented the elevation of plasma corticosterone following restraint, but not cold stress. It also attenuated the restraint, as well as cold, triggered elevation of adrenomedullary DBH, but not TH mRNA. However, the effect of these stressors on TH and DBH mRNA in the LC were unaltered by this nicotine pretreatment. Administration of 8 mg/kg/day nicotine for 7 days inhibited the elevation of adrenomedullary, TH, but not DBH, mRNA with a single restraint, and the response of DBH mRNA in the LC to a second daily restraint.

The effect of nicotine pretreatment on plasma corticosterone differed with the two stressors examined. Nicotine infusion did not alter the basal level of plasma corticosterone but effectively attenuated response to single restraint stress. However, similar stress-evoked changes in corticosterone levels were found in rats with and without nicotine infusion exposed to 24 h of cold. The differences might be due to differential mechanisms involved in the regulation of these two stressors (Pacak and Palkovits, 2001). However, corticosterone was only measured 3 h after the restraint stress. Therefore the level is lower than expected immediately after or during the stress, while with cold stress it was measured immediately afterwards. We cannot distinguish whether nicotine elicited a diminution of the elevation in corticosterone in response to restraint stress, or

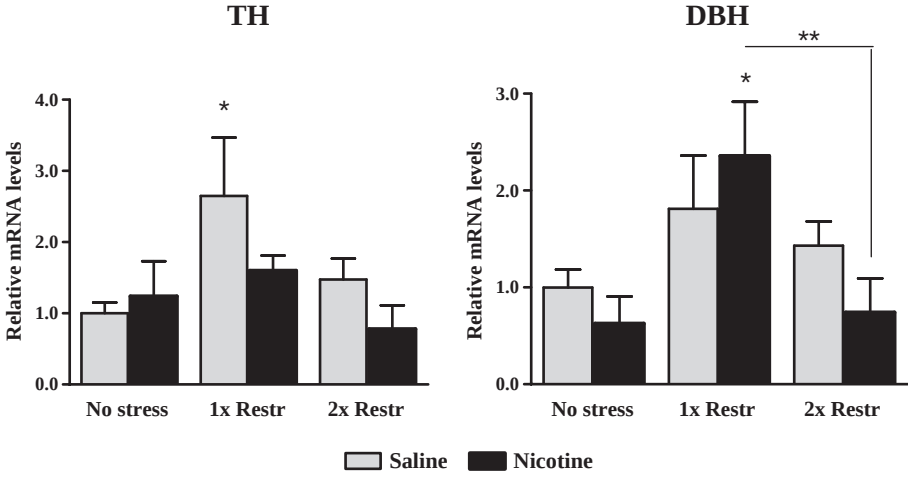

Fig. 5. Effect of 8 mg/kg/day nicotine infusion on restraint-elicited changes in TH and DBH mRNA levels in adrenal medulla. Rats were infused for 7 days with saline or nicotine (8 mg/kg/day) and exposed to a single 2 h restraint (Restr 1×) or 2 daily 2 h restraint on two consecutive days (Restr 2×). For TH, $F_{2,18}=3.65$ ($p<0.05$) for saline-treated and $F_{2,24}=1.58$ ($p>0.05$) for nicotine-treated rats and for DBH, $F_{2,18}=1.76$ ($p>0.05$) for saline-treated and $F_{2,24}=12.90$ ($p<0.05$) for nicotine treated rats. Data are expressed as mean±S.E.M, relative to the mean of the unstressed saline-infused animals taken as 1, *$p<0.05$ relative to respective control (No stress); **$p<0.05$ relative to Restr 1× group.

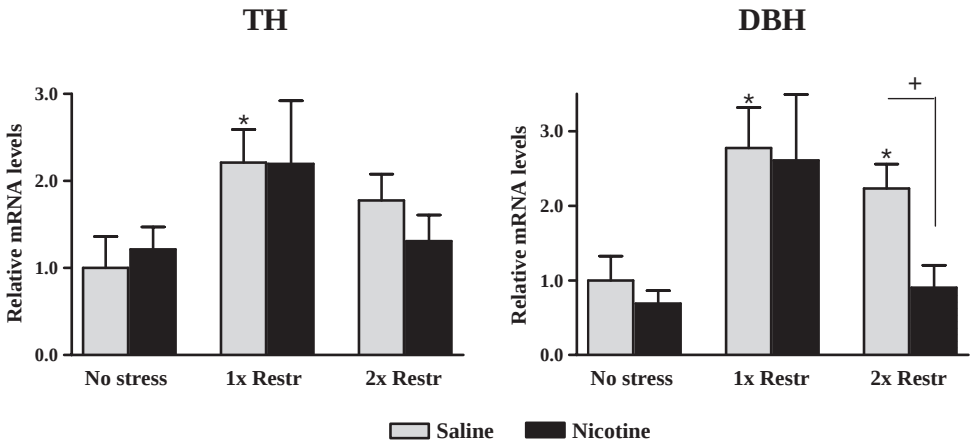

Fig. 6. Effect of 8 mg/kg/day nicotine infusion on restraint-elicited changes in TH and DBH mRNA levels in locus coeruleus. Animals were treated as described in Fig. 5. For TH, $F_{2,21}=4.40$ ($p<0.05$) for saline-treated and $F_{2,18}=1.36$ ($p>0.05$) for nicotine-treated rats and for DBH, $F_{2,21}=7.75$ ($p<0.05$) for saline-treated and $F_{2,21}=3.96$ ($p<0.05$) for nicotine treated rats. Data are expressed as mean ± S.E.M, relative to the mean of the unstressed saline-infused animals taken as 1, *$p<0.05$ relative to respective control (No stress); +$p<0.05$ relative to Restr 2× saline-infused group.

whether the changes reflect a more rapid return towards basal levels. Examination of a detailed time course of changes in corticosterone in the future will be needed to distinguish between these possibilities. Nicotine-elicited attenuations were also observed in plasma aldosterone levels with restraint and cold stress (Stier et al., 2004).

Overall, the stress triggered changes in DBH mRNA appear to be more sensitive to nicotine than the changes in TH gene expression. Thus, infusion of 5 mg/kg nicotine for 14 days prevented the elevation in DBH mRNA in adrenal medulla in response to either restraint or cold stress, without altering the stress triggered responses in TH mRNA. Similarly, in the locus coeruleus, 8 mg/kg nicotine for 7 days, attenuated the response of DBH mRNA to a second daily restraint, but had no effect on TH mRNA levels. Our previous study revealed that nicotine infusion was more effective in inhibiting the elevation of DBH mRNA levels than TH mRNA levels in response to immobilization stress (Serova et al., 1999). DBH is generally not considered rate limiting in the regulation of catecholamine biosynthesis. However, under certain conditions DBH can be rate limiting in catecholamine biosynthesis (Cubells and Zabetian, 2004). Gene expression of DBH is regulated by many of the treatments which have been shown to induce elevated TH gene expression including stress elevated cAMP and glucocorticoids (e.g. McMahon and Sabban, 1992; Kvetnansky and Sabban, 1993; Hwang et al., 1997; Sabban and Kvetnansky, 2001), and may be necessary to replenish the DBH enzyme stores released during neurosecretion.

Nevertheless in some cases nicotine infusion can change response of TH expression to stress. Nicotine infusion was found to attenuate the immobilization stress (a strong physical and emotional stress) triggered TH mRNA elevation in the adrenal medulla, but not in the locus coeruleus or the substantia nigra (Serova et al., 1999). In the current work, while a single restraint stress significantly elevated adrenomedullary TH mRNA levels in saline infused rats, it was unaltered in nicotine (8 mg/kg, 7 days) treated rats.

The critical role of TH and DBH genes in nicotine addiction was supported by investigations of associations between polymorphism of these genes and smoking. It has been shown that two different alleles (K1 and K4) of the human TH intron-1 tetranucleotide repeat polymorphism on chromosome 11 are associated with the number of cigarettes smoked per day. The K1 allele was associated with higher smoking usages, whereas the K4 allele was associated with lower smoking usages. The data suggested that regular smokers carrying the K4 allele are approximately 85% less likely to be addicted to smoking (Anney et al., 2004). An association between DBH polymorphism and tobacco consumption has also been found. It was shown that smokers with the 1368DBH A allele on chromosome 9 tended to smoke more cigarettes than those with the more common 1368DBH G allele (McKinney et al., 2000). The same polymorphism was associated with the effectiveness of nicotine patch (Johnstone et al., 2004).

Differences between the effects of various types of stress on TH and DBH mRNA levels were observed. Animals subjected to cold stress showed more resistance to the effect of nicotine infusion compared to restraint or immobilization stress. Prolonged cold treatment has been shown to increase the total cytochrome *P*450 (CYP) content (Gromova et al., 2004). Since CYP 2A6 is the major enzyme involved in nicotine metabolism, the high CYP content may cause an increased rate of nicotine metabolism. Therefore, higher nicotine dosage may be needed to observe an attenuation of the response to cold. The neuronal pathways activated by cold stress, as shown by c-*fos* immunocytochemisty differs from those which respond to immobilization (reviewed by Pacak and Palkovits, 2001; Palkovits, 2002). Rats which received nicotine infusions still responded to 1 day cold stress with a similar rise in plasma corticosterone and with similar increase in adrenomedullary TH and DBH mRNA levels as animals which received saline infusions.

The response to cold stress in the locus coeruleus was similar in both the saline and nicotine infused groups, with about one-third of the animals displaying very low TH and

DBH mRNA levels and two thirds of them displaying high levels following 1 day of cold stress. Part of this variation may result from the use of Sprague–Dawley rats, an outbred strain. We deliberately used an outbred strain of rats, in the hope of obtaining results more representative of the general population than might be found with an inbred strain. These results would indicate that there may be individual differences in the response of the LC to cold stress.

There are conflicting reports regarding the effect of chronic exposure to cold on expression of TH mRNA in the LC of Sprague–Dawley rats. Five hours of cold stress (4 °C) is reported to elicit a significant elevation in TH mRNA levels in the LC (Rusnak et al., 2001) and chronic cold results in persistent alterations in the function of the ascending noradrenergic efferents from the LC, as well as potentiation of CRF-evoked increased in electrophysiological activity of LC neurons (Jedema et al., 2001; Jedema and Grace, 2003). On the other hand, chronic cold stress (5 °C) for 21 days is reported to lead to a significant decline in TH mRNA levels in the LC (Featherby and Lawrence, 2004). These investigators did not examine changes at a shorter time. Rat strain differences have been reported in nicotine's behavioral effects. While nicotine did not affect anxiety behaviors in Sprague–Dawley rats (Benwell et al., 1994), anxiolytic effects of nicotine were observed in Wistar and Fisher 344 rats (Brioni et al., 1993; Onaivi et al., 1994). Further studies are needed to determine more carefully the time course of the changes in TH and DBH expression by cold stress in the LC, and whether the dichotomous response observed here reflect individual differences in the stress-induced sensitization to cold stress (Changeux et al., 1998). It remains to be determined whether the second mode of nicotine administration (8 mg/kg/day for 7 days) will affect the response to cold stress. This was not carried out in the present study, since an extremely large number of animals would be required to clarify the underlying mechanism of this dichotomous response observed earlier.

One of the difficulties in the present experiments was in determining the conditions which would be appropriate to evaluate response of catecholamine biosynthetic enzyme gene expression in the AM and LC in the same animals. In this study, nicotine was delivered by osmotic minipumps. Osmotic minipumps are a convenient and efficacious alternative to multiple injections. It is somewhat analogous to delivery by a nicotine patch to humans. Chronic nicotine administration by minipumps, as well as multiple injections, can up-regulate the nicotinic receptor levels in central and peripheral nervous tissues; although nicotine infusion causes significantly higher levels of nicotinic receptor compared to multiple injections (Ulrich et al., 1997). Infusion can avoid the hypoxic–ischemic episodes that accompany nicotine injections, and also prevent the inherent problems of repeated handling stress and episodic withdrawal between injections. Since this study is trying to understand the paradoxical relationship between nicotine and stress, it was deemed essential to eliminate the unnecessary physiological stress caused by injections. In this study, we administrated nicotine (5 mg/kg/day) as previously (Serova et al., 1999) for 14 days and 8 mg/kg/day for 7 days. Despite the 60% increase in the dose of nicotine in the pumps, the level of plasma nicotine was 6 times higher. These differences might reflect different pharmacokinetics, changes in stability with long-term infusions in the pumps, or perhaps a decline in delivery towards the end of the length of time recommended for the osmotic pumps.

The concentration of nicotine (22 ng/ml) observed here in rat plasma after 14 days of 5 mg/kg nicotine are similar to that observed in human plasma (15 ng/ml) with 21 mg/day transdermal nicotine for 5 days (Gorsline et al., 1993). Continuous intravenously infusion of 8 mg/kg/day nicotine is reported to be more effective than lower concentrations (1 and 3 mg/kg/day) to suppress nicotine self administration (LeSage et al., 2003). Although these continuous administration paradigms and dosages differ markedly from human tobacco smokers, they produced results on body weight, food consumption, sensory gating and physical activity (e.g. Acri et al., 1991; Grunberg, 1992; Faraday et al., 1998, 1999).

Although 5 mg/kg nicotine infusion for 14 days was effective in preventing the response of DBH mRNA in the adrenal medulla with restraint or cold stress, these conditions were not appropriate for the LC. This mode of infusion did not alter the changes observed with restraint or cold stress, and tended to elevate basal levels of mRNAs in the LC without stress. This tissue specificity could be explained by the diversity of neuronal nicotinic acetylcholine receptor (nAChR) subtypes with varied affinities for nicotine, expressed in different locations (reviewed by Dani, 2001; Leonard and Bertrand, 2001). Receptors containing the α3 subunit are the predominated nicotinic acetylcholine receptors (nAChR) in adrenal medulla (Free and McKay, 2003). Nicotinic receptors comprising the α4β2 nAChR are the major high affinity nAChR in the brain (Whiting et al., 1987). The homopentomers of the α7 nAChR subtype constitute the major low affinity receptor in the brain (Chen and Patrick, 1997). Many LC neurons express the relatively rare α6 nAChR subtype, which is not expressed in the adrenal medulla (reviewed by Lena et al., 1999). Although most of the subunits are present in both tissues their relative proportion and subunit assembly generates different variety of nAChR that differ in their pharmacological and biophysical properties, such as nicotine sensitivity and rate of desensitization (Le Novere et al., 2002). During the chronic nicotine exposure some of the nicotine receptors undergo a transition to a reversible desensitized state which is resistant to activation (Quick and Lester, 2002) and the rate of desensitization depends on composition of nAChRs, which is specific for the different cell types.

The hypothetical mechanism of the attenuation of the stress response by nicotine infusion could involve desensitization of specific nicotinic receptors. In this regard constant infusion of nicotine (at 1 and 4 but not 0.25 mg/kg/day) was found to abolish the sensitized dopamine response in the nucleus accumbens to an injection of nicotine (Benwell et al., 1995). While acute nicotine self administration elevated NE release in the amygdala, with long-term administration there appeared to be desensitization of the pharmacologic effects of nicotine and decline in the amygdaloid NE secretion and also partial

desensitization of NE secretion from the paraventricular nucleus (PVN) (Fu et al., 2001, 2003). Such changes might mediate an attenuation of some of the responses to stress following prolonged exposure to nicotine.

It is also possible that stress-triggered responses on the locus coeruleus (or adrenal medulla) do not necessarily involve nicotinic receptors on these structures, but rather effects on neuronal afferents regulating them. For example, prolonged nicotine might modulate CRH (corticotrophin releasing hormone) neurons from the PVN, which have been shown to participate in the regulation of LC neurons and are functionally important for LC activation during exposure to several stressors, such as cold and immobilization (Melia and Duman, 1991; Valentino et al., 1993; Smagin et al., 1997).

The results of this study, although restricted in the target genes examined, may help to shed light on the ameliorative effects of nicotine in smokers during the stress and in understanding the greater propensity for relapse during stressful situations. The findings indicate that constant release of nicotine can modulate, or even prevent, some of stress responses at the level of the HPA axis and gene expression of catecholamine biosynthetic enzymes in the CNS and periphery. The response of DBH was more sensitive than of TH to modulation by nicotine, which might indicate greater vulnerability of the noradrenergic systems. The tissue specific differences observed with the two durations and concentrations of nicotine infusion might lead to different patterns of behavioral responses. It remains to be determined which receptor subtypes are involved in the selectivity of the modulation of the stress responses to nicotine.

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References

Abrams DB, Monti PM, Pinto RP, Elder JP, Brown RA, Jacobus SI. Psychosocial stress and coping in smokers who relapse or quit. Health Psychol 1987;6:289–303.

Acri JB. Nicotine modulates effects of stress on acoustic startle reflexes in rats: dependence on dose, stressor and initial reactivity. Psychopharmacology (Berl) 1994;116:255–65.

Acri JB, Grunberg NE, Morse DE. Effects of nicotine on the acoustic startle reflex amplitude in rats. Psychopharmacology (Berl) 1991;104:244–8.

Anney RJ, Olsson CA, Lotfi-Miri M, Patton GC, Williamson R. Nicotine dependence in a prospective population-based study of adolescents: the protective role of a functional tyrosine hydroxylase polymorphism. Pharmacogenetics 2004;14:73–81.

Balfour DJ. Studies on the biochemical and behavioural effects of oral nicotine. Arch Int Pharmacodyn Ther 1980;245:95–103.

Baruchin A, Weisberg EP, Miner LL, Ennis D, Nisenbaum LK, Naylor E, et al. Effects of cold exposure on rat adrenal tyrosine hydroxylase: an analysis of RNA, protein, enzyme activity, and cofactor levels. J Neurochem 1990;54:1769–75.

Benwell ME, Balfour DJ, Khadra LF. Studies on the influence of nicotine infusions on mesolimbic dopamine and locomotor responses to nicotine. Clin Investig 1994;72:233–9.

Benwell ME, Balfour DJ, Birrell CE. Desensitization of the nicotine-induced mesolimbic dopamine responses during constant infusion with nicotine. Br J Pharmacol 1995;114:454–60.

Brioni JD, O'Neill AB, Kim DJ, Decker MW. Nicotinic receptor agonists exhibit anxiolytic-like effects on the elevated plus-maze test. Eur J Pharmacol 1993;238:1–8.

Changeux JP, Bertrand D, Corringer PJ, Dehaene S, Edelstein S, Lena C, et al. Brain nicotinic receptors: structure and regulation, role in learning and reinforcement. Brain Res Brain Res Rev 1998;26:198–216.

Chen D, Patrick JW. The alpha-bungarotoxin-binding nicotinic acetylcholine receptor from rat brain contains only the alpha7 subunit. J Biol Chem 1997;272:24024–9.

Cryer PE, Haymond MW, Santiago JV, Shah SD. Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. N Engl J Med 1976;295:573–7.

Cubells JF, Zabetian CP. Human genetics of plasma dopamine beta-hydroxylase activity: applications to research in psychiatry and neurology. Psychopharmacology (Berl) 2004;174:463–76.

Dani JA. Overview of nicotinic receptors and their roles in the central nervous system. Biol Psychiatry 2001;49:166–74.

Dani JA, De Biasi M. Cellular mechanisms of nicotine addiction. Pharmacol Biochem Behav 2001;70:439–46.

Faraday MM, O'Donoghue VA, Grunberg NE. Effects of nicotine and stress on startle amplitude and sensory gating depend on rat strain and sex. Pharmacol Biochem Behav 1999;62:273–84.

Faraday MM, Rahman MA, Scheufele PM, Grunberg NE. Nicotine administration impairs sensory gating in Long–Evans rats. Pharmacol Biochem Behav 1998;61:281–9.

Featherby T, Lawrence AJ. Chronic cold stress regulates ascending noradrenergic pathways. Neuroscience 2004;127:949–60.

Fossom LH, Sterling C, Tank AW. Activation of tyrosine hydroxylase by nicotine in rat adrenal gland. J Neurochem 1991;57:2070–7.

Free RB, McKay DB. Surface and intracellular nicotinic receptors expressed in intact adrenal chromaffin cells: direct measurements using [3H]epibatidine. Brain Res 2003;974:60–9.

Fu Y, Matta SG, Brower VG, Sharp BM. Norepinephrine secretion in the hypothalamic paraventricular nucleus of rats during unlimited access to self-administered nicotine: an in vivo microdialysis study. J Neurosci 2001;21:8979–89.

Fu Y, Matta SG, Kane VB, Sharp BM. Norepinephrine release in amygdala of rats during chronic nicotine self-administration: an in vivo microdialysis study. Neuropharmacology 2003;45:514–23.

George TP, Verrico CD, Roth RH. Effects of repeated nicotine pre-treatment on mesoprefrontal dopaminergic and behavioral responses to acute footshock stress. Brain Res 1998;801:36–49.

Gorsline J, Gupta SK, Dye D, Rolf CN. Steady-state pharmacokinetics and dose relationship of nicotine delivered from Nicoderm (Nicotine Transdermal System). J Clin Pharmacol 1993;33:161–8.

Gromova OA, Grishanova AY, Perepechaeva ML, Kolosova NG, Kolpakov AR. Effect of long-term cold exposure on activities of cytochrome *P*450-containing monooxygenases and glutathione S-transferase in rat liver microsomes. Bull Exp Biol Med 2004;138:237–9.

Grunberg NE. Cigarette smoking and body weight: a personal journey through a complex field. Health Psychol 1992;11:26–31.

Haass M, Kubler W. Nicotine and sympathetic neurotransmission. Cardiovasc Drugs Ther 1997;10:657–65.

Hiremagalur B, Sabban EL. Nicotine elicits changes in expression of adrenal catecholamine biosynthetic enzymes, neuropeptide Y and immediate early genes by injection but not continuous administration. Brain Res Mol Brain Res 1995;32:109–15.

Hofle G, Weiler R, Fischer-Colbrie R, Humpel C, Laslop A, Wohlfarter T, et al. Stimulation of rat adrenal medulla can induce differential changes in the peptide and mRNA levels of chromogranins, neuropeptides and other constituents of chromaffin granules. Regul Pept 1991; 32:321–31.

Hwang O, Park SY, Kim KS. Protein kinase A coordinately regulates both basal expression and cyclic AMP-mediated induction of three catecholamine-synthesizing enzyme genes. J Neurochem 1997;68:2241–7.

Jedema HP, Grace AA. Chronic exposure to cold stress alters electrophysiological properties of locus coeruleus neurons recorded in vitro. Neuropsychopharmacology 2003;28:63–72.

Jedema HP, Finlay JM, Sved AF, Grace AA. Chronic cold exposure potentiates CRH-evoked increases in electrophysiologic activity of locus coeruleus neurons. Biol Psychiatry 2001;49:351–9.

Johnstone EC, Yudkin PL, Hey K, Roberts SJ, Welch SJ, Murphy MF, et al. Genetic variation in dopaminergic pathways and short-term effectiveness of the nicotine patch. Pharmacogenetics 2004;14:83–90.

Kvetnansky R, Sabban EL. Stress-induced changes in tyrosine hydroxylase and other cathecolamine biosynthetic enzymes. In: Parvez SH, editor. Tyrosine hydroxylase: from discovery to cloning. Utrecht, The Natherlands: VSP; 1993.

Kvetnansky R, Rusnak M, Dronjak S, Krizanova O, Sabban EL. Effect of novel stressors on tyrosine hydroxylase gene expression in the adrenal medulla of repeatedly immobilized rats. Neurochem Res 2003;28:625–30.

Kvetnansky R, Nankova B, Hiremagalur B, Viskupic E, Vietor I, Rusnak M, et al. Induction of adrenal tyrosine hydroxylase mRNA by single immobilization stress occurs even after splanchnic transection and in the presence of cholinergic antagonists. J Neurochem 1996;66:138–46.

Langone J, VanVunakis H. Radioimmunoassay of nicotine, cotinine, and gamma-(3-pyridyl)-gamma-oxo-*N*-methylbutyramide. Methods Enzymol 1982;84:628–40.

Langone J, Gjika H, Van Vunakis H, 1993. Use of Immunoassay Techniques for the Determination of Nicotine and its Metabolites, vol. 12. Lyon: World Health Organization International Agency for Research on Cancer; 1993. p. 293–9.

Le Novere N, Corringer PJ, Changeux JP. The diversity of subunit composition in nAChRs: evolutionary origins, physiologic and pharmacologic consequences. J Neurobiol 2002;53:447–56.

Lena C, de Kerchove D'Exaerde A, Cordero-Erausquin M, Le Novere N, del Mar Arroyo-Jimenez M, Changeux JP. Diversity and distribution of nicotinic acetylcholine receptors in the *locus ceruleus* neurons. Proc Natl Acad Sci U S A 1999;96:12126–31.

Leonard S, Bertrand D. Neuronal nicotinic receptors: from structure to function. Nicotine Tob Res 2001;3:203–23.

LeSage MG, Keyler DE, Collins G, Pentel PR. Effects of continuous nicotine infusion on nicotine self-administration in rats: relationship between continuously infused and self-administered nicotine doses and serum concentrations. Psychopharmacology (Berl) 2003;170:278–86.

Malin DH. Nicotine dependence: studies with a laboratory model. Pharmacol Biochem Behav 2001;70:551–9.

Matta SG, Foster CA, Sharp BM. Selective administration of nicotine into catecholaminergic regions of rat brainstem stimulates adrenocorticotropin secretion. Endocrinology 1993;133:2935–42.

McKinney EF, Walton RT, Yudkin P, Fuller A, Haldar NA, Mant D, et al. Association between polymorphisms in dopamine metabolic enzymes and tobacco consumption in smokers. Pharmacogenetics 2000;10:483–91.

McMahon A, Sabban EL. Regulation of expression of dopamine beta-hydroxylase in PC12 cells by glucocorticoids and cyclic AMP analogues. J Neurochem 1992;59:2040–7.

Melia KR, Duman RS. Involvement of corticotropin-releasing factor in chronic stress regulation of the brain noradrenergic system. Proc Natl Acad Sci U S A 1991;88:8382–6.

Mitchell SN. Role of the locus coeruleus in the noradrenergic response to a systemic administration of nicotine. Neuropharmacology 1993;32:937–49.

Mitchell SN, Smith KM, Joseph MH, Gray JA. Increases in tyrosine hydroxylase messenger RNA in the locus coeruleus after a single dose of nicotine are followed by time-dependent increases in enzyme activity and noradrenaline release. Neuroscience 1993;56:989–97.

Nankova B, Kvetnansky R, McMahon A, Viskupic E, Hiremagalur B, Frankle G, et al. Induction of tyrosine hydroxylase gene expression by a nonneuronal nonpituitary-mediated mechanism in immobilization stress. Proc Natl Acad Sci U S A 1994;91:5937–41.

Onaivi ES, Payne S, Brock JW, Hamdi A, Faroouqui S, Prasad C. Chronic nicotine reverses age-associated increases in tail-flick latency and anxiety in rats. Life Sci 1994;54:193–202.

Pacak K, Palkovits M. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. Endocr Rev 2001;22:502–48.

Palkovits M. Stress-related central neuronal regulatory circuits. In: Kvetnansky R, editor. Stress: nerual, endocrine and molecular studies. London: Taylor and Francis; 2002. p. 1–11.

Quick MW, Lester RA. Desensitization of neuronal nicotinic receptors. J Neurobiol 2002;53:457–78.

Rose JE, Ananda S, Jarvik ME. Cigarette smoking during anxiety-provoking and monotonous tasks. Addict Behav 1983;8:353–9.

Rusnak M, Kvetnansky R, Jelokova J, Palkovits M. Effect of novel stressors on gene expression of tyrosine hydroxylase and monoamine transporters in brainstem noradrenergic neurons of long-term repeatedly immobilized rats. Brain Res 2001;899:20–35.

Sabban EL, Kvetnansky R. Stress-triggered activation of gene expression in catecholaminergic systems: dynamics of transcriptional events. Trends Neurosci 2001;24:91–8.

Schachter S. Pharmacological and psychological determinants of smoking A New York University honors program lecture. Ann Intern Med 1978; 88:104–14.

Schachter S, Silverstein B, Kozlowski LT, Perlick D, Herman CP, Liebling B. Studies of the interaction of psychological and pharmacological determinants of smoking. J Exp Psychol Gen 1977;106:3–12.

Serova L, Danailov E, Chamas F, Sabban EL. Nicotine infusion modulates immobilization stress-triggered induction of gene expression of rat catecholamine biosynthetic enzymes. J Pharmacol Exp Ther 1999;291: 884–92.

Seyler Jr LE, Fertig J, Pomerleau O, Hunt D, Parker K. The effects of smoking on ACTH and cortisol secretion. Life Sci 1984;34:57–65.

Shiffman S. Relapse following smoking cessation: a situational analysis. J Consult Clin Psychol 1982;50:71–86.

Silverstein B. Cigarette smoking, nicotine addiction, and relaxation. J Pers Soc Psychol 1982;42:946–50.

Smagin GN, Zhou J, Harris RB, Ryan DH. CRF receptor antagonist attenuates immobilization stress-induced norepinephrine release in the prefrontal cortex in rats. Brain Res Bull 1997;42:431–4.

Smith KM, Mitchell SN, Joseph MH. Effects of chronic and subchronic nicotine on tyrosine hydroxylase activity in noradrenergic and dopaminergic neurones in the rat brain. J Neurochem 1991;57:1750–6.

Stier Jr CT, Serova LI, Singh G, Sabban EL. Stress triggered rise in plasma aldosterone is lessened by chronic nicotine infusion. Eur J Pharmacol 2004;495:167–70.

Ulrich YM, Hargreaves KM, Flores CM. A comparison of multiple injections versus continuous infusion of nicotine for producing up-regulation of neuronal [3H]-epibatidine binding sites. Neuropharmacology 1997;36: 1119–25.

USDHHS CM. The health consequences of Smoking. A report of the surgeon general. Wasington, DC: US Governemnt Printing Office; 1998.

Valentino RJ, Foote SL, Page ME. The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses. Ann N Y Acad Sci 1993;697:173–88.

Whiting PJ, Liu R, Morley BJ, Lindstrom JM. Structurally different neuronal nicotinic acetylcholine receptor subtypes purified and characterized using monoclonal antibodies. J Neurosci 1987;7:4005–16.