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Opposing actions of chronic stress and chronic nicotine on striatal function in mice

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Abstract

Stress is a major risk factor in drug addiction development and relapse. Virtually all drugs of abuse act by increasing extracellular dopamine levels in the striatum. To gain an understanding of the interaction between stress and drug exposure, we studied the effects of concomitant chronic nicotine and chronic stress exposure on mouse striatal dopamine levels.

C57Bl6/J mice were treated with nicotine in the drinking water or control solution for at least 6 weeks. Some mice were chronically stressed by daily exposure to cold, shaking or restrain.

Nicotine treated mice showed up-regulation of epibatidine binding in several brain regions. In mice treated with both chronic nicotine and stress, epibatidine binding was increased in all studied areas except the dorsal striatum. Therefore, microdialysis was used to measure extracellular dopamine levels in the dorsal striatum of mice chronically treated with nicotine, stress, or both. To have a measure of striatal response to different challenges, we performed microdialysis after acute injection of saline, nicotine, and cocaine. Chronic nicotine enhanced nicotine-dependent dopamine release, while chronic stress blunted the response to cocaine. When mice were subjected to both chronic nicotine and chronic stress, nicotine- and cocaine-dependent dopamine release was undistinguishable from that of control animals.

In conclusion, our data suggest that chronic stress and chronic nicotine counteract each other's effect on dopamine release in the striatum. This effect might be mediated by changes in nicotinic acetylcholine receptor upregulation. This "normalization" of striatal function when both nicotine and stress are present might help explain the comorbidity between stress-related disorders and drug abuse.

Keywords

Nicotinic acetylcholine receptors; nicotine; stress; dopamine; microdialysis; striatum

Tobacco smoking is a leading cause of death in the world (Peto et al., 2000). The major addictive component of tobacco is nicotine which acts at nicotinic acetylcholine receptors (nAChRs) in the nervous system. Understanding how certain risk factors promote nicotine addiction will be beneficial for the development of improved anti-smoking therapies. Stress is one of the most prominent risk factors for the development of drug addiction (Sinha, 2001).

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Striatal dopamine (DA) is a critical modulator of both motor coordination and behavioral reinforcement. Thus, dopaminergic mechanisms mediate several properties of addictive drugs (Graybiel et al., 1994; Breiter et al., 1997; Kalivas and Nakamura, 1999; Berke and Hyman, 2000). The nicotinic cholinergic system and $\beta 2$ subunit-containing nAChRs in particular, is a major player in the regulation of extracellular dopamine levels. (Zhou et al., 2001). nAChRs containing the $\beta 2$ subunit have been shown to be up-regulated by chronic nicotine treatment (Flores et al., 1992a). Therefore, we used radioactive epibatidine binding to study the levels of expression of $\beta 2$ -containing nAChRs in the striatum of mice chronically treated with nicotine and/or stress. Epibatidine is a ligand that binds to various heteromeric nAChR subtypes (Zoli et al., 1998; Whiteaker et al., 2000). In addition, we performed microdialysis to study the dopaminergic response to acute injections of drug of abuse (nicotine and cocaine) after chronic nicotine and chronic stress treatments.

In this report, we show that chronic nicotine and chronic stress affect drug-induced dopamine levels in the striatum and that the interaction between stress and nicotine treatment yields normal striatal DA levels in response to nicotine and cocaine injection. As the effects of cocaine on the dorsal striatum have already been characterized (Gehrke et al.), we used acute injection of cocaine as a positive control for striatal function and the microdialysis experiment.

Experiments were conducted on 2 to 6 month old female and male C57Bl6/J mice. All the procedures complied with the directives of Baylor's Center for Comparative Medicine and the NIH Guide for the Care and Use of Laboratory Animals. Mice had *ad libitum* access to water and food pellets (Labdiet 5001, PMI[®], Brentwood, MO), were maintained on a 12-h light/dark cycle, and were tested between 8 am and 4 pm. Nicotine was administered chronically through the drinking water following a previously described protocol (Sparks and Pauly, 1999; Brunzell et al., 2003). Control mice had unlimited access to a solution of tap water containing 2% saccharine, while nicotine-treated mice drank water containing 2% saccharine plus 200 mg/ml nicotine. This treatment was continued for at least 6 weeks before either epibatidine binding assay or microdialysis were conducted. Chronic stress was achieved by exposing the mice daily to one of three different stressors (one hour at 4 °C, one hour of shaking at approximately 50 rpm, or 2 hours of restraint in a ventilated plastic tube) in an unpredictable pattern.

Slide-mounted brain sections for [¹²⁵I]epibatidine autoradiography were processed as previously described (Salas et al., 2004). Sections were incubated at 22°C for 1 h in buffer (50 mM Tris-base, pH 7.4, 120 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂ and 1 mM MgCl₂) containing 500 pM [¹²⁵I]epibatidine (specific activity=2200 Ci/mmol, NEN). Brain sections were then washed twice for 3 min each in buffer, dipped once in water, air dried, and apposed to Biomax film (Kodak) for 3 to 12 h.

After a minimum of 5 weeks of chronic treatment, mice were implanted with a microdialysis cannula guide (either CMA7, CMA Microdialysis AB, Solna, Sweden or MAB 4.15.1 PES, Microbiotech, Stockholm, Sweden) into the dorsal striatum (coordinates AP 0, DL 2.0, V -2.0 from Bregma). At least 3 days after surgery mice were placed in a regular cage with bedding but no lid for a few hours, and a microdialysis probe (6 KDa cut off, 1 mm long, CMA or Microbiotech) was inserted into the dorsal striatum. Artificial cerebrospinal fluid was perfused overnight, and the next day, samples were collected (40 μ l in 20 minutes, 3 samples per condition) before and after saline injection, after nicotine injection (1 mg/kg, ip), and after cocaine injection (10 mg/kg, ip). A minimum of 3 h elapsed between nicotine and cocaine injections. Samples were analyzed by HPLC (ESA, Chelmsford, MA), with the dopamine peak appearing at about 4.5 min. All data were normalized by taking the average of the saline injection samples as 100% for each mouse. Since the effect of nicotine and cocaine was heavily time-dependent, only the first sample after injection was used for analysis of acute nicotine and cocaine conditions.

X-ray films were analyzed using computer-assisted densitometry (NIH Image program, developed at the U.S. National Institutes of Health) without changing the contrast or brightness of the images. Relative optical densities (ROD) from discrete brain regions were measured and presented as a percentage of readings from control mice in the same film (no background subtraction was performed as we used control mouse binding as 100% in each case). Data were examined by ANOVA, followed by Duncan's post-hoc comparisons. Microdialysis data were analyzed using ANOVA followed by a paired Student's t-test to compare the effect of acute nicotine or cocaine to saline injection on each mouse (a priori planned comparison).

According to the literature, nicotine-treated mice should have elevated levels of $\beta 2$ -containing nAChRs (Flores et al., 1992b). To confirm that our treatment was effective and to study the possibility of an interaction between nicotine and stress at the level of $\beta 2$ -containing nAChRs, we performed binding studies with [125 I]-epibatidine. As shown in Figure 1, chronic nicotine treatment increased [125 I]-epibatidine binding in several areas of the brain, including the striatum, cingulate cortex, somatosensory cortex and the dentate gyrus of the hippocampus. No statistically significant difference was found between the epibatidine binding in control and stress-treated mice. Interestingly, animals treated with nicotine plus stress showed nicotine-induced up regulation of epibatidine binding in all areas examined, except for the striatum (Fig. 1).

As seen in Figure 2, in control mice acute nicotine (1 mg/kg) had no effect on extracellular dopamine levels in the dorsal striatum. In contrast, acute cocaine (10 mg/kg) induced a small but significant increase in dopamine levels. In mice chronically treated with nicotine, nicotine injection seemed to have a stronger effect on nicotine-induced dopamine levels than in nicotine-naïve mice. Neither acute nicotine nor acute cocaine produced an increase in dopamine levels in chronically stressed mice. Interestingly, in mice treated with both acute nicotine and acute stress, dopamine levels after nicotine and cocaine injections were indistinguishable from those on control mice.

Stress has been shown to have a major impact on addiction, including nicotine addiction. DA levels in mesolimbic areas are determined in part by the activity of $\beta 2$ subunit-containing nicotinic acetylcholine receptors (Zhou et al., 2001). In addition, it has been repeatedly shown that in both humans and animals, chronic nicotine increases $\beta 2$ -containing nAChR levels in several brain areas of mice chronically treated with nicotine (Flores et al., 1992a; Nguyen et al., 2003). Our binding studies with [125 I]-epibatidine (which binds $\beta 2$, $\beta 3$ and $\beta 4$ containing receptors) in the brains of C57Bl6/J mice treated with chronic nicotine and/or stress confirmed that [125 I]-epibatidine binding increases upon chronic nicotine treatment. Interestingly, the combination of chronic nicotine and chronic stress induced similar receptor up-regulation in all studied areas except the striatum. The microdialysis studies conducted to assess the levels of dopamine in the striatum of chronically treated mice, after acute injections of nicotine and cocaine are interesting for several reasons. First, the data suggests that there are not only effects of nicotine alone and stress alone on drug-induced dopamine levels, but that the combination of chronic nicotine and chronic stress returns the values of drug-induced dopamine levels back to control levels. There is, thus, an interaction between chronic nicotine and chronic stress at the level of striatal extracellular dopamine concentrations. Second, we confirmed previous data in the literature showing that chronic stress can induce a blunted response to cocaine in dopamine levels in the nucleus accumbens and prefrontal cortex (Mangiacavchi et al., 2001), which is in agreement with our results in the striatum. Third, we confirmed that repeated nicotine enhances nicotine-induced dopamine levels in mesolimbic areas (Benwell and Balfour, 1992). Our data agrees with similar experiments (Pawlak et al., 2000) in rats treated with chronic nicotine and acute stress. In that report, rats injected with nicotine once daily for 2 weeks showed increased DA levels in the striatum 3 h after the last nicotine injection. In control rats, acute stress treatment also increased DA levels. In rats exposed to both chronic

nicotine and chronic stress, stress did not have an affect beyond nicotine's effect. In fact, these authors show a trend toward a "normalizing" effect of acute stress and nicotine, although they did not reach statistical significance. It is possible that chronic stress has an effect in the same direction but more pronounced. In conclusion, we have shown for the first time that when chronic nicotine and stress are combined, the dopamine response of the striatum to acute nicotine or cocaine challenge returns to control values. It is therefore tempting to speculate that smokers who are under chronic stress tend to smoke more to try to maintain a certain homeostasis in their striatal dopaminergic activity.

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Figure 1. Effects of chronic nicotine and stress on [¹²⁵]I-epibatidine binding

After chronic treatment for 6 weeks (control, nicotine, stress, or nicotine plus stress), epibatidine binding was studied in brain sections. ROD: relative optical density. Data is expressed as average ± sem. *p<0.05.

DG, dentate gyrus; Som Cx, somatosensory cortex; STR, striatum, CgCx, cingulate cortex.

Figure 2. Dopamine microdialysis in the striatum of nicotine and stress treated mice

After a minimum of 6 weeks of chronic treatment mice were implanted with microdialysis cannulae and dopamine levels in the striatum were studied under basal conditions (saline injection) and after ip injections of nicotine (1 mg/kg) and cocaine (10 mg/kg). Dopamine levels are expressed as % of saline injection for each mouse $\pm$ sem. N=10, 9, 10, 4 in control, Nic, Stress and Nic + Stress groups, respectively.

* $p < 0.05$ compared to saline injection, same chronic treatment

$p < 0.05$ compared to control mice, same acute treatment.