ELSEVIER

Effects of Repeated Nicotine Administration and Footshock Stress on Rat Mesoprefrontal Dopamine Systems: Evidence for Opioid Mechanisms

Tony P. George, M.D., Christopher D. Verrico, B.S., Li Xu, and Robert H. Roth, Ph.D.

We have examined the effects of nicotine pre-treatment on mesoprefrontal dopamine (DA) function in the presence and absence of acute stress, and the involvement of endogenous opiate peptide systems (EOPS). Acute electrical footshock stress preferentially increases DA utilization in medial prefrontal cortex (mPFC) compared to nucleus accumbens (NAS) and striatal terminal fields, and this is correlated with profound locomotor immobility. Our recent studies have demonstrated that repeated, but not acute, nicotine pre-treatment significantly reduced mPFC DA utilization and footshock stress-induced immobility responses. There is increasing evidence that the biochemical and behavioral effects of nicotine are mediated by EOPS, and we hypothesized that the stress-reducing effects of repeated nicotine administration in these studies were mediated by EOPS. Accordingly, rats pre-treated subcutaneously with repeated nicotine were given a single dose of the opiate receptor antagonist naloxone (0.1–10.0 mg/kg, i.p.) or saline as a co-treatment with nicotine or saline 10 min prior to acute footshock stress. Naloxone had no effects on non-stressed or acute footshock stress-induced mPFC DA utilization, but dose-dependently antagonized repeated nicotine's attenuation of stress-induced mesoprefrontal DA utilization and immobility responses. Furthermore, naloxone dose-dependently blocked repeated nicotine's augmentation of accumbal DA utilization. These results suggest that EOPS may be involved in mediating repeated nicotine administration effects on mesoprefrontal dopaminergic and immobility responses to acute footshock stress.
[Neuropsychopharmacology 23:79–88, 2000] *© 2000 American College of Neuropsychopharmacology. Published by Elsevier Science Inc. All rights reserved*

KEY WORDS: *Nicotine; Dopamine; Prefrontal cortex; Opiates; Naloxone*

Nicotine exerts diverse psychopharmacologic effects and is thought to be the key ingredient which is responsible for the initiation and maintenance of tobacco smoking behaviors (Henningfield et al. 1995). Furthermore, nicotine cessation leads to a well-defined withdrawal syndrome in animals (Malin et al. 1993, 1996a,b; Hildebrand et al. 1997, 1998) and humans (Balfour and Fagerstrom 1996). The mechanisms subserving nicotine's actions appear to be mediated, initially, by nicotinic acetylcholine receptors (nAChRs), which are present in at the neuromuscular junction, autonomic ganglia and in the central nervous system (Boyd 1997). The subsequent steps which lead to nicotine's biobehavioral effects are less clear, but nicotine is known to interact with numerous monoamine pathways including dopamine (DA), serotonin and norepinephrine, as well as glutamatergic, GABAergic and opiatergic path-

From the Division of Substance Abuse (TPG), Department of Psychiatry (TPG, LX, RHR), and Department of Pharmacology (CDV, RHR), Yale University School of Medicine, New Haven, CT.

Address correspondence to: Tony P. George, M.D., Division of Substance Abuse, Department of Psychiatry, Yale University School of Medicine, Rm. S-109, Substance Abuse Center, Connecticut Mental Health Center, 34 Park Street, New Haven, CT 06519.

Received June 14, 1999; revised October 11, 1999; accepted December 6, 1999.

© 2000 American College of Neuropsychopharmacology
Published by Elsevier Science Inc. All rights reserved
655 Avenue of the Americas, New York, NY 10010
0893-133X/00/$–see front matter
PII S0893-133X(99)00165-7

ways (McGehee and Role 1995). These effects are thought to be facilitated by activation and/or desensitization of pre-synaptic nAChRs present on neurons secreting these transmitters (McGehee and Role 1995; Pidoplichko et al. 1997).

Several studies have documented the involvement of endogenous opiate peptide systems (EOPS) in the biobehavioral effects of nicotine (Corrigall et al. 1988; Malin et al. 1993, 1996a,b). Nicotine is known to activate EOP release (Eiden et al. 1984; Davenport et al. 1990; Houdi et al. 1991). In rats chronically pre-treated with nicotine, the opioid receptor antagonists naloxone (Malin et al. 1993) and neuropeptide FF (Malin et al. 1996b) can precipitate nicotine abstinence symptoms and nicotine rechallenge can attenuate nicotine abstinence symptoms (Malin et al. 1996a). Nicotine can attenuate naloxone-precipitated jumping behavior in morphine-dependent mice (Zarrindast and Farzin 1996). Furthermore, nicotine has potent anti-nociceptive properties (Christensen and Smith 1990) which can be antagonized by naloxone (Aceto et al. 1993). Finally, in human smokers, β-endorphin levels positively correlate with plasma nicotine levels (Pomerleau et al. 1983) and naloxone may decrease smoking (Karras and Kane 1980; Gorelick et al. 1988), particularly in combination with the nicotine patch (O'Malley et al. 1997), though other studies (Nemeth-Coslett and Griffiths 1986; Sutherland et al. 1995) have found no effect of opioid antagonists on smoking consumption. The study by Sutherland et al. (1995) suggests that naltrexone administration can produce a clinical syndrome similar to nicotine withdrawal in dependent smokers. In support of this observation, in a recent human laboratory study, intravenous naloxone challenge was found to significantly increase "opioid-like" withdrawal symptoms compared to saline infusion in nicotine-dependent tobacco smokers (Krishnan-Sarin et al. 1999). Accordingly, these lines of evidence strongly implicate EOPS in the biochemical and behavioral effects of nicotine.

The DA neurons originating in the ventral tegmental area (A10) in the midbrain and projecting to the prefrontal cortex are known to have unique functional characteristics, including higher turnover rates compared to mesolimbic and nigrostriatal DA neurons, the presence of enhanced burst firing, and high responsivity to mild stressors (Thierry et al. 1976). This may be due to reduced autoreceptor regulation at A10 terminal projections and by complex afferent modulation (Horger and Roth 1996).

Studies by our group (George et al. 1998) and others (Vezina et al. 1992; Nisell et al. 1996) have demonstrated that systemic nicotine administration leads to complex acute and repeated effects on central DA systems. In our recent studies (George et al. 1998), acute administration of nicotine (0.15 mg/kg) produces activation of dopamine neuron terminal fields (assessed by tissue DA turnover) in the nucleus accumbens (NAS) and medial prefrontal cortex (mPFC). However, this is dependent on dose and is region-specific, as its has been shown that higher doses (0.60 mg/kg) produce no acute effects in the mPFC. Desensitization of nAChRs in the mPFC at higher nicotine doses may explain these effects (George et al. 1998). Similarly, repeated nicotine administration produces differential effects on DA systems, with mesolimbic projections (NAS) showing sensitization with enhanced DA turnover, and mesocortical projections showing tolerance to acute nicotine effects.

Further, we have shown that repeated, but not acute, nicotine pre-treatment reduces the responsivity of these mesoprefrontal projections to acute electrical footshock (physical) stress and this is correlated with reductions in stress-induced immobility responses (George et al. 1998). However, with conditioned fear (psychological) stress, nicotine pre-exposure reduces the biochemical activation of mPFC DA systems, but not the immobility response to conditioned fear stress (George et al. 1997). This suggests that nicotine differentially effects the expression of the immobility response to physical and psychological stressors.

A number of studies have documented that enkephalin-containing projections innervate the VTA (Finley et al. 1981; Khachaturian et al. 1983) and synapse on dopaminergic neurons (Sesack and Pickel 1992), and it has been shown that local infusion of μ-opioid receptor agonists into the VTA leads to increased DA release in the NAS (Spanagel et al. 1992; Devine et al. 1993). However, opioid regulation of the mesoprefrontal DA projections is unclear.

In the present study, we tested the hypothesis that modulation of mesoprefrontal dopaminergic and behavioral responses to acute footshock stress by repeated nicotine administration is mediated by EOPS with the use of the μ-opioid receptor antagonist naloxone.

MATERIALS AND METHODS

Materials

A total of 80 male Sprague-Dawley rats initially weighing 250–275 g were obtained from CAMM (Rutgers, NJ). The final weight of rats prior to sacrifice on Day 5 was 300–325 g. S-(-)nicotine bitartarate and naloxone hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest grade commercially available.

Methods

The protocol used has been described in a previous study (George et al. 1998). Briefly, animals were housed in pairs in plexiglass cages, and maintained in an animal room with *ad libitum* availability of food. Lights were on at 0700 h and off at 1900 h. All handling of rats

and experiments were carried out between 0900 h and 1700 h. Rats were given daily injections for five days with either saline (1 cc/kg) or (-)nicotine bitartarate (0.15 mg/kg; expressed as the freebase). Naloxone hydrochloride (NLX, 0.1–10.0 mg/kg) was given intraperitoneally as a co-treatment with the challenge injections (10 min prior to footshock sessions). All injection solutions were freshly prepared on a daily basis, and the pH of the solutions was adjusted to approximately pH 7.4 with NaOH.

On Day 5, rats were given a final injection of saline and nicotine (co-administered with naloxone 10 min prior to footshock; 30 min prior to sacrifice), and then placed (up to two per session) into paired 12″ (L) × 10″ (W) × 12″ (H) cm sound-attenuating chambers illuminated with red-light, with standard grid floors connected to a shock generator (BRS/LVE Division of Tech Serv Inc., Beltsville, MD) and a pulse stimulator (Grass Medical Instruments, Quincy, MA). Background noise was masked using white noise broadcast in the chambers. Footshocks were delivered (0.8 mA shocks of 160 ms duration, according to Davis and Astrachan (1978)) every 10 seconds for 20 minutes . Rats received either the footshock paradigm or no shocks. The shock chambers were cleaned with 70% ethanol between rat pairs to remove olfactory cues (i.e., urine, feces).

At the conclusion of the footshock period (30 min after final injections), rats were sacrificed by decapitation, and brains were rapidly removed from the calvarium, placed in saline chilled on ice, and dissected into coronal slices with a brain mold on a refrigerated stage (FTS Systems Inc., Stone Ridge, NY). Samples from mPFC (+2.7–1.2 mm from bregma) were harvested by block dissection, and samples of NAS (+1.2–0.2 mm, including core and shell) and dorsolateral striatum (+0.2 to −0.8 mm) were obtained by tissue punch (Paxinos and Watson 1986). All samples were stored frozen at −70°C until prepared for extraction.

All footshock sessions were recorded by videotaping. The percentage of time spent immobile with only observable respirations for each one minute interval during the 20 min of the footshock procedure (% immobility) was scored by blinded examiners who manually rated the taped sessions post-hoc (TPG, CDV). There was excellent interrater reliability ($\kappa = 0.80$) in the scoring of immobility (George et al. 1998).

Dopamine (DA) and its major metabolite, dihydroxy-O-phenylacetic acid (DOPAC), were quantitated in rat brain using high performance liquid chromatography with electrochemical detection (HPLC-ED) using a glassy carbon electrode set at +0.7 V and an Ag/AgCl reference electrode. The procedure involved alumina extraction prior to HPLC analysis as described previously (Elsworth et al. 1996). A reverse phase 3 μ C18 HPLC column (Ranin Instruments, Woburn, MA) was utilized. The mobile phase, delivered at 0.65 ml/min, was comprised of sodium citrate (30 mM), sodium dihydrogen phosphate (14 mM), sodium octanesulphonate (2.3 mM), EDTA (0.025 mM), acetonitrile (6.5%), tetrahydrofuran (0.6%), and diethylamine (0.1%), adjusted to pH 3.1 with concentrated phosphoric acid.

Dihydroxybenzamine was used as an internal standard. Results are expressed as the ratio of DOPAC to DA, with tissue levels calculated in ng/mg protein. Protein determination was done using the method of Lowry et al. (1957) with bovine serum albumin as standard.

Comparisons between groups across dose-response and immobility acquisition studies were done with repeated measures ANOVA, while one- or two-factor ANOVA were used for other comparisons (Super ANOVA; Abacus Concepts, Berkeley, CA). Post-hoc comparisons were done with Fisher's least significant differences procedure; differences were considered significant when $p < .05$.

RESULTS

Effects of Naloxone on DA Utilization in Saline and Nicotine Pre-Treated Unstressed Rats

The effects of naloxone (NLX) co-treatment on DA utilization in mPFC, NAS, and dorsolateral striatum are presented in Table 1.

Medial Prefrontal Cortex. In mPFC, there were no effects of nicotine pre-treatment ($F = 0.03$, df = 1,24, $p = .87$) or naloxone co-treatment ($F = 1.32$, df = 1,24, $p = .26$), but nearly significant effects of nicotine challenge ($F = 3.53$, df = 1,24, $p = .07$). In rats repeatedly treated with saline (1 cc/kg), NLX (1.0 mg/kg, i.p.) did not alter basal DA utilization in the mPFC. While mesoprefrontal DA metabolism was significantly increased by acute nicotine challenge ($p < .05$, post-hoc comparison), this was not significantly altered by NLX co-treatment. NLX co-treatment did not significantly alter the effects of repeated nicotine on mPFC DA utilization.

Nucleus Accumbens. In NAS, there were significant effects of nicotine pre-treatment ($F = 21.2$, df = 1,24, $p < .01$), nicotine challenge ($F = 39.5$, df = 1,24, $p < .01$), and a nearly significant effect of naloxone co-treatment ($F = 3.42$, df = 1,24, $p = .08$). NLX pre-treatment did not alter accumbal DA utilization in saline pre-exposed rats. Acute nicotine significantly increased mesoaccumbal DA utilization ($p < .05$), an effect which was not altered by NLX co-treatment. Repeated nicotine, however, produced a slight, but significant ($p < .05$) augmentation of accumbal DA utilization, which was attenuated by NLX co-treatment.

Dorsolateral Striatum. There were no significant effects of acute or repeated nicotine on dorsolateral striatum DA utilization, and NLX co-treatment did not

Table 1. Effects of Naloxone on Regional DA Utilization in Saline and Nicotine Pre-Treated Non-Stressed Rats

	Dopamine Utilization (% SAL/SAL Control)	
	Saline (1 cc/kg)	Naloxone (1 mg/kg)
Medial Prefrontal Cortex		
SAL/SAL	100.00 ± 4.19	95.03 ± 19.54
SAL/NIC	134.53 ± 13.66[a]	112.62 ± 21.27
NIC/NIC	113.49 ± 8.41	108.07 ± 21.60
NIC/SAL	110.25 ± 6.98	112.07 ± 10.45
Nucleus Accumbens		
SAL/SAL	100.00 ± 7.13	91.87 ± 7.15
SAL/NIC	150.00 ± 24.41[a]	134.26 ± 14.27
NIC/NIC	111.79 ± 5.34[a,b]	97.55 ± 4.85[c]
NIC/SAL	90.25 ± 6.98	92.07 ± 10.45
Dorsolateral Striatum		
SAL/SAL	100.00 ± 9.30	106.57 ± 8.76
SAL/NIC	120.29 ± 19.01	124.00 ± 14.33
NIC/NIC	103.52 ± 3.90	110.40 ± 8.34
NIC/SAL	88.26 ± 4.92	84.85 ± 7.03

Data are presented as a percentage (± SD) of saline-treated controls. The first condition under treatment groups represents the four-day pre-treatment, the second condition is the challenge type.

Tissue levels for dopamine in each region were as follows: mPFC, 0.890 ± 0.138; NAS, 139.15 ± 13.32; Striatum, 199.17 ± 13.90 ng/mg protein.

$N = 8$ animals for each treatment group.

[a] $p < 0.05$ vs. SAL/SAL control.

[b] $p < 0.05$ vs. SAL/NIC control.

[c] $p < 0.05$ vs. NIC/NIC control.

modify DA utilization in dorsolateral striatum in either saline or nicotine pre-treated rats.

There was a slight, but non-significant reduction in DA utilization in NAS and striatum in repeated nicotine pre-treated rats challenged with saline (NIC/SAL), and this nicotine withdrawal effect was not modified by NLX co-treatment.

Effects of Nicotine Pre-treatment on Acute Footshock Stress-Induced Mesoprefrontal DA Utilization and Immobility Responses: Blockade by Naloxone

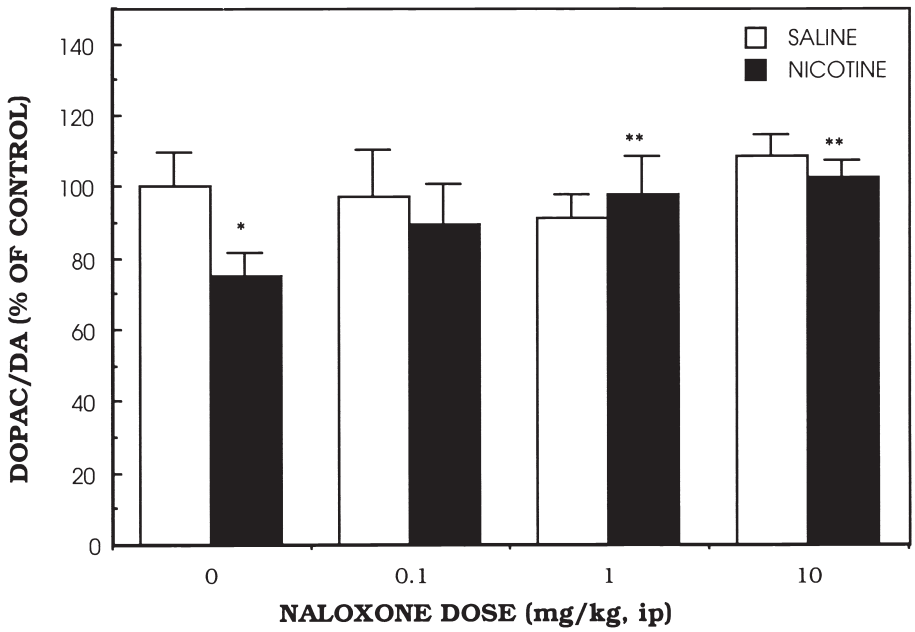
Medial Prefrontal Cortex. NLX administration (0.1–10.0 mg/kg) alone did not affect stress-induced mPFC DA utilization (Figure 1). Repeated measures ANOVA revealed nearly significant effects of nicotine pre-treatment ($F = 4.08$, df = 1,30, $p = .07$), and there was a significant reduction of stress-induced mPFC DA activity in repeated nicotine pre-treated rats compared to saline pre-treated controls ($p < .01$). There was a significant effect of NLX dose ($F = 6.43$, df = 3,30, $p < .01$) and a significant pre-treatment × dose interaction ($F = 4.75$, df = 3,30, $p < .01$). NLX dose-dependently blocked the reduction in stress-induced mesoprefrontal DA activity produced by nicotine pre-treatment, with a return to the stress control levels at NLX doses of 1.0–10.0 mg/kg (Figure 1).

Nucleus Accumbens and Dorsolateral Striatum. The effects of NLX (0.1–10.0 mg/kg) on the effects of repeated saline or nicotine pre-treatment (0.15 mg/kg, s.c.) on stress-induced DA utilization in NAS and striatum are shown in Figure 2. In NAS (Figure 2A), there was no effect of NLX alone on DA utilization. There were significant effects of nicotine pre-treatment ($F = 7.95$, df = 1,39, $p < .01$), and repeated nicotine produced an augmentation of DA utilization, which was dose-dependently blocked by NLX, particularly at the 1.0 and 10.0 mg/kg doses. In striatum (Figure 2B), there were no effects of repeated nicotine or NLX on DA utilization in the dorsolateral striatum, and no significant nicotine pre-treatment × NLX interactions ($F = 0.83$, df = 1,38, $p = .37$).

Acquisition of Immobility Responses. The acquisition curve for acute footshock stress-induced immobility responses over the 20-min footshock session is presented in Figure 3. For immobility responses to acute footshock stress, repeated measures ANOVA revealed significant effects of nicotine pre-treatment ($F = 18.9$, df = 3,120, $p < .01$), time ($F = 50.2$, df = 6,120, $p < .01$) and pre-treatment × time interactions ($F = 4.7$, df = 18,120, $p < .01$).

Saline pre-treated rats rapidly acquired immobility responses, and this was not significantly modified by NLX co-treatment (1.0 mg/kg), indicating that NLX by itself did not modify immobility thresholds in these animals. Repeated nicotine pre-treatment reduced immobility responses to footshock stress, and post-hoc analysis demonstrated differences from stressed controls at the 1-, 10-, and 15-min time points ($p < .05$). NLX co-treatment (1 mg/kg, i.p.) blocked the reduction by repeated nicotine of footshock-induced immobility responses. Similar results were obtained at the 10.0 mg/kg NLX dose (data not shown).

Effects of Repeated Nicotine and Naloxone on Immobility Responses at 1 minute. The effects of NLX co-treatment on immobility responses at 1 min, where differences between saline and nicotine pre-treated rats was greatest, is presented in Figure 4. NLX administration (0.1–10.0 mg/kg) did not affect stress-induced immobility responses at 1 min. Repeated measures ANOVA revealed significant effects of nicotine pre-treatment ($F = 30.41$, df = 1,30, $p < .01$), and there was a significant reduction of the 1 min immobility response in nicotine pre-treated controls ($p < .01$; post-hoc comparison). There was a significant effect of NLX dose ($F = 24.39$, df = 3,30, $p < .01$) and a significant pre-treatment × dose interaction ($F = 8.63$, df = 3,30, $p < .01$). NLX co-treatment dose-dependently blocked the reduction in stress-induced immobility responses at 1 min produced by nicotine pre-treatment (Figure 4).



* p<0.05 vs. saline control
** p<0.05 vs. nicotine control

Figure 1. Effects of naloxone on repeated nicotine modulation of stress-induced mesoprefrontal dopamine utilization. The effects of repeated saline and nicotine pre-treatment on mPFC dopaminergic responses to acute footshock stress are presented (n = 6 animals for each treatment group).

Relationship between Immobility Responses and Stress-Induced Mesoprefrontal DA Utilization: Effects of Naloxone Co-Treatment

The relationship between stress-induced immobility responses at 1 min and mesoprefrontal DA utilization and the effects of repeated nicotine and NLX co-treatment is depicted in Figure 5. In general, repeated nicotine pre-treatment (NIC/SAL) appeared to produce a parallel reduction in both immobility responses and mPFC DA activity compared to saline controls (SAL/SAL). NLX co-treatment (1 mg/kg) appeared to block the reduction by repeated nicotine (NIC/NLX) of both immobility and cortical dopaminergic responses to footshock stress, with a preferential effect on immobility, while having no effect on mesoprefrontal DA and immobility responses by itself (SAL/NLX). The correlation coefficient in this sample was modest ($F = 9.21$, df = 1,26, $p < .01$; $r = 0.544$), consistent with our previous findings (George et al. 1998).

DISCUSSION

Effects of Naloxone on Repeated Nicotine's Modulation of Dopaminergic and Behavioral Responses to Acute Footshock (Physical) Stress

Our previous studies have suggested that nicotine has dose-dependent effects on footshock stress-induced mesoprefrontal DA responsivity and immobility responses (George et al. 1998). In the present study, we found that naloxone (co-administered with saline or nicotine challenge) prior to acute footshock stress dose-dependently blocked repeated nicotine's reduction of footshock stress-induced mesoprefrontal DA utilization and immobility responses. In contrast, naloxone had no effects on mesoprefrontal DA and immobility responses to acute stress by itself. This suggests a role for the EOPS in mediating repeated nicotine's effects on cortical dopaminergic and behavioral responses to acute footshock stress. In addition, naloxone by itself did not modify DA utilization in mPFC, NAS or striatum under non-stressed conditions and did not block acute nicotine stimulation of DA utilization in mPFC and NAS.

Previous results suggest that naloxone does not alter footshock stress-induced mesoprefrontal DA utilization (Tam 1988), though one study reported that naloxone pre-exposure (0.4 mg/kg, i.p.) reduced stress-induced mPFC DA metabolism (Miller et al. 1984). Methodological differences between these studies may explain the discrepancy. Kalivas and Abhold (1987) found that the μ-opioid antagonist, naltrexone methobromide, microinjected into the VTA blocked footshock stress-induced DA metabolism in the mPFC and NAS, suggesting that stress potentiates mesoprefrontal DA metabolism through enkephalin release in the VTA. Thus, the interactions between nicotinic and EOPS, presumably at the

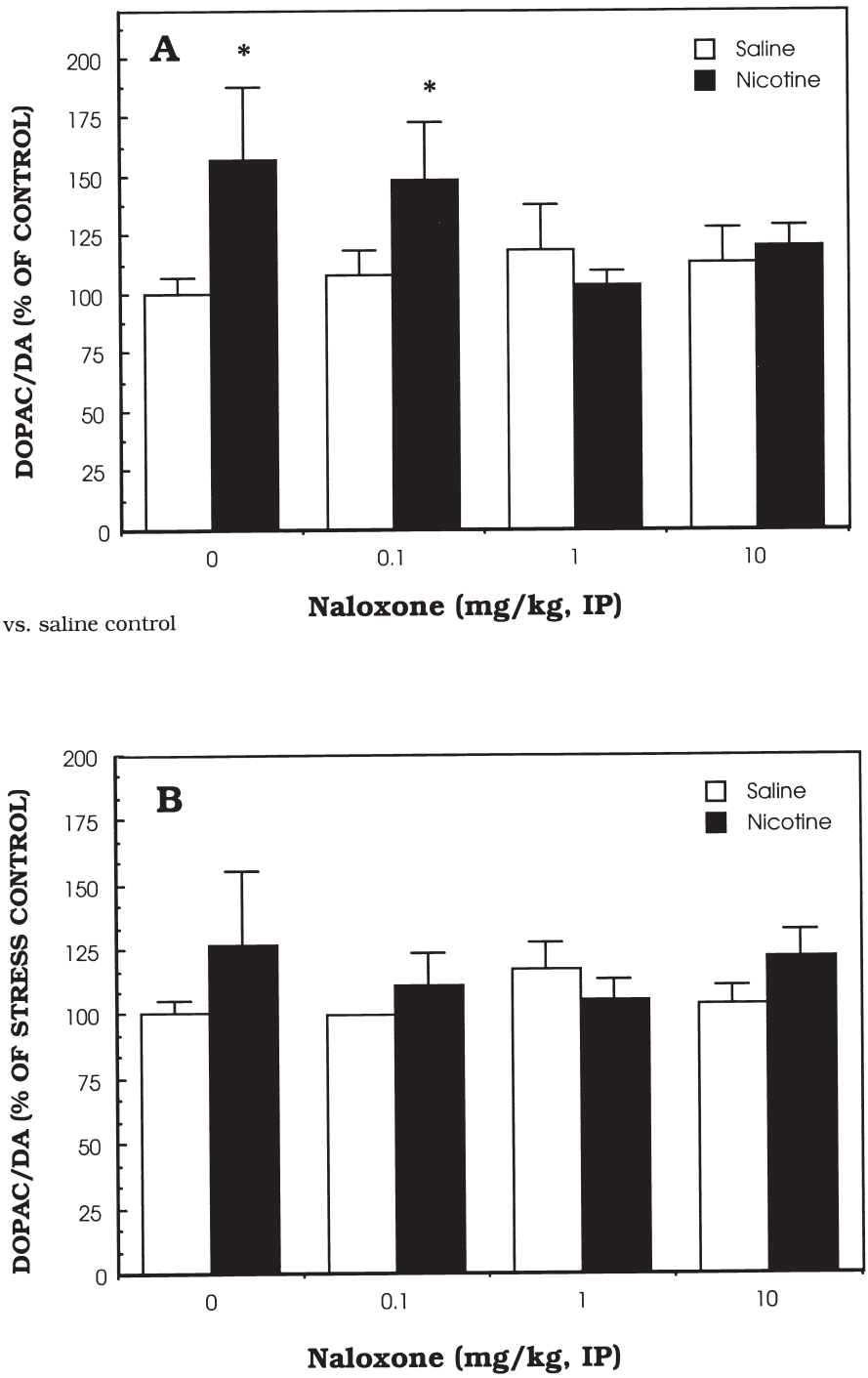


Figure 2. Effects of naloxone on repeated nicotine modulation of stress-induced nucleus accumbens and striatal dopamine utilization. Effects of repeated saline and nicotine pre-treatment on NAS (A) and striatal (B) dopaminergic responses to acute footshock stress are presented ($n = 6$ animals for each treatment group).

level of the VTA, are complex and will require further investigation. Adding to this complexity, it has been demonstrated that nicotine's inhibition of tuberoinfundibular DA activity is EOP-dependent, and that this varies with diurnal activity of the EOPS, which control prolactin release (Shieh and Pan 1997).

Naloxone dose-dependently blocked repeated nicotine's enhancement of accumbal DA activity, suggesting and important role for EOPS in the modulation of repeated nicotine effects on the mesoaccumbal DA pathway under both non-stressed and stress-induced states (Spanagel et al. 1992). Our findings also suggest that EOP

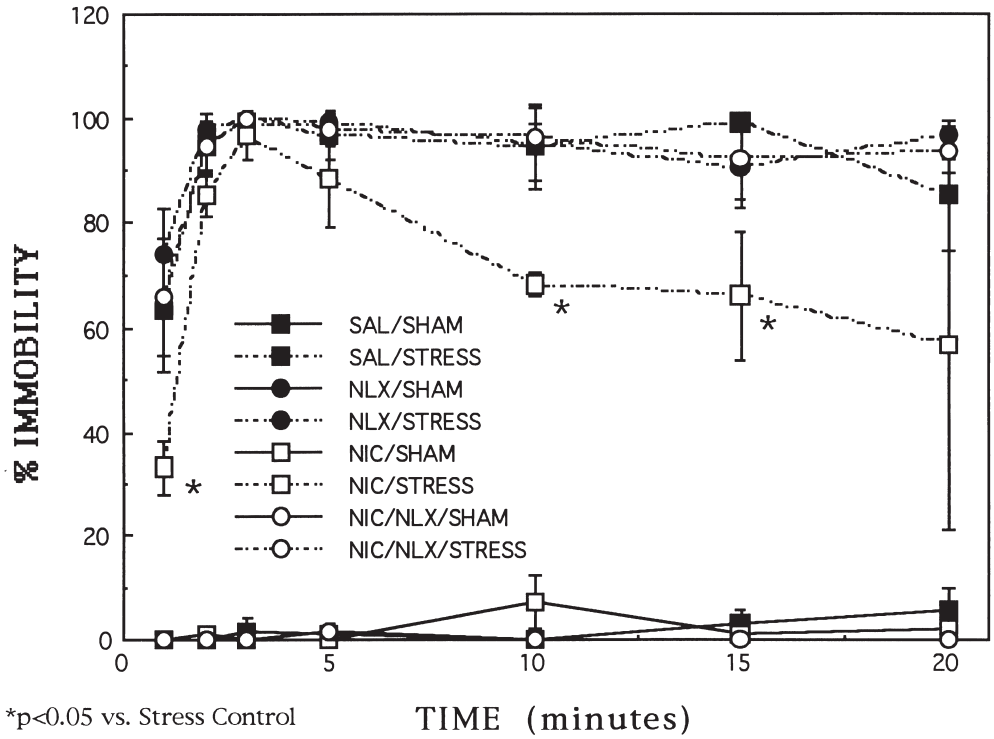


Figure 3. Acquisition of immobility responses to acute footshock stress: Effects of repeated nicotine and naloxone. The acute footshock stress-induced immobility curve for the various pre-treatment and stress conditions is presented ($n = 6$ animals for each treatment group). SAL/SHAM, saline pre-treatment and challenge, no stress; SAL/STRESS, saline pre-treatment and challenge, footshock stress; NLX/SHAM, saline pre-treatment and naloxone challenge, no stress; NLX/STRESS, saline pre-treatment and naloxone challenge, footshock stress; NIC/SHAM, nicotine pre-treatment and saline challenge, no stress; NIC/STRESS, nicotine pre-treatment and saline challenge, footshock stress; NIC/NLX/SHAM, nicotine pre-treatment and naloxone challenge, no stress; NIC/NLX/STRESS, nicotine pre-treatment and naloxone challenge, footshock stress.

modulation of the mesoaccumbal system is recruited with repeated, but not acute, nicotine administration.

Effects of Repeated Nicotine on Physical versus Psychological Stressors

Our preliminary results with a conditioned fear (CF) paradigm (George et al. 1997) imply that although the nicotine pre-treatment regimen reduces mesocorticolimbic DA responses to CF stress, it does not modify the expression of CF immobility responses (a *psychological* stressor). This supports the notion that the neuroanatomic substrates mediating expression of acute footshock- and CF-induced immobility responses are distinct; nicotine pre-treatment appears to differentially effect behavioral, but not dopaminergic, responses in these two types of stress paradigms. Based on the results of the present study, EOPS are probably involved in both the mesoprefrontal dopaminergic and immobility responses to *physical* stressors. Whether smoking has beneficial effects (i.e., anti-nociceptive, anxiolytic) during different types of (stressful) situations is an open question, which will require carefully controlled studies in both habitual smokers and nicotine-naive subjects.

Thus, our results suggest that mesoprefrontal DA responsivity and associated behavioral responses are regulated by opioid mechanisms, presumably by endogenous opioids acting on μ-opiate receptors in either the VTA or mPFC. The results of experiments involving local infusion of naloxone or its hydrophilic congener methylnaloxonium into the VTA or mPFC could clarify whether repeated nicotine administration produces its effects on mesoprefrontal DA systems through EOP circuitry at the level of the VTA or mPFC.

Clinical Implications

Taken together, the present experiments suggest that nicotinic modulation of footshock stress-induced mesoprefrontal DA activation and associated behavioral responses are modulated by EOPS. Naltrexone, the long-acting congener of naloxone, may have some efficacy as a pharmacotherapeutic agent in smoking cessation (Karras and Kane 1980; Gorelick et al. 1988; Sutherland

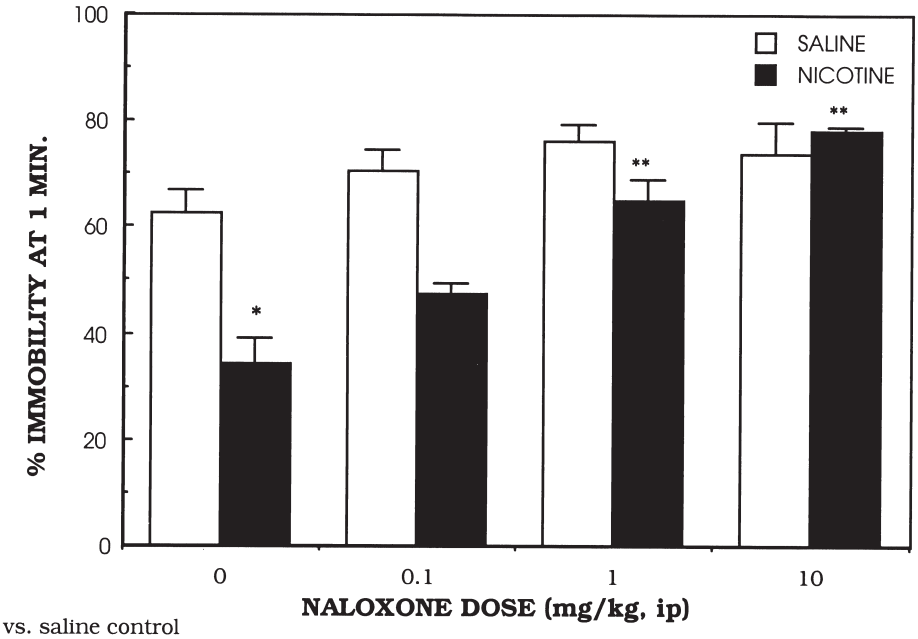


Figure 4. Effects of naloxone on repeated nicotine modulation of stress-induced immobility responses at 1 minute. The effects of repeated saline and nicotine pre-treatment on stress-induced immobility responses at 1 minute are shown. Data are derived from Figure 3 ($n = 6$ animals in each treatment group).

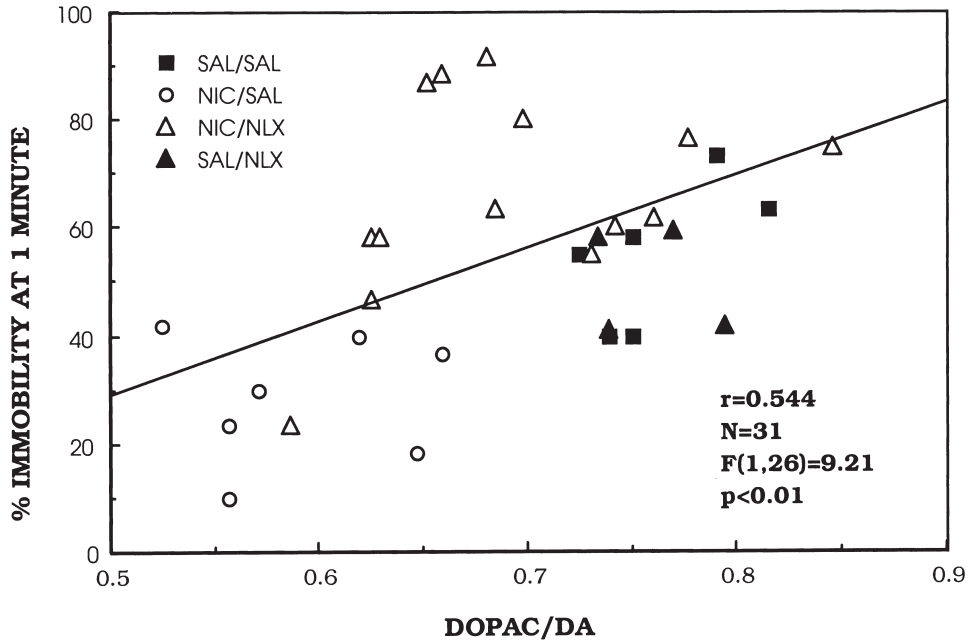


Figure 5. Correlation between stress-induced immobility responses and mesoprefrontal dopamine utilization: Effects of naloxone. This figure shows the relationship between stress-induced cortical DA utilization and immobility responses and the coordinate effects of repeated nicotine and naloxone combination treatments. The figure is constructed from data presented in Figures 1 and 4. The naloxone dose used was 1.0 mg/kg. Groups are as follows: SAL/SAL, saline pre-treatment, saline challenge; NIC/SAL, nicotine pre-treatment, saline challenge; SAL/NLX, saline pre-treatment, naloxone challenge; NIC/NLX, nicotine pre-treatment, naloxone challenge. The correlation coefficient was r = 0.544 ($F(1,26) = 9.21$, $p < .01$).

et al. 1995; O'Malley et al. 1997) and our results may have implications for understanding the putative antinociceptive and stress-reducing effects of smoking, which may contribute to the nicotine dependence state. Furthermore, our results may have implications for our understanding of neuropsychiatric disorders such as schizophrenia, which has high rates of co-morbid nicotine dependence, stress-responsive symptom exacerbation, and prefrontocortical dysfunction.

ACKNOWLEDGMENTS

This study was supported in part by a Lucille P. Markey Physician-Scientist Award and a NARSAD Young Investigator Award (TPG) and USPHS Grants K12-DA00167 (to TPG) and MH-14092 (to RHR). We thank Jane R. Taylor, Ph.D., Suchitra Krishnan-Sarin, Ph.D., and Marina R. Picciotto, Ph.D. for their helpful comments on the manuscript.

REFERENCES

Aceto MD, Scates SM, Ji Z, Bowman ER (1993): Nicotine's opioid and anti-opioid interactions: proposed role in smoking behavior. Eur J Pharmacol 248:333–335

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

Boyd RT (1997): The molecular biology of neuronal nicotinic acetylcholine receptors. Crit Rev Toxicol 27:299–318

Christensen M-K, Smith DF (1990): Antinociceptive effects of the stereoisomers of nicotine given intrathecally in spinal rats. J Neural Transm 80:189–194

Corrigall WA, Herling S, Coen KM (1988): Evidence for opioid mechanisms in the behavioral effects of nicotine. Psychopharmacology 96:29–35

Davenport KE, Houdi AA, Van Loon GR (1990): Nicotine protects against μ-opioid receptor antagonism by β-funaltrexamine: Evidence for nicotine-induced release of endogenous opiods in the brain. Neurosci Lett 113:40

Davis M, Astrachan DI (1978): Conditioned fear and startle magnitude: Effects of different footshock or barshock intensities used in training. J Exp Psychol 4:95–103

Devine DP, Leone P, Pocock D, Wise RA (1993): Differential involvement of ventral tegmental *mu*, *delta*, and *kappa* opioid receptors in modulation of basal mesolimbic dopamine release: In vivo microdialysis studies. J Pharmacol Exp Ther 244:1067–1080

Eiden LE, Giraud P, Dave JT, Hotchkiss AJ, Affolter H (1984): Nicotinic receptor stimulation activates enkephalin release and biosynthesis in adrenal chromaffin cell. Nature 312:661

Elsworth JD, Brittan MS, Taylor JR, Sladek JR, Al-Tikriti MS, Zea-Ponce Y, Innis RB, Redmond DE Jr, Roth RH (1996): Restoration of dopamine transporter density in the striatum of fetal ventral mesencephalon-grafted, but not sham-grafted, MPTP-treated parkinsonian monkeys. Cell Transpl 5:315–325

Finley JCW, Maderdrut JL, Petrusz P (1981): The immunocytochemical localization of enkephalin in the central nervous system of the rat. J Comp Neurol 198:541–565

George TP, Verrico CD, Roth RH (1997): Effects of nicotine and stress on rat mesoprefrontal dopamine systems: Evidence for opiate mechanisms. Soc Neurosci Abstr 23:1359

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

Gorelick DA, Rose J, Jarvik ME (1988): Effects of naloxone on cigarette smoking. J Subst Abuse 1:153–159

Henningfield JE, Schuh LM, Jarvik ME (1995): Pathophysiology of tobacco dependence. In: Bloom FE, Hupfer DJ (eds), Psychopharmacology: The Fourth Generation of Progress. New York, Raven Press, pp 1715–1729

Hildebrand BE , Nomikos GG, Bondjers C, Nisell M, Svensson TH (1997): Behavioral manifestations of the nicotine abstinence syndrome in the rat: Peripheral versus central mechanisms. Psychopharmacology 129:348–356

Hildebrand BE, Nomikos GG, Panagis G, Hertel P, Schilstrom B, Svensson TH (1998): Reduced dopamine output in the nucleus accumbens but not in the medial prefrontal cortex in rats displaying a mecamylamine-precipitated nicotine withdrawal syndrome. Brain Res 779:214–225

Horger BA, Roth RH (1996): The role of mesoprefrontal dopamine neurons in stress. Crit Rev Neurobiol 10:395–418

Houdi AA, Pierzchala K, Marson M, Palkovits M, Van Loon GR (1991): Nicotine-induced alteration in Tyr-Gly-Gly and Met-enkephalin in discrete brain nuclei reflects altered enkephalin neuron activity. Peptides 12:161

Kalivas PW, Abhold R (1987): Enkephalin release into the ventral tegmental area in response to stress: Modulation of mesocorticolimbic dopamine. Brain Res 414:339–348

Karras A, Kane JM (1980): Naloxone reduces cigarette smoking. Life Sci 27:1541–1545

Khachaturian H, Lewis ME, Watson SJ (1983): Enkephalin systems in diencephalon and brainstem of the rat. J Comp Neurol 220:310–320

Krishnan-Sarin S, Rosen MI, O'Malley SS (1999): Evidence for an opioid component in nicotine dependence. Arch Gen Psychiatry 56:663–668

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1957): Protein measurement with the phenol-folin reagent. J Biol Chem 193:265–275

Malin DH, Lake JR, Carter VA, Cunningham JS, Wilson OB (1993): Naloxone precipitates nicotine abstinence syndrome in the rat. Psychopharmacology 112:339–342

Malin DH, Lake JR, Payne MC, Short PE, Carter VA, Cunningham JS, Wilson OB (1996a): Nicotine alleviation of nicotine abstinence syndrome is naloxone-reversible. Pharmacol Biochem Behav 53:81–85

Malin DH, Lake JR, Short PE, Blossman JB, Lawless BA, Schopen CK, Sailer EE, Burgess K, Wilson OB (1996b): Nicotine abstinence syndrome precipiated by an analog of neuropeptide FF. Pharmacol Biochem Behav 54:581–585

McGehee DS, Role LW (1995): Physiological diversity of nic-

otinic acetylcholine receptors expressed by vertebrate neurons. Ann Rev Physiol 57:521–546

Miller JD, Speciale SG, McMillen BA, German DC (1984): Naloxone antagonism of stress-induced augmentation of frontal cortex dopamine metabolism. Eur J Pharmacol 98:437–439

Nemeth-Coslett R, Griffiths RR (1986): Naloxone does not affect cigarette smoking. Psychopharmacology 89:261–264

Nisell M, Nomikos GG, Hertel P, Panagis G, Svennson TH (1996): Condition-independent sensitization of locomotor stimulation and mesocortical dopamine release following chronic nicotine treatment in the rat. Synapse 22:369–381

O'Malley SS, Krishnan-Sarin S, Meandzija B (1997): Naltrexone treatment of nicotine dependence: A preliminary study. Presented at the Annual Meeting of the American Psychiatric Association, San Diego, CA, May 1997

Paxinos G, Watson C (1986): The Rat Brain in Stereotaxic Coordinates, 2nd ed. San Diego, CA, Academic Press

Pidoplichko VI, DeBias M, Williams JT, Dani JA (1997): Nicotine activates and desensitizes midbrain dopamine neurons. Nature 390:401–404

Pomerleau OF, Fertig JB, Seyler LE, Jaffe J (1983): Neuroendocrine reactivity to nicotine in smokers. Psychopharmacology 81:61–67

Sesack SR, Pickel VM (1992): Dual ultrastructural localization of enkephalin and tyrosine hydroxylase immunoreactivity in the rat ventral tegmental area: Multiple substrates for opiate-dopamine interactions. J Neurosci 12:1335–1350

Shieh K-R, Pan J-T (1997): Nicotinic control of tuberoinfundibular dopaminergic neuron activity and prolactin secretion: Diurnal rhythm and involvement of endogenous opiodergic system. Brain Res 756:266–272

Spanagel R, Herz A, Shippenberg TS (1992): Opposing tonically active endogenous opiate systems modulate the mesolimbic dopaminergic pathway. Proc Natl Acad Sci USA 89:2046–2050

Sutherland G, Stapleton JA, Russell MA, Feyerabend C (1995): Naltrexone, smoking behavior and cigarette withdrawal. Psychopharmacology 120:418–425

Tam S-Y (1988): Regulation of Mesoprefrontal Dopamine Neurons. Ph.D. Thesis. New Haven, CT, Yale University

Thierry AM, Tassin J-P, Blanc G, Glowinski J (1976): Selective activation of the mesocortical dopamine system by stress. Nature 263:2420–2424

Vezina P, Blanc G, Glowinski J, Tassin J-P (1992): Nicotine and morphine differentially activate brain dopamine in prefrontocortical and subcortical terminal fields: Effects of acute and repeated injections. J Pharmacol Exp Ther 261:484–490

Zarrindast M-R, Farzin D (1996): Nicotine attenuates naloxone-induced jumping behavior in morphine-dependent mice. Eur J Pharmacol 298:1–6