Nicotine Infusion Modulates Immobilization Stress-Triggered Induction of Gene Expression of Rat Catecholamine Biosynthetic Enzymes

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ABSTRACT
The relationship between nicotine and stress is complex and paradoxical. Although people claim they smoke because it relaxes them, nicotine can trigger some of the effects observed with stress, including the release and synthesis of the catecholamines and their biosynthetic enzymes. This study examined one aspect of this confusing relationship between nicotine and stress. Multiple injections of nicotine bitartrate (5 mg/kg) elevated mRNA levels for the catecholamine biosynthetic enzymes, tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH), and phenylethanolamine N-methyltransferase, and of preproneuropeptide Y in rat adrenal medulla more than did 1 mg/kg of nicotine bitartrate. In the locus ceruleus, substantia nigra, and ventral tegmental area both doses equally induced TH mRNA levels. Nicotine infusion (15 mg/kg/day) did not affect adrenal mRNA levels for any of the genes of interest and did not increase plasma corticosterone levels. However, in rats preexposed to nicotinic infusions, the response to a single immobilization (IMO) stress was markedly attenuated with respect to changes in adrenomedullary TH, DBH, and phenylethanolamine N-methyltransferase mRNA levels and in c-Fos protein levels. In the central nervous system, the chronic infusion of nicotine prevented the induction of TH mRNA by repeated IMO stress in the ventral tegmental area (but not in substantia nigra) and of DBH mRNA by single IMO in the locus ceruleus. These findings may explain some of the complex interactions between stress and exposure to nicotine.

Nicotine as well as stress is known to promote catecholamine synthesis by activating tyrosine hydroxylase (TH), the first and major rate-limiting enzyme in the catecholamine biosynthetic pathway, and other downstream enzymes. Physiological stress, when prolonged and/or repeated, also activates gene expression for catecholamine biosynthetic enzymes in the adrenal medulla, sympathetic ganglia, and a number of brain locations (reviewed in Sabban et al., 1995; Kvetnansky and Sabban, 1998; Serova et al., 1999). Likewise, the injection of nicotine into rats was found to elicit elevations of gene expression for catecholamine biosynthetic enzymes as well as for several neuropeptides [neuropeptide Y (NPY) and enkephalin] and other constituents coreleased with the stress-responsive hormone, adrenocorticotropic, as well as other pituitary hormones, leading to elevated plasma adrenocorticotropic and glucocorticoid levels. The administration of nicotine increases heart rate and blood pressure, as well as activates the electroencephalogram of both humans and experimental animals (United States Department of Health and Human Services, 1988). These cardiovascular effects are largely attributed to the direct stimulation of catecholamine release from peripheral sympathetic nerve endings and the adrenal medulla (Haass and Kubler, 1997).

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The relationship between stress and nicotine, the major pharmacological agent in cigarettes, is puzzling. On one hand, smoking is reported to be calming, and on the other hand, it can mimic some of the physiological effects of stress. The generation of these normally incompatible physiological and psychological responses to smoking is termed Nesbitt's Paradox (Nesbitt, 1973).

Cigarette smokers report that smoking helps them cope with stress. The most common reason for relapse to smoking after quitting is exposure to stress. Notably, smokers increased the intensity of their smoking when confronted with a variety of environmental stressors. In addition, when chronic smokers were allowed to smoke, they seemed to have a higher threshold for stress than smokers who were not allowed to smoke or who were given cigarettes low in nicotine content (Silverstein, 1982).

Like stress, nicotine taken in doses similar to those obtained in smoking has broad effects on the neuroendocrine system. It triggers the release of the stress-responsive hormone, adrenocorticotropic, as well as other pituitary hormones, leading to elevated plasma adrenocorticotropic and glucocorticoid levels. The administration of nicotine increases heart rate and blood pressure, as well as activates the electroencephalogram of both humans and experimental animals (United States Department of Health and Human Services, 1988). These cardiovascular effects are largely attributed to the direct stimulation of catecholamine release from peripheral sympathetic nerve endings and the adrenal medulla (Haass and Kubler, 1997).

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ABBREVIATIONS: TH, tyrosine hydroxylase; DBH, dopamine β-hydroxylase; IMO, immobilization stress; LC, locus ceruleus; NPY, neuropeptide Y; PNMT, phenylethanolamine N-methyltransferase; SN, substantia nigra; VTA, ventral tegmental area; CNS, central nervous system.
with catecholamines (Slotkin and Seidler, 1976; Fossom et al., 1991; Hiremagalur and Sabban, 1995; Jahng et al., 1997).

In the central nervous system (CNS), the activation of nicotine acetylcholine receptors in locus ceruleus (LC) noradrenergic, as well as the nigrostriatal and mesolimbic dopaminergic pathways, also elevates both catecholamine synthesis and release, and TH gene expression (Smith et al., 1991; Vezina et al., 1992; Mitchell et al., 1993; Nisell et al., 1996).

Several hypotheses have been advanced to explain how nicotine nevertheless reduces the effect of stress. The failure of the habitual smoker to stop smoking while stressed may result in physiological or psychological withdrawal symptoms, which may add to the intensity of the stress response. The stress may alter the relative bioavailability or effective dose of nicotine. This could induce a condition of "relative" nicotine withdrawal or craving. Another possible explanation is that smoking may decrease the sympathetic autonomic arousal associated with the stress response. Alternatively, the stress-relieving effects of smoking may be psychological and provide the smoker with an alternate focus of attention, thus reducing the stress.

There is conflicting evidence regarding whether or not nicotine can ameliorate the physiological effects of stress. Some studies revealed that nicotine administration did not reduce, but in some cases enhanced, the physiological effects of stress (Benwell and Balfour, 1982; Morse, 1989). Conversely, other studies demonstrated that nicotine attenuated some of the effects of stress (Fuxe et al., 1983; Sharp et al., 1987; Acri, 1994; George et al., 1998).

We hypothesize that these different responses to nicotine may depend on the mode of administration as well as on the cell type involved. In the rat adrenal medulla, we showed previously that although nicotine injections induced the expression of the immediate early gene c-fos, increased the phosphorylation of cAMP response element-binding protein, and elevated mRNA levels for several catecholamine biosynthetic enzymes, a constant infusion of nicotine for 1 to 27 days did not elicit any of these effects (Hiremagalur and Sabban, 1995).

The present study aimed to clarify the confusing relationship between nicotine and stress. We compared the effect of nicotine on the expression of the immediate early gene c-fos, increased the phosphorylation of cAMP response element-binding protein, and elevated mRNA levels for several catecholamine biosynthetic enzymes in adrenal medulla and in the major catecholaminergic neurons of the CNS. We further examined whether nicotine infusion altered the stress-evoked activation of the hypothalamic-pituitary-adrenal axis and the subsequent elevation of mRNAs for catecholamine biosynthetic enzymes in these different locations.

**Materials and Methods**

**Animals, stress, drug treatment, and doses.** The Animal Care and Use Committee approved all animal experiments. Adult, pathogen-free, male Sprague-Dawley rats (250–300 g) were purchased from Taconic Farms (Germantown, NY) and housed four per cage. Animals were maintained under controlled conditions of a 12 h light/dark cycle at 23 ± 2°C and given food and water ad libitum.

Nicotine was administered by two different methods: injections, which deliver a large but short-lived bolus dose of nicotine, or by continual infusion with an osmotic pump, which delivers a controlled dosage over a longer time period. The (-)-nicotine-d3-tartrate (Research Biochemicals Inc., Natick, MA) was freshly dissolved in saline. For injections, nicotine-d3-tartrate (1 or 5 mg/kg b.w.t.) or the vehicle (saline) was administered to the rats as five s.c. injections in the nape of the neck every 12 h. Although on the high side, the dose of nicotine used in this study parallels those previously reported to increase the expression of catecholamine biosynthetic enzymes in rat adrenal medulla (Slotkin and Seidler, 1976; Fossom et al., 1991; Hoftle et al., 1991; Hiremagalur and Sabban, 1995; Jahng et al., 1997). The rats were then euthanized by decapitation 3 h after the last injection. For delivery by infusion, osmotic pumps (model 2002; Azlet, Palo Alto, CA) were implanted s.c. below the neck fold under anesthesia with 50 mg/kg phenobarbital to deliver nicotine-d3-tartrate (15 mg/kg b.wt./day) or a comparable volume of saline for 7 or 12 days. In some experiments, rats with the osmotic pumps were then subjected to immobilization stress (IMO) for 2 h/day on 1 or 2 consecutive days as described previously in detail (Nankova et al., 1994; Kvetnansky et al., 1996). The immobilizations were carried out between 8 AM and 1 PM. All groups were composed of six to eight animals each.

Rats were euthanized 3 h after the last injection or immediately after the last episode of stress. Blood was collected into EDTA-containing tubes on ice and centrifuged 20 min at 4000g. The resulting plasma was kept at −70°C for the determination of corticosterone levels. The adrenal medulla and a number of catecholaminergic brain regions were immediately dissected and separately frozen in liquid nitrogen. Dissection of the brain was performed by using a tissue slicer with digital micrometer (Stoelting, Wood Dale, IL). Frontal sections 9.2 to 10.4 or 4.8 to 5.5 mm from the bregma were taken for LC, ventral tegmental area (VTA), and substantia nigra (SN) sampling, respectively, and chilled in ice-cold saline. The regions of the LC, and VTA were isolated by taking bilateral punches with a 15-gauge needle.

**Isolation of RNA and Northern blots.** The relative levels of mRNAs for TH, dopamine β-hydroxylase (DBH), phenylethanolamine-N-methyltransferase (PNMT), or NPY were determined by performing Northern blot analyses. The adrenal medulla or brain punches from each separate animal were homogenized in RNAzol (Tel-Test, Friendswood, TX). The total amount of 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, DBH, PNMT, and NPY cDNA probes or with a probe for 18S rRNA (Nankova et al., 1994; Serova et al., 1999). The probes were labeled with [35S]dCTP (6000 Ci/mmol; New England Nuclear) by using the random primer method (Megaprime; Amersham, Arlington Heights, IL) and purified on Nuc Trap column (Stratagene, La Jolla, CA). Hybridization was performed at 42°C in a solution containing 5× standard saline citrate phosphate/EDTA (0.15 M NaCl, 10 mM Na2HPO4, and 1 mM EDTA), 50% formamide, 5× Denhardt’s solution, and 1% SDS, and 10 6 dpm of 35S-labeled probes. The blots were stripped in a boiling solution of 10 mM Tris-HCl (pH 8), 1 mM EDTA, and 1% SDS, and then rebiohybridized with subsequent cDNA probes. The hybridizations with the DNA probes and the washing of the filters were done as described previously (Nankova et al., 1994; Kvetnansky et al., 1996). Following exposure to X-ray film (Kodak, Rochester, NY) within the linear range of the signal, autoradiograms were scanned, analyzed by using the Image-Pro-Analysis software (Media Cybernetics, Silver Springs, MD) and normalized to 18S rRNA.

**Determination of Plasma Corticosterone Concentrations.** Plasma was freshly prepared and kept at −70°C until assayed. Corticosterone levels were determined by using the Corticosterone 125I RIA kit (ICN, Costa Mesa, CA) according to the manufacturer’s instructions. The standard curve was determined by using corticosterone concentrations that ranged from 25 to 1000 ng/mL. The interassay coefficients of variation were less than 5%. Plasma from saline- or nicotine-treated rats was diluted 1:200, 0.2 ml 125I-labeled corticosterone and 0.2 ml corticosterone antiserum were added, and the tubes were incubated at room temperature for 2 h. After centrifugation and precipitation, the 125I-labeled precipitates were analyzed by using the 125I RIA kit (ICN, Costa Mesa, CA) according to the manufacturer’s instructions. The standard curve was determined by using corticosterone concentrations that ranged from 25 to 1000 ng/mL. The interassay coefficients of variation were less than 5%.
lyzed by gamma scintillation counting and compared with the standard curve for quantification.

**Immunoblot for c-Fos.** Adrenal medullae punched from three to four adrenal glands were pooled and homogenized in SDS buffer (Tris, 50 mM, pH 7.4, 1% SDS, 2% mercaptoethanol, and 0.1% sodium vanadate) and boiled immediately for 5 min. From these samples, proteins (100 μg/well) were resolved on a 10% SDS polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). After transfer, the membrane was blocked for 2 to 4 h with blocking buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5% BSA, and 0.05% Tween 20). Subsequently, the membrane was incubated for 5 to 16 h at 4°C with rabbit polyclonal IgG (Santa Cruz Biotechnology, Santa Cruz, CA) directed against rat c-Fos, diluted 1:3000 in blocking buffer. The membrane was then washed three times for 5 min each, with Tris buffered saline with Tween 20. The membrane was then incubated for 1 to 2 h with anti-rabbit IgG conjugated to horseradish peroxidase (Promega, Madison, WI). This antibody was diluted 1:40,000 in blocking buffer. The membrane was then washed with Tris buffered saline with Tween 20 (10 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween 20). The membrane was then incubated for 1 to 2 h with a supersignal antibody directed against rat IgG (Promega, Madison, WI) according to the manufacturer’s instruction. The membrane was then exposed to X-ray film (Kodak) for 1 to 5 min visualization.

**Statistical Analyses.** Data are expressed as mean ± S.E. 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. A level of P < .05 was accepted as statistically significant.

### Results

#### Changes in Plasma Corticosterone Levels by Nicotine

The effect of the two different modes of nicotine administration on plasma corticosterone levels was examined. Nicotine injections but not infusions were found to increase plasma corticosterone levels (Fig. 1A). Injections of nicotine significantly raised plasma corticosterone levels relative to saline injections. However, saline injections raised plasma corticosterone to levels approximately 5-fold beyond those observed with saline infusions. Strikingly, the infusion of nicotine for 12 days did not raise plasma corticosterone levels above those observed in the vehicle-infused animals.

When the animals treated with nicotine infusion were subjected to a single immobilization (IMO) stress, the rise in corticosterone tended to be lower (P < .08) than that in saline-infused rats. Exposure to two daily immobilizations raised plasma corticosterone levels to the same degree in the nicotine- or saline-infused rats (Fig. 1B).

**Effect of Nicotine Injection on Catecholamine Biosynthetic Enzymes and NPY Gene Expression**

Injections of 5 mg/kg nicotine bitartrate were previously shown to elevate rat adrenomedullary TH, DBH, and NPY mRNA levels (Hiremagalur and Sabban, 1995). Therefore, we examined whether this relatively high concentration is needed for maximal effect and if PNMT mRNA levels are also elevated by these amounts of injected nicotine. To study this event, rats were injected with 1 or 5 mg/kg nicotine or the vehicle five times, at 12-h intervals, and the levels of RNA from each individual animal were analyzed by performing Northern blots (Fig. 2). Although 1 mg/kg nicotine significantly induced TH, DBH, and NPY mRNA levels, this dose was not sufficient to elicit a maximal increase. However, treating the animals with 5 mg/kg further elevated the mRNA levels for all genes of interest. Interestingly, significant elevations of PNMT mRNA were only observed at the higher dose of nicotine.

Nicotine injections also increased TH mRNA levels in the major catecholaminergic cell bodies in the brainstem (Fig. 3). In the brain, TH mRNA was even more sensitive to injections of nicotine than was observed in the adrenal medulla. The injection of nicotine at 1 mg/kg was similar in effect to 5 mg/kg, raising TH mRNA levels in the LC, SN, and VTA to values above 200% of those in the control group.

**Nicotine Infusion Modulates Stress-Triggered Changes in Gene Expression in Adrenal Medulla**

In contrast to injections, our earlier study found that delivery of nicotine by infusion failed to elevate adrenomedul-
lary TH, DBH, and NPY mRNA levels (Hiremagalur and Sabban, 1995). In light of these findings, we examined whether the infusion of nicotine would alter the stress-triggered activation of their gene expression. Rats receiving a constant infusion of saline or nicotine then were subjected to a 2-h IMO on 1 or 2 consecutive days. Nicotine infusion by minipump did not alter the steady-state level of mRNAs for the above genes or for PNMT (Fig. 4). When the rats with the

Fig. 2. Changes in mRNA levels for adrenomedulary catecholamine biosynthetic enzymes and NPY with nicotine injection. Representative Northern blots and summary data (mean ± S.E.) are shown for TH, DBH, PNMT, and NPY mRNA levels. Rats were injected five times at 12-h intervals with 1 or 5 mg/kg nicotine or saline (0). Total RNA was isolated from the adrenal medullae of each animal 3 h after the last injection and was analyzed by Northern blot analysis. Results are expressed relative to values for 18S rRNA. Data are expressed as mean ± S.E. (n = six to eight rats/group). *P < .05; **P < .01 versus saline.

Fig. 3. Effect of nicotine injections on TH mRNA levels in LC, SN, and VTA. Representative Northern blots and summary data (mean ± S.E.) are shown for TH mRNA levels. Rats were injected five times at 12-h intervals with 1 or 5 mg/kg nicotine or saline (0). The total mRNA from brain tissue punches obtained from individual rats was analyzed separately by Northern blot. Data are expressed as mean ± S.E. (n = six to eight rats/group). *P < .05 versus saline.
implanted nicotine minipump were exposed to a single IMO, this stress response in the adrenal medulla was markedly attenuated compared with that in the saline-infused group. There was a marked diminution in the stress-elicited elevation of TH, DBH, and PNMT mRNA levels (Fig. 4). However, unlike the catecholamine biosynthetic enzymes, in the same tissue, the induction of NPY mRNA beyond baseline levels was equally high in the nicotine- and saline-infused rats (Fig. 4D).

Although nicotine infusion was found to reduce the stress response of adrenomedullary TH, DBH, and PNMT mRNAs caused by a single IMO, the second IMO overcame this inhibition. Two daily IMOs induced each of the genes to a similar extent in the presence or absence of nicotine.

The stress-elicited rise in TH mRNA is correlated with induction of c-Fos and heightened activator protein-1 binding to the TH promoter (Nankova et al., 1994; Sabban et al., 1995). Therefore, we also examined whether nicotine infu-
sion would alter the IMO-stimulated induction of c-fos. The changes in c-Fos protein levels were determined by performing immunoblotting. As shown in Fig. 5, after a single IMO, levels of the 62-kDa c-Fos protein were markedly elevated in the adrenal medullae of saline-infused rats. The infusion of nicotine did not induce c-Fos protein, as observed previously (Hiremagalur and Sabban, 1995). However, it did diminish the IMO stress-evoked induction of c-fos.

**Effect of Nicotine Infusion and Stress on Gene Expression of Catecholamine Biosynthetic Enzymes in CNS**

**LC.** In striking contrast to the lack of an effect of nicotine infusion on gene expression in the adrenal medulla, in the LC, infusion of nicotine elicited about a 2- to 3-fold increase in TH mRNA levels compared with rats infused with saline. This elevation in TH mRNA levels is as large as that observed in response to IMO stress (Fig. 6A). When the animals receiving nicotine infusions were subjected to IMO stress, there was no further elevation in TH mRNA levels by either single or repeated stress.

DBH mRNA in the LC responded differently than did TH. In this instance, similar to the effects in the adrenal medulla, nicotine infusion alone did not significantly alter DBH mRNA levels. Importantly, nicotine infusion ablated the response to a single IMO but not to the twice repeated IMO (Fig. 6B).

**SN.** In the substantia nigra, nicotine infusion failed to modulate the effect of IMO stress on TH mRNA levels. Thus, the rise in TH mRNA by single IMO or twice repeated IMO stress was similar in animals regardless of saline or nicotine infusions (Fig. 7A).

**VTA.** The TH mRNA levels in the dopaminergic cell bodies of the mesolimbic system in the VTA were not altered by nicotine infusion. IMO stress elevated TH mRNA amounts in this brain region only when it was repeated. Thus, a single IMO had no effect on TH mRNA levels, either in saline- or nicotine-infused animals. However, two daily IMO stresses led to a small (about 50%), but significant, rise in TH mRNA levels ($P < .01$). The nicotine infusion abolished this stress response (Fig. 7B).

**Discussion**

**Nicotine Infusion and Stress Response.** One of the major findings of this study is that nicotine infusion can modulate or attenuate some of the physiological effects of stress. In rat adrenal medulla, nicotine infusion reduced the IMO stress-triggered induction of c-Fos protein in the mRNA levels for catecholamine biosynthetic enzymes. However, in the CNS, the effect of nicotine on the stress response was more complex: nicotine prevented the induction of TH mRNA by repeated IMO stress in the VTA and of DBH mRNA by a single IMO, in the locus ceruleus. Summary of changes in mRNA levels for genes of interest are shown in Table 1.

Our findings suggest that the reported calming effects of nicotine may indeed be a physiological response. Similar to our findings, Acri (1994) reported that nicotine infusion attenuated the stress response. With conditions similar to those in the present study, unstressed and restrained rats displayed similar acoustic startle reflexes. Hence, nicotine administration, or smoking, may ameliorate the stress response by decreasing the stress-triggered activation of the sympathoadrenal system. However, this conclusion conflicts with data by Morse (1989) who found that the administration of nicotine actually enhanced the restraint stress-stimulated increase in plasma corticosterone or epinephrine levels. These discrepancies may result from using a different dose and mode of nicotine administration. In Morse's experiments, nicotine was administered in a pulsatile fashion in dose $\leq 0.1$ mg/kg, which may be insufficient to affect the response to restraint stress.

Interestingly, the ability of nicotine infusion to attenuate the stress response in the periphery was only evident with a single IMO stress. Subjection of the rats to a second immobilization overcame this inhibitory effect. These findings are consistent with smoking being a mild stress-reducing agent.

In contrast to the attenuation of the stress-elicted induction of mRNAs for the adrenomedullary catecholamine biosynthetic enzymes, nicotine infusion did not alter NPY mRNA in the same animals. This finding argues against a nonspecific effect of nicotine infusion on gene expression. The mechanism by which the infusion of nicotine alters the adrenomedullary response for catecholamine biosynthetic enzymes to stress remains to be determined. Based on previous findings, we postulate that the response is likely indirect and does not simply involve the desensitization of the nicotinic receptors on the chromaffin cells. Splanchnic nerve section, or the administration of chlorisondamine, did not prevent the induction of adrenomedullary TH mRNA by single IMO stress, although elevation of NPY mRNA was blocked (Hiremagalur et al., 1994; Nankova et al., 1994; Kvetnansky et al., 1996). Interestingly, nicotine infusion is one of the only treatments to inhibit the elevation of TH gene expression by IMO stress.

IMO stress triggers increased c-Fos immunoreactivity and elevated c-Fos/c-Jun binding to an activator protein-1-like site on the TH promoter (Nankova et al., 1994; Sabban et al., 1995). In this study, the nicotine infusion was found to block the IMO-elicted induction of c-fos, supporting the involvement of c-fos in the stress-elicted activation of gene expression for the adrenal catecholamine biosynthetic enzymes. However, the finding that repeated IMO increased TH and PNMT mRNA levels in c-fos-deficient mice indicates that the situation is more complex (Serova et al., 1998).

It is important to note that, although nicotine-infused rats exhibited an average reduction in TH and PNMT mRNA levels of more than 50% with stress, there was considerable variation among animals. In some of the rats in a given experiment, nicotine completely ablated the induction of TH and PNMT gene expression normally seen with stress, whereas in other animals in the cohort, it exhibited no inhi-
bition at all. Although we do not currently know the source of the variations in these Sprague-Dawley rats, an outbred strain, it probably is important. For example, inbred mouse strains vary in number and sensitivity of brain nicotinic receptors (Marks et al., 1996). In humans, varied responses to nicotine (Benowitz et al., 1982) and the effectiveness of the nicotine patch for smoking cessation also exist.

**Effects of Nicotine on Catecholaminergic Systems in CNS.** The central catecholamine neurons were all sensitive to lower concentrations of injected nicotine to elicit maximal changes in TH mRNA levels. The increase in TH mRNA observed with 1 mg/kg nicotine in the LC is consistent with the previous report that a single injection of 0.8 mg/kg nicotine increased TH activity and mRNA levels in the LC (Smith et al., 1991; Mitchell et al., 1993) but not in dopaminergic cell bodies in the VTA and SN (Clarke et al., 1985). Transient increases in TH activity in SN and VTA were found after 3 days of nicotine injections (Smith et al., 1991). Our data show...
that repeated nicotine injections elevate TH mRNA levels in the VTA and SN, brain centers involved in the pathogenesis of idiopathic Parkinson’s disease or tardive dyskinesia. Thus, increased TH gene expression by nicotine could be associated with the observation that smokers are less likely to develop these conditions (Kirch et al., 1988). Interestingly, there is an inverse dose-response relationship between Parkinson’s disease and smoking (Gorell et al., 1999).

Although the nicotine infusion alone failed to increase mRNA levels for any of the genes of interest in the adrenal medulla and did not increase plasma corticosterone levels, it raised TH mRNA levels in the LC, similar to the levels attained with IMO stress or repeated injections.

Numerous studies have shown that nicotine administration by different modes increases the nicotine receptor density in the CNS in a region specific fashion (Wonnacott, 1990; Marks et al., 1992; Rowell and Li, 1997). These studies argue for agonist-induced receptor up-regulation as a consequence of receptor desensitization. Longer lasting receptor “inactivation”, which might be achieved only at high nicotine concentrations, has been proposed to be involved in the up-regulation of nicotinic receptors in the CNS (Rowell and Li, 1997). An increase in central nicotinic receptors has also been shown to occur in the brains of cigarette smokers (Benwell et al., 1988) implying that high levels of nicotine may “inactivate” these CNS receptors.

Effect of Nicotine Infusion on Stress-Induced Changes in mRNA Levels for Catecholamine Biosynthetic Enzymes in CNS. The effect of nicotine infusion on the IMO-triggered rise in TH and DBH mRNA levels differed in the LC compared with the adrenal medulla. These results indicate that the assessment of peripheral neurologic changes with nicotine administration may not be sufficient when evaluating CNS effects. Although nicotine infusion or IMO stress alone each increased TH mRNA levels in LC, the combined treatment did not display an additional activation. We speculate that the high basal TH mRNA levels, and presumably high norepinephrine levels in terminals with prolonged nicotine infusion and, perhaps with smoking and the absence of an additional rise with stress, may give the impression that smoking is attenuating the anxiety of the stress.

Nicotine infusion prevented the induction of DBH mRNA levels caused by stress in the LC. Although TH is generally considered the rate-limiting enzyme in norepinephrine biosynthesis under some situations, β-hydroxylation can be rate limiting in LC neurons (Scatton et al., 1984). It would be interesting to determine the physiological importance of inhibition of stress-triggered induction of DBH mRNA.

In the SN, the cell bodies of the nigrostriatal system, nicotine did not attenuate the induction of TH mRNA by IMO. In general, TH mRNA levels in the SN are more resistant to regulation than in other locations. A number of studies, including our own (unpublished data), found that restraint stress did not significantly change TH mRNA levels in the SN. However, immobilization was found to consistently elicit about a 2-fold elevation of steady-state TH mRNA levels in the SN.

The sensitivity to stress and to nicotine in the VTA is different from in other catecholaminergic locations. In the VTA cell bodies for the mesolimbic dopaminergic pathway, as we constantly observed (manuscript in preparation), only repeated, not single, IMO elevated TH gene expression in line with the role of these neurons in addictive behavior and adaptation to drug usage. Recently, a reduction in foot shock stress-induced immobility was found after repeated 0.15 mg/kg nicotine injections (George et al., 1998). In the nicotine-pretreated animals, however, the biochemical index of acute cortical dopamine system activation (3,4-dihydroxyphenylacetic acid/dopamine ratio) remained significantly attenuated compared with stressed controls. These results and our finding suggest that nicotine pretreatment can modulate the stress response in the VTA. Further studies remain to determine whether the inhibition persists with more than two daily repeated episodes of IMO.

In summary, our studies reveal that continual infusion of nicotine can attenuate some of the responses to stress. We postulate that infusion of nicotine, such as with the nicotine patch, may also modulate the stress response in humans, and could therefore be a part of its ability to help many individuals overcome the urge to smoke.

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References


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