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Changes in Sensitivity to Nicotine and Brain Nicotinic Receptors Following Chronic Nicotine and Corticosterone Treatments in Mice

S. F. ROBINSON,* E. U. GRUN,* J. R. PAULY† AND A. C. COLLINS*[1]

*Institute for Behavioral Genetics, Campus Box 447, University of Colorado, Boulder, CO 80309
†Department of Pharmacology, Medical College of Georgia, Augusta, GA 30912-2300

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ROBINSON, S. F., E. U. GRUN, J. R. PAULY AND A. C. COLLINS. *Changes in sensitivity to nicotine and brain nicotinic receptors following chronic nicotine and corticosterone treatments in mice.* PHARMACOL BIOCHEM BEHAV **54**(3) 587-593, 1996.—Chronic nicotine treatment often results in tolerance to nicotine as well as increases in brain [^{3}H]-nicotine binding and [^{125}I]-α-bungarotoxin (α-BTX) binding. Chronic corticosterone (CCS) treatment also produces tolerance to nicotine, but it does not change [^{3}H]-nicotine binding; decreases in α-BTX binding are observed, which suggests that tolerance to nicotine may be related to decreases in the number of this nicotinic receptor subtype. In the studies reported here, C57BL/6 mice were implanted subcutaneously with cholesterol or 60% CCS/40% cholesterol-containing pellets and were infused continuously with saline (control) or nicotine for a total of 9 days. Effects of acute nicotine challenge on Y-maze crossing and rearing activities, heart rate, and body temperature were measured. Both chronic nicotine and CCS treatment resulted in tolerance to nicotine for all of the measures, and some evidence for additivity was seen in the animals that were cotreated with CCS and nicotine. Chronic nicotine infusion increased brain nicotine binding and CCS treatment reduced α-BTX binding. Decreases in α-BTX binding were not detected in the cotreated animals. The latter finding argues that changes in α-BTX binding are not reliable predictors of or a cause of tolerance to nicotine.

Nicotine Nicotinic receptors Corticosterone Locomotor activity Heart rate Body temperature

TOLERANCE, a decrease in sensitivity to a challenge dose of a drug, frequently occurs following chronic nicotine treatment. For example, chronic nicotine treatment results in tolerance to a wide range of nicotine's effects in the rat (2,3,5, 6,9,13,35,38,39) and mouse (17–19, 22–25).

Many studies have attempted to assess the potential role of changes in brain nicotinic receptor binding in regulating tolerance to nicotine. The most abundant brain nicotinic receptor binds agonists such as [^{3}H]-nicotine and [^{3}H]-acetylcholine with high affinity (4,16,34,37) and is probably made up of a mixture of α4 and β2 subunits (10,40). Chronic treatment with nicotine evokes what has come to be known as a paradoxical upregulation of brain nicotinic receptors [reviewed in (41)]. Several studies using the mouse have demonstrated that tolerance to nicotine increases with the chronic treatment dose, and in some, but not all, mouse strains this tolerance is paralleled by dose-dependent increases in brain [^{3}H]-nicotine binding (17–19,24). Similarly, the time courses for upregulation of [^{3}H]-nicotine binding and return to control after chronic nicotine treatment is stopped, correlate with the development and loss of tolerance to low dose effects of nicotine (23).

Other studies have suggested a role for the adrenal hormone, corticosterone (CCS), in regulating response to nicotine. Adrenalectomy results in increased sensitivity to nicotine (31). The supersensitivity to nicotine seen in adrenalectomized mice is reversed by chronic treatment with CCS, and chronic CCS treatment results in tolerance to nicotine in mice with an

[1] To whom requests for reprints should be addressed.

intact adrenal (28). Adrenalectomy also results in an increase in the number of nicotinic receptors that bind α-bungarotoxin (α-BTX) with high affinity (28); these changes are seen most readily in the hippocampus. In contrast, chronic treatment with high doses of CCS evokes decreases in α-BTX binding in many brain regions (27,28,31). [^{3}H]-Nicotine binding is not altered by adrenalectomy or by chronic CCS treatment (27,28, 31). These results suggest that increases in α-BTX binding lead to increased sensitivity to nicotine and decreases in binding lead to decreases in sensitivity (tolerance).

The results obtained with chronic nicotine and chronic CCS treatment suggest that tolerance to nicotine may be evoked by upregulating the nicotinic receptor that binds [^{3}H]-nicotine and by downregulating the α-BTX binding site. If separate mechanisms are truly involved, cotreatment with nicotine and CCS should result in additive tolerance. The experiments described here compared the effects of chronic treatment with nicotine or CCS alone with the effects of chronic cotreatment with CCS and nicotine. Response to nicotine and brain nicotinic receptor binding were measured in an attempt to provide explanations for changes in sensitivity (tolerance) to nicotine that were observed.

METHOD

Animals

Female mice of the C57BL/6 strain were used in this study. This strain was selected for these studies because it develops marked dose-dependent tolerance to nicotine to the behavioral and physiological responses that were used in the present study (18). In addition, in C57BL/6 mice tolerance is closely paralleled by changes in [^{3}H]-nicotine binding (18). This strain has been maintained for over 30 generations at the Institute for Behavioral Genetics. Mice were housed under a 12 L : 12 D cycle, with lights on at 0700 h and were provided with unlimited access to water and pelleted food. All animals were between 60 and 90 days of age at the initiation of the experiment.

Surgical Procedures

Mice were anesthetized with pentobarbital (54 mg/kg) and catheters were implanted in the jugular vein according to the methods of Barr et al. (1). Hormone pellets were also implanted at this time as described by Pauly et al. (28). The pellets were made by melting cholesterol and hormone mixtures and contained either 60% corticosterone (CCS) or 100% cholesterol (control). Following surgery, the animals were housed individually and the catheters were attached to 1 ml syringes that were mounted on Harvard infusion pumps. The animals were infused with sterile saline (35 μl/h) for 1 day. Following this recovery period, saline treatment continued for control animals or nicotine treatment was initiated. Animals were infused for 1 day with 2.0 mg/kg/h nicotine (base). This was increased by 2 mg/kg/h each day until a final dose of 6.0 mg/kg/h was achieved. The mice were infused with this dose for 7 days. Thus, the mice were treated chronically with nicotine for a total of 9 days. Previous studies from our laboratory have demonstrated that this treatment regimen produces maximal changes in nicotinic cholinergic receptor binding and tolerance to nicotine as measured by the responses used in the present study (18).

Tolerance Testing

Animals were tested for sensitivity to nicotine starting 2 h after chronic infusion was stopped. The 2-h time point was chosen because all of the residual nicotine should have been metabolized and eliminated from the mouse before 2 h had passed; the half-life for nicotine in control C57BL/6 mice is 6.9 min (32).

The animals were challenged with (−)nicotine base (Sigma) that had been dissolved in physiological saline, and neutralized with HCl. It was injected in a volume of 0.01 ml/g body weight. Doses of nicotine (0–2.0 mg/kg) were altered by changing the concentration of nicotine in the injection solution. The responses to acute nicotine challenge were measured using a battery of behavioral and physiological responses (21). The effects of nicotine injection on locomotor activity were measured in a symmetrical Y-maze; each arm was divided into two sections and crosses from one arm/section to another were counted during a 3-min test. The number of rears was also counted. Y-maze activity was measured starting 4.5 min following acute nicotine administration. Heart rate was determined 8.5 min following nicotine injection, using an E and M Physiograph. Rectal temperature was measured 15 min postinjection using a Bailey Instruments digital thermometer. The time points for these measures reflect the time of maximal nicotine response of each test (21).

Receptor Binding

The animals were decapitated following behavioral testing and the brains were dissected into eight anatomical regions (cortex, striatum, hypothalamus, midbrain, hippocampus, colliculi, hindbrain, and cerebellum). Brain regions were placed in 10 vol of Krebs-Ringers HEPES buffer (pH, 7.5) and stored at −70°C until assayed. On the day of the assay the tissue was thawed and homogenized using a Teflon pestle. Membranes were prepared using the method of Romano and Goldstein (34). L-[^{3}H]-Nicotine binding was determined at 4°C using the method of Romano and Goldstein (34), as modified by Marks et al. (26). Equilibrium binding was measured by a filtration assay using 10 nM [^{3}H]-nicotine and a 90-min incubation. Blanks were determined by the inclusion of 10 μM unlabeled (−)nicotine in the binding assay. Kinetic analysis (K_D and B_{max} measurement) of receptor binding was determined in cortical tissue using six increasing concentrations of nicotine (0.5–20 nM). The binding of [^{125}I]-α-BTX was determined as described by Marks et al. (26). Membranes were incubated for 3 h at 37°C using 1 nM [^{125}I]-α-BTX. Kinetic analyses were performed using six concentrations of labeled toxin (0.1–2.0 nM). Nonspecific binding was determined by the inclusion of 1 mM nicotine in the binding assays.

Determination of Radioactivity

After filtration of tissue to which [^{3}H]-nicotine had been bound, the glass fiber filters were placed in polypropylene vials (6 ml) and 2.5 ml of scintillation fluid (Budget Solve, Research Products International, Mt. Prospect, IL) was added. The samples were mixed by shaking for 30–60 min and radioactivity was determined on a Packard 1600CA Liquid Scintillation Spectrometer. Tritium was counted at 53% efficiency. After filtration of tissue, to which [^{125}I]-α-BTX had been bound, the glass fiber filters were placed in 12 × 75 test tubes and radioactivity was determined on a Packard 5000 Series Gamma Counter. Efficiency of counting was 71%.

Protein Assay

Protein was measured using the method of Lowry et al. (15) with bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as the standard.

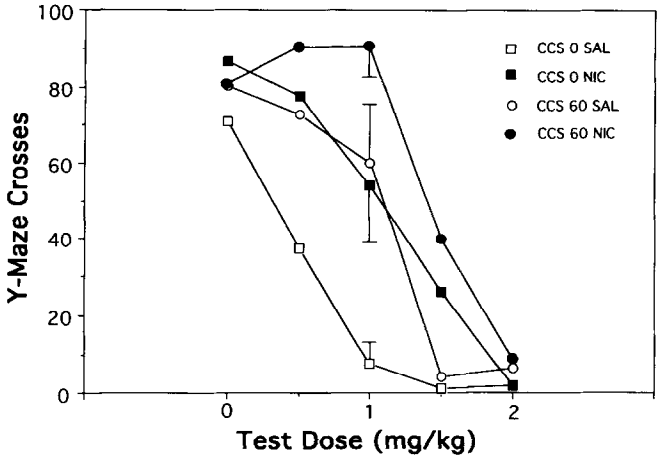

FIG. 1. Dose-response curves for nicotine effects on Y-maze crossing activity. C57BL/6 mice were implanted with cholesterol pellets (CCS0) and infused with saline the first day, saline (CCS0-SAL) or 2.0 mg/kg/h nicotine the second day, 4.0 mg/kg/h nicotine the third day, and 6.0 mg/kg/h nicotine (CCS0-NIC) for 7 days. Other animals were implanted with 60% (w/w) CCS-containing pellets (CCS60) and infused with either saline (CCS 60 SAL) or nicotine (CCS60-NIC) for the same number of days. Two hours after infusion was stopped, the animals were injected with either saline or one of the indicated doses of nicotine, and Y-maze crossing activity was measured for a 3-min period starting 4.5 min after injection. Each point represents the mean activity obtained from five to six animals. Standard error bars are presented for only one dose for the sake of clarity.

Statistical Analysis

The behavioral and physiological response data were analyzed by three-way analysis of variance (ANOVA) (pellet × nicotine infusion dose × nicotine challenge dose) with *t*-tests used for posthoc analyses. Binding data were analyzed by two-way ANOVA (pellet × nicotine infusion dose). The dose-response data were also analyzed by calculating nicotine doses that produced a standard effect: ED_{50}, nicotine dose that decreased Y-maze crossing and rearing activities by 50%; ED_{-100}, nicotine dose that decreased heart rate by 100 beats/min; $ED_{-2°}$, nicotine dose that decreased body temperature by 2°C. The ED values were calculated and the dose-response curves compared for each measure using a computerized regression comparison that follows the method devised by Diem and Lentner (8). The program sequentially tests for: a) differences in homogeneity of variance (an *F*-test); b) differences in slope (a *t*-test); and c) whether the lines may be superimposed (a *t*-test). This analysis takes into consideration all points on the linear portions of the dose-response curves and is more powerful than using a *t*-test or 95% confidence limits to test for differences in ED values.

RESULTS

Nicotine challenge produced dose-dependent decreases in Y-maze crossing activity, $F(4, 118) = 49.34$, $p < 0.001$ (Fig. 1). The dose that produced a 50% decrease in activity in control animals (cholesterol pellet, chronic saline infused, CCS 0 SAL) was 0.66 ± 0.21 mg/kg. Chronic nicotine infusion produced tolerance to nicotine's locomotor depressant effects as indicated by a significant main effect of nicotine infusion on Y-maze crossing activity, $F(1, 118) = 26.65$, $p < 0.001$. Similarly, chronic CCS treatment produced tolerance to nicotine; the three-way ANOVA detected a significant overall effect of CCS treatment, $F(1, 118) = 17.68$, $p < 0.001$. The ED_{50} values for nicotine's locomotor depressant effects in chronic nicotine-infused animals and chronic CCS-treated animals were virtually identical (1.15 ± 0.07 mg/kg and 1.11 ± 0.17 mg/kg, respectively). Those animals that were treated chronically with both nicotine and CCS were even more tolerant to the locomotor depressant effects of nicotine; the ED_{50} of 1.51 ± 0.06 mg/kg was significantly greater than the ED_{50} values obtained following chronic treatment with nicotine or CCS alone.

Figure 2 illustrates the results obtained with the Y-maze rearing test. Consistent with the effects on Y-maze crossing activity, nicotine challenge produced a dose dependent decrease in activity, $F(4, 118) = 29.85$, $p < 0.001$, and both chronic nicotine infusion, $F(1, 118) = 22.22$, $p < 0.001$, and chronic CCS treatment, $F(1, 118) = 19.09$, $p < 0.001$, produced overall decreases in sensitivity to nicotine. These changes were also evident from the ED_{50} calculations where the control ED_{50} of 0.40 ± 0.29 mg/kg was significantly less than the ED_{50} values calculated for the chronic nicotine (1.09 ± 0.17 mg/kg) and chronic CCS-treated (1.11 ± 0.11 mg/kg) animals. Once again, some evidence for additivity of effect was seen in those animals that were cotreated with nicotine and CCS; the ED_{50} for this group was 1.33 ± 0.04 mg/kg.

The data presented in Fig. 3 show that acute challenge with nicotine produced dose-dependent decreases in heart rate, $F(4, 118) = 17.56$, $p < 0.001$, and both chronic nicotine infusion, $F(1, 118) = 11.14$, $p < 0.001$, and chronic CCS treatment, $F(1, 118) = 25.71$, $p < 0.001$, decreased the response to nicotine challenge. The nicotine dose that decreased heart rate by 100 beats per minute (ED_{-100}) was 1.04 ± 0.15 mg/kg in controls, 1.74 ± 0.16 mg/kg in chronic nicotine-infused ani-

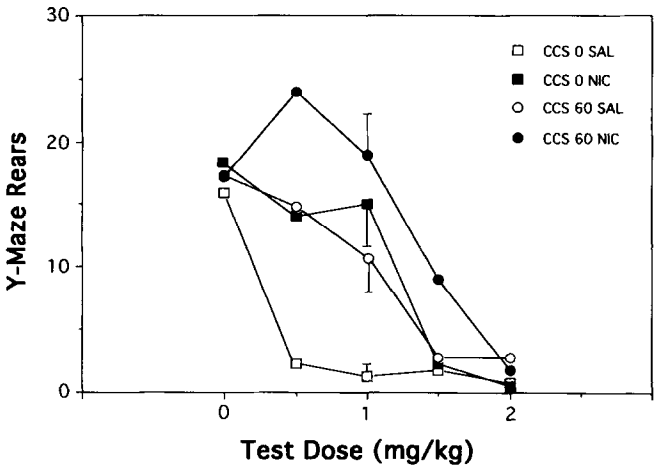

FIG. 2. Dose-response curves for nicotine effects on Y-maze rearing activity. C57BL/6 mice were treated chronically with CCS or nicotine as described in the Method section and in the legend to Fig. 1 to produce four treatment groups: cholesterol pellet–saline infused (CCS0-SAL), cholesterol pellet–nicotine infused (CCS0-NIC), CCS pellet–saline infused (CCS60-SAL), CCS pellet–nicotine infused (CCS60-NIC). Two hours after infusion was stopped the animals were injected with either saline or one of the indicated doses of nicotine, and Y-maze rearing activity was measured for a 3-min period starting 4.5 min after injection. Each point represents the mean activity obtained from five to six animals. Standard error bars are presented for only one dose for the sake of clarity.

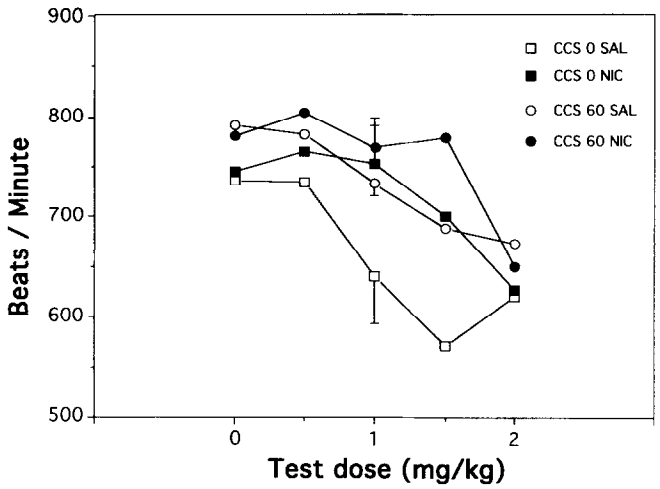

FIG. 3. Dose-response curves for nicotine effects on heart rate. C57BL/6 mice were treated chronically with CCS or nicotine as described in the Method section and in the legend to Fig. 1 to produce four treatment groups: cholesterol pellet – saline infused (CCS0-SAL), cholesterol pellet – nicotine infused (CCS0-NIC), CCS pellet – saline infused (CCS60-SAL), CCS pellet – nicotine infused (CCS60-NIC). Two hours after infusion was stopped, the animals were injected with either saline or one of the indicated doses of nicotine, and heart rate was measured 9 min after injection. Each point represents the mean activity obtained from five to six animals. Standard error bars are presented for only one dose for the sake of clarity.

mals, 1.66 ± 0.13 mg/kg in chronic CCS-treated animals, and 1.51 ± 0.08 mg/kg in animals that had been treated with both nicotine and CCS. Cotreatment did not, in this case, produce any evidence for additivity in the development of tolerance.

Similar results were obtained for the body temperature test (Fig. 4). Once again, acute nicotine challenge produced a dose-dependent decrease in the measure, $F(4, 118) = 52.30$, $p < 0.001$, and both chronic nicotine, $F(1, 118) = 39.17$, $p < 0.001$, and chronic CCS, $F(1, 118) = 91.37$, $p < 0.001$, produced overall changes in response to nicotine challenge. The ED_{-2° value (nicotine dose that decreased body temperature by 2°C) was nearly tripled from control (0.52 ± 0.15 mg/kg) by chronic nicotine (1.50 ± 0.19 mg/kg) or chronic CCS (1.36 ± 0.14 mg/kg) treatment. The ED_{-2° value for cotreated animals (1.71 ± 0.22 mg/kg) was not significantly greater than the ED^{-2° value obtained with animals that had been chronically treated with nicotine or CCS alone.

Chronic nicotine infusion produced significant overall effects on [^{3}H]-nicotine binding in virtually every brain region (Fig. 5). Significant F values, $F(1, 31)$, were obtained in cortex (13.01), cerebellum (14.98), midbrain (5.86), hindbrain (24.19), hippocampus (11.93), striatum (9.60), hypothalamus (12.60), and colliculli (13.57). In contrast, no significant overall effects of CCS treatment were detected nor was a significant interaction term obtained that indicates that only chronic nicotine treatment produced changes in [^{3}H]-nicotine binding. These changes in binding were obtained using a single ligand concentration, but these changes probably reflect changes in receptor number since chronic nicotine infusion increased the B_{max} for [^{3}H]-nicotine binding in cortex, $F(1, 31) = 13.24$, $p < 0.001$, from 38.7 ± 4.1 fmol/mg (control) to 57.9 ± 2.9 fmol/mg (nicotine treated) without changing the K_d [control = 5.7 ± 0.41 nM and nicotine-infused = 5.0 ± 0.74 nM, $F(1, 31) = 0.31$]. Nicotine infusion also elicited significant, $F(1, 31) = 12.99$, $p < 0.001$, increases in B_{max} in chronic CCS-treated mice without changes in K_d; the B_{max} in saline-infused CCS-treated mice was 42.2 ± 2.4 fmol/mg, whereas the B_{max} in nicotine-infused CCS-treated mice was 63.9 ± 2.7 fmol/mg protein.

Chronic drug treatment also produced changes in [^{125}I]-α-BTX binding, but the pattern was different from that seen for [^{3}H]-nicotine binding (Fig. 6). The ANOVA detected significantly ($p < 0.001$) greater [^{125}I]-α-BTX binding in cortex, $F(1, 31) = 12.59$, cerebellum, $F(1, 31) = 11.26$, midbrain, $F(1, 31) = 8.34$, hippocampus, $F(1, 31) = 13.54$, striatum, $F(1, 31) = 14.26$, and colliculli, $F(1, 31) = 10.88$, following nicotine infusion, but this did not arise because of a chronic nicotine-induced increase in binding over that seen in control animals. Instead, chronic nicotine treatment blocked or reversed the decrease in [^{125}I]-α-BTX binding produced by chronic CCS treatment. CCS treatment produced significant decreases in [^{125}I]-α-BTX binding in five of the eight brain regions [the $F(1, 31)$ values are: cortex, 19.72; hindbrain, 5.93; hippocampus, 12.48; striatum, 29.52; colliculli, 6.33], but no significant changes from control were seen in animals that were cotreated with CCS and nicotine.

Saturation binding analyses using cortex also detected significant overall effects of nicotine, $F(1, 31) = 8.34$, and CCS, $F(1, 31) = 26.46$, on B_{max}. The B_{max} in the control (CCS0-SAL) group (32.0 ± 1.2 fmol/mg) was not significantly different from that obtained in the cholesterol pellet- (CCS0) nicotine-infused animals (35.1 ± 1.3 fmol/mg), but it was significantly greater than that seen in the CCS60-SAL group (24.1 ± 1.6 fmol/mg) [^{125}I]-α-BTX binding did not differ from control following cotreatment with CCS and nicotine (B_{max} = 28.9 ± 1.4 fmol/mg in cortex obtained from nico-

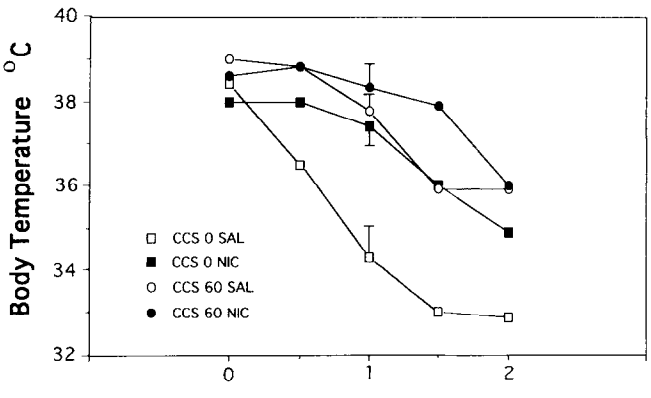

FIG. 4. Dose-response curves for nicotine effects on body temperature. C57BL/6 mice were treated chronically with CCS or nicotine as described in the Method section and in the legend to Fig. 1 to produce four treatment groups: cholesterol pellet – saline infused (CCS0-SAL), cholesterol pellet – nicotine infused (CCS0-NIC), CCS pellet – saline infused (CCS60-SAL), CCS pellet – nicotine infused (CCS60-NIC). Two hours after infusion was stopped the animals were injected with either saline or one of the indicated doses of nicotine, and body temperature was measured 15 min after injection. Each point represents the mean activity obtained from five to six animals. Standard error bars are presented for only one dose for the sake of clarity.

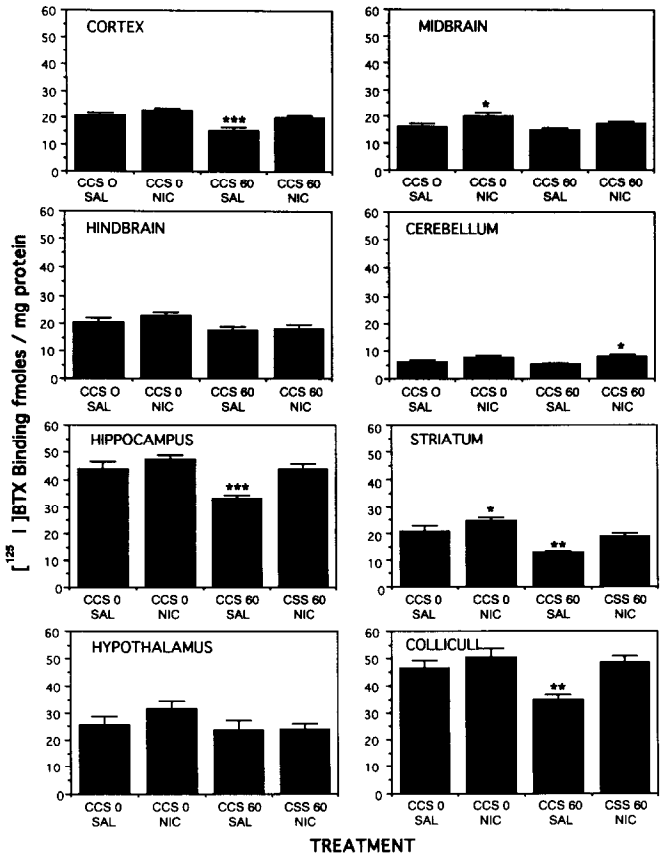

FIG. 5. Effects of chronic CCS and/or nicotine treatment on brain [^{3}H]-nicotine binding. C57BL/6 mice were implanted with cholesterol (CCS0) or 60% CCS-containing (CCS60) pellets and infused with saline or nicotine for 9 days. The animals were sacrificed after behavioral testing and regional brain [^{3}H]-nicotine binding measured as described in the Method section. Each bar represents the means ± standard error of measurements made on eight separate animals. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

tine-infused CCS-treated animals). No effects on K_d of CCS or nicotine treatment were detected, which suggests that changes in binding reflect changes in the number of binding sites. The K_d values (nM) for cortical [^{125}I]-α-BTX binding were 0.57 ± 0.03 (CCS0-SAL), 0.54 ± 0.04 (CCS0-NIC), 0.53 ± 0.02 (CCS60-SAL), and 0.48 ± 0.02 (CCS60-NIC).

DISCUSSION

Chronic treatment with nicotine, CCS, or both drugs resulted in tolerance to nicotine; the potency for nicotine was reduced when compared with the control group, as demonstrated by the observation that effective doses (ED values) were increased. The tolerance that developed following chronic treatment with nicotine alone and CCS alone were virtually identical for each of the measures, and some evidence for additivity was seen in those animals that were treated chronically with both CCS and nicotine, particularly for the Y-maze measures. Because the dose of nicotine used in this study elicits the maximal tolerance to nicotine evoked by chronic nicotine treatment (22), the finding that the CCS60-NIC-treated animals exhibited more tolerance to nicotine than did the CCS0-NIC-treated animals suggests that CCS elicits tolerance to nicotine by a different mechanism(s) than does nicotine.

The finding that chronic nicotine infusion resulted in tolerance to nicotine, as well as increases in mouse brain [^{3}H]-nicotine binding, replicates several previous studies (17-20,22-25). However, it is not clear whether these changes in [^{3}H]-nicotine binding explain tolerance to nicotine because we have detected several circumstances where tolerance to nicotine can be dissociated from changes in nicotinic receptor binding (5,6,18,30). Similarly, the observation that chronic CCS treatment results in tolerance to nicotine that is accompanied by decreases in α-BTX binding has been made previously (28,29). We have interpreted this finding as suggesting that decreases in α-BTX binding may contribute to reduced sensitivity to nicotine. This interpretation seems less likely given that the mice cotreated with CCS and nicotine were more tolerant to nicotine than were those animals treated with nicotine or CCS alone even though they did not exhibit decreases in α-BTX binding. Thus, unless those brain regions that modulate all four of the responses measured exhibit an unusual pattern of receptor change, it seems most probable that mechanisms other than changes in α-BTX binding explain tolerance to nicotine.

One of the other mechanisms that may explain tolerance to nicotine is altered brain nicotinic receptor function. We have recently demonstrated that chronic nicotine infusion results in dose-dependent inhibition of two assays that measure the function of two different brain nicotinic receptors (19). This alteration in brain nicotinic receptor function parallels dose-dependent changes in sensitivity to nicotine (19). Perhaps chronic CCS treatment also alters brain nicotinic receptor function, and perhaps this effect is additive to that produced by nicotine alone.

Another possible explanation for tolerance is that the binding or function of brain nicotinic receptors that do not bind [^{3}H]-nicotine or [^{125}I]-α-BTX may be altered. [^{3}H]-Nicotine binds to receptors made up of α4 and β2 subunits (10,40) and [^{125}I]-α-BTX binds to the protein encoded by the α7 nicotinic receptor subunit gene (36). However, at last count, mammalian brain contains a total of seven α and three β nicotinic receptor subunits (14). Although the α4 and α7 receptor subtypes are clearly the most widespread in mouse brain (20), it is entirely possible that changes in these rarer subtypes could play a role in the altered response to nicotine elicited by either chronic nicotine or chronic CCS treatment.

Tolerance to nicotine might also occur if the rate of nicotine metabolism is changed. Studies done in mice (11,17) have not detected effects of chronic nicotine treatment on the rate of nicotine metabolism. However, altered metabolism might underlie the CCS-induced changes in sensitivity to nicotine since glucocorticoids can induce one or more of the cytochrome P-450 enzymes (7,12,33). Clearly, a direct analysis of chronic CCS effects on nicotine metabolism should be done to address this issue.

In conclusion, the data presented here demonstrate that tolerance to nicotine is induced by both chronic nicotine and chronic CCS treatment, and animals that are treated with both CCS and nicotine show enhanced tolerance, at least for some measures. Although chronic CCS and nicotine treatments have primary effects on two different nicotinic receptors in brain, it does not seem likely that these differences explain the additive tolerance seen when animals are cotreated with CCS and nicotine. At this point, it seems more likely that chronic

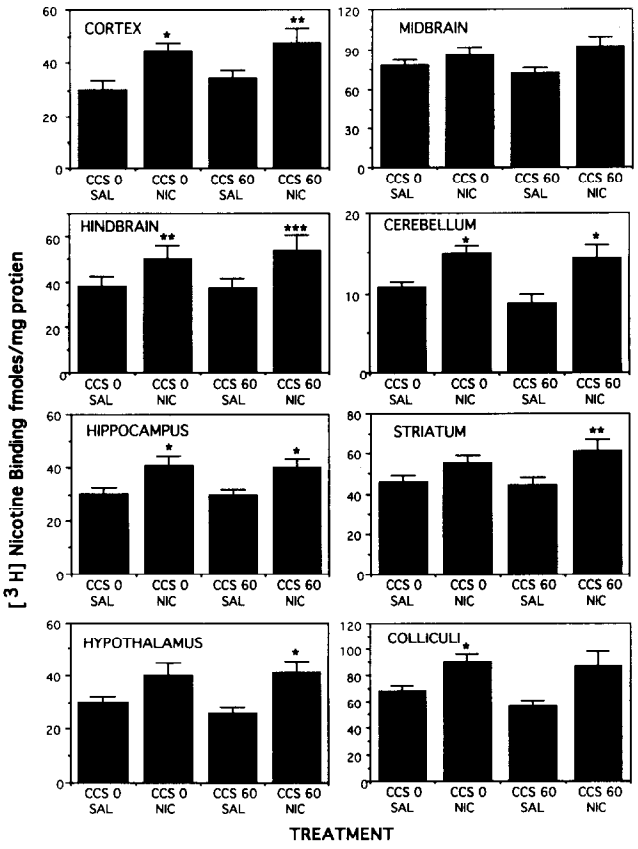

FIG. 6. Effects of chronic CCS and/or nicotine treatment on brain [^{125}I]-α-BTX binding. C57BL/6 mice were implanted with cholesterol (CCS0) or 60% CCS-containing (CCS60) pellets and infused with saline or nicotine for 9 days. The animals were sacrificed after behavioral testing and regional brain [^{125}I]-α-BTX binding measured as described in the Method section. Each bar represents the mean ± standard error of measurements made on eight separate animals. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

nicotine treatment evokes tolerance to nicotine, at least in part, by changing function of brain nicotinic receptors. The mechanisms underlying tolerance to nicotine evoked by chronic CCS treatment probably do not involve changes in α-BTX binding. Perhaps CCS also alters brain nicotinic receptor function or, given the well-established effects of CCS on cytochrome P-450 activities, perhaps chronic CCS treatment produces tolerance to nicotine because of an effect on nicotine metabolism.

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