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Corticosterone Reversibly Alters Brain α-Bungarotoxin Binding and Nicotine Sensitivity

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GRUN, E. U., J. R. PAULY, A. E. BULLOCK AND A. C. COLLINS. *Corticosterone reversibly alters brain α-bungarotoxin binding and nicotine sensitivity.* PHARMACOL BIOCHEM BEHAV 52(3) 629–635, 1995.—Previous studies have shown that chronic corticosterone (CCS) treatment via subcutaneous pellets elicits reduced sensitivity to many actions of nicotine in mice as well as decreased brain α-bungarotoxin (α-BTX) binding. We report here the time courses of altered sensitivity to nicotine, as measured by acoustic startle, Y-maze crossing and rearing activities, heart rate, and body temperature, and α-BTX binding during and after CCS treatment. CCS treatment resulted in rapid decreases in sensitivity to nicotine for four of the five responses that were measured, as well as rapid changes in α-BTX binding. Sensitivity to nicotine returned to control levels within 3 days following pellet removal, but α-BTX binding returned to control levels in most brain regions 9–11 days after pellet removal. Because the restoration of control sensitivity to nicotine occurred long before α-BTX binding returned to control levels, it seems likely that factors other than changes in α-BTX binding cause chronic CCS-induced changes in sensitivity to nicotine.

Nicotine Nicotinic receptors Corticosterone Locomotor activity Hypothermia

MULTIPLE nicotinic receptor subunits have been identified in mammalian brain. The most abundant receptors are identified by the high affinity binding of agonists such as L-[^{3}H]-nicotine, [^{3}H]-acetylcholine, [^{3}H]-methylcarbachol, or [^{3}H]-cytisine (1,25,31,35). Another site is labelled by the snake toxin α-[^{125}I]-bungarotoxin (α-BTX) (23,24,36). These two binding sites are differentially distributed in brain (2,9) and are encoded for by different genes. The [^{3}H]-nicotine binding site is most probably made up of the proteins produced by the α4 nicotinic receptor gene (4,38) and the α-BTX binding site is the product of the α7 nicotinic receptor gene (3,34).

The role of these two classes of nicotinic receptors in regulating behavioral and physiologic responses to an acute dose of nicotine has been studied using quantitative genetic analyses (13,17,20–22) and by chronic treatment (tolerance) studies (8,9,11,14–16). The genetic studies suggest that nicotine effects on measures such as acoustic startle, locomotor activity, heart rate, and body temperature correlate highly with the number of brain L-[^{3}H]-nicotine binding sites, whereas sensitivity to nicotine-induced seizures is significantly correlated with the α-BTX binding site. Similarly, the chronic treatment studies argue that tolerance to the locomotor activity and temperature depressant effects of nicotine are best explained by changes in the number or function of the [^{3}H]-nicotine binding sites (8,9,11,14–16), and changes in α-BTX binding seem to correlate with the development of tolerance to nicotine-induced seizures (19).

Recent studies that have assessed the role of glucocorticoid hormones in regulating response to nicotine (5,27–30) have demonstrated that adrenalectomy (ADX) results in increased sensitivity to most of the behavioral and physiologic responses to nicotine that we have routinely measured (e.g., acoustic startle, locomotor activity, heart rate, body temperature). Adrenalectomy had no effect on [^{3}H]-nicotine binding, but produced increases in α-BTX binding, although this was largely restricted to hippocampus. In contrast, chronic corticosterone (CCS) administration to adrenalectomized and sham-operated mice resulted in mice that were very insensitive to nicotine-

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induced changes in acoustic startle, activity in a Y-maze, heart rate, and body temperature (27,29). Curiously, chronic CCS administration was virtually without effect on L-[^{3}H]-nicotine binding, as measured in regionally dissected tissue, but the number of brain α-BTX sites was dramatically decreased throughout the brain following chronic CCS administration.

The glucocorticoid studies argue that the α-BTX binding site is important in regulating sensitivity to many of nicotine's effects, whereas the genetic studies and chronic nicotine studies argue that the [^{3}H]-nicotine binding site is most important in regulating these same responses. However, the responses to nicotine of chronic CCS-treated animals were measured at a time when the plasma concentrations of CCS were high. Consequently, it may be that elevated CCS levels, rather than changes in α-BTX binding, explain the subsensitivity to nicotine seen in chronic CCS-treated animals. The present study was performed in an attempt to ascertain whether increased plasma levels of CCS or decreased brain α-BTX binding underlie the reduced sensitivity to nicotine seen in chronic CCS-treated mice. Mice were adrenalectomized and implanted with CCS pellets for 1 week and the time courses for the induction of tolerance to nicotine and changes in brain α-BTX binding were examined. After 1 week, the pellets were removed and the return to normal of α-BTX binding and nicotine sensitivity were monitored. The results suggest that the reduced sensitivity to nicotine detected in CCS-treated animals occurs primarily because of high plasma CCS levels, not because of a reduction of brain α-BTX binding sites.

METHODS

Animals

Adult (60–90 days of age) female mice of the C3H strain were used for these studies. This strain was used for these studies because it is the most sensitive of several inbred strains that have been tested to CCS-induced modification of sensitivity to nicotine (30). These mice were maintained in the breeding colony at the Institute for Behavioral Genetics for more than 20 generations. The animals were housed under a 12 L : 12 D cycle with lights on at 0700 h, and were provided with pelleted food (Wayne Lab Blox, Madison, WI) and water ad lib.

Surgical Procedures and Corticosterone Replacement

Animals were anesthetized with pentobarbital (54 mg/kg), administered intraperitoneally (IP). Adrenalectomy (ADX) was performed using a dorsal approach. Steroid pellets (60–70 mg) were implanted subcutaneously in the nape of the neck (approximately 2.0 cm rostral to the dorsal incision) at the time of adrenalectomy or sham operation. The pellets were made as described in Pauly et al. (27) and contained 60% CCS, 40% cholesterol by weight (CCS60). Control pellets contained only cholesterol (CCS0). Molten hormone mixtures were pipetted into reservoirs of a one-grain pharmaceutical pellet mold. A small amount of peanut oil was added to the hormone mixture to make the pellet less brittle; the pellet mold was also lubricated with peanut oil to make the pellets easier to remove. In some experiments, CCS pellets were removed 1 week after implantation; these animals were used to examine the recovery of sensitivity to nicotine as well as the recovery of α-BTX binding.

Behavioral Testing

Animals were tested for nicotine sensitivity 1, 3, 5, and 7 days following surgery or at 1 or 3 days following pellet removal. All behavioral testing was performed between the hours of 0800 and 1300.

(−)-Nicotine base (Sigma Chemical Co., St. Louis, MO), purified by distillation, was dissolved in physiologic saline, neutralized with HCl, and injected in a volume of 0.01 ml/g body wt. Animals were tested following IP injection with saline or a single dose of nicotine (1.0 mg/kg). This dose of nicotine was chosen because it produces maximal, or near maximal, depression of Y-maze activity in the C3H strain (17).

Nicotine sensitivity was measured in a battery of tests that hae been previously described (12). Acoustic startle response was measured 1 min following nicotine (or saline) administration using a Columbus Instruments (Columbus, OH) Responder Startle Reflex Monitor. Ten auditory stimuli (6250 Hz, 120 db, 50 ms) were delivered in each 90-s test. A relative scale was assigned such that the total startle response for each animal was scored between 0 and 20. Nicotine effects on locomotor activity were measured in a symmetrical Y-maze, with each arm being 26.0 cm long, 6.1 cm wide, and 10.2 cm high. Each arm was divided into two sections and crosses from one arm or section to another were counted. The number of rears (animal standing on its hind legs) were also counted during the 3-min test. Y-maze activity was measured 4.5 min after nicotine administration. Heart rate was measured 8.5 min postinjection using an E and M Physiograph (Narco Biosystems, Houston, TX). We measured body temperature (rectal) 15 min following nicotine injection, using a Baily Instruments digital thermometer. The time points chosen for these measures reflect the times of maximal nicotine effect on each test that have previously been determined in our laboratory (12). A minimum of three animals from each treatment group were studied on each test day.

Corticosterone Radioimmunoassay

Immediately following behavioral testing, venous blood samples were collected by retro-orbital sinus puncture to verify successful adrenalectomy and the level of glucocorticoid replacement. The CCS radioimmunoassay described by Gwosdow-Cohen (6) was used as previously described (29). CCS antibody was obtained from G. Niswender (Department of Physiology and Biophysics, Colorado State University, Fort Collins, CO).

To establish control levels of CCS, the circadian variation in levels was determined. Blood samples were taken from control (no pellet) mice as described before at one of the following time points: 0300, 0700, 1100, 1500, 1900, or 2300 h. Each animal was used only once (n = 5/time point).

Tissue Preparation

After the collection of blood samples that were used for the CCS assay, an animal was killed by cervical dislocation and its brain was removed, washed, and then dissected into cortex, cerebellum, striatum, hypothalamus, hippocampus, colliculi (inferior and superior), hindbrain (pons-medulla), and midbrain (tissue remaining after dissection of other regions). Brain regions were placed in 10 vol. of ice-cold buffer (Krebs-Ringer's Hepes: NaCl, 118 mM; KCl, 4.8 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 2.5 mM; Hepes, 20 mM; pH adjusted to 7.5 with NaOH). Tissue preparation was essentially similar to that of Romano and Goldstein (31) as described by Marks et al. (18).

[^{3}H]-Nicotine Binding

The binding of L-[^{3}H]-nicotine (*N*-methyl-[^{3}H], specific activity, 60 Ci/mmol; New England Nuclear, Boston, MA) was determined at 4°C using the method of Romano and Goldstein (31) as modified by Marks and Collins (10). The final incubation volume was 250 μl and each sample contained 150–500 μg of tissue protein. Kinetic analysis (K_d and B_{max} determinations) was performed in cortical tissue using six increasing concentrations of radiolabeled nicotine (ranging from 0.6–20.0 nM). In nonkinetic experiments, nicotine binding was measured in each of the brain regions using a single concentration of labeled ligand (5.0 nM).

α-BTX Binding

The binding of α-[^{125}I]-BTX (Tyr-[^{125}I], initial specific activity, 219 Ci/mmol; Amersham, Buckinghamshire, UK) was performed at 37°C using the methods of Marks and Collins (10). Single-concentration assays were performed in each of the eight brain regions using 1.0 nM labeled α-BTX. Scatchard analysis of cortical tissue used six concentrations of labeled toxin (0.08–1.60 nM).

Protein Determination

Protein determinations were performed using the method of Lowry et al. (7) with bovine serum albumin as the standard.

Data Analysis

Data were analyzed by two- and one-way analyses of variance (ANOVA). When significant effects were detected, *t*-tests were used to make specific comparisons.

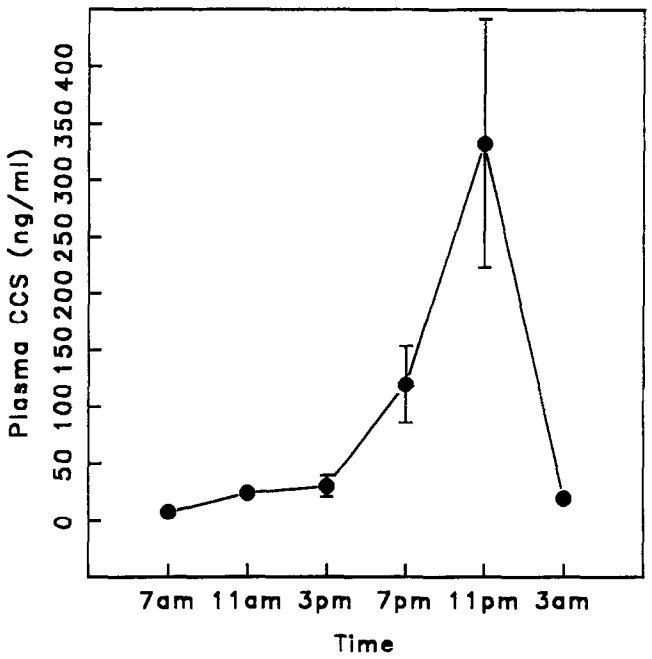

FIG. 1. Circadian variation in plasma CCS levels. Control mice were housed in groups of five and plasma CCS levels were determined at one of six time points during the day. Each point represents the mean ± SE obtained from five animals.

TABLE 1

PLASMA CCS LEVELS (ng/ml)

Pellet In			Pellet Out	
Time	CCS	Cholesterol	Time	Pellet Out
Day 1	858.5 ± 28.8	7.2 ± 0.4	Day 1	13.3 ± 1.4
Day 3	671.0 ± 61.4	10.8 ± 1.9	Day 3	14.3 ± 1.2
Day 5	397.2 ± 44.8	9.5 ± 1.2	Day 5	12.2 ± 1.2
Day 7	340.6 ± 36.4	21.0 ± 6.5	Day 7	12.9 ± 2.0
			Day 9	23.3 ± 3.4
			Day 11	12.2 ± 1.0
			Day 13	10.7 ± 0.9

Mice were adrenalectomized and treated with 60% CCS- or cholesterol-containing pellets for 1–7 days, and plasma CCS levels were measured as described in Methods. Separate animals were adrenalectomized and treated with CCS-containing pellets for 7 days. The pellets were then removed and CCS levels were measured 1–13 days after pellet removal. Data are presented as mean ± SE of 11–13 animals.

RESULTS

Plasma CCS Levels

Figure 1 depicts the results of the experiment in which plasma CCS levels were determined at six different time points using control (no pellet) animals. The ANOVA detected a significant [$F(5, 24) = 7.16$, $p < 0.001$] effect of the time of day on CCS levels. For most of the daylight hours plasma CCS levels were low, but at night (the animals' active period) levels rose over 300 ng/ml.

Plasma CCS levels were determined on each test day. In the first experiment, animals were adrenalectomized and implanted with 60% CCS/40% cholesterol (CCS60) or cholesterol (CCS0) pellets and tested for nicotine sensitivity/receptor binding on days 1, 3, 5, and 7 following pellet implantation. On the 8th day of the experiment the animals were anesthetized and the pellets were removed. Animals were tested for nicotine sensitivity 1 and 3 days after pellet removal and binding was measured on days 1, 3, 5, 7, 9, 11, and 13 following pellet removal. Plasma CCS levels on each of these days are presented in Table 1. CCS supplementation significantly increased plasma CCS levels on each test day, and immediately following pellet removal plasma CCS levels returned to levels seen in adrenalectomized mice. These levels were determined during the daylight hours and exceeded the levels seen in control animals measured at the same time. However, the levels measured at days 5 and 7 of CCS treatment were not significantly different from the levels seen in control animals at night.

Nicotine Sensitivity

Sensitivity to nicotine was determined on each of the days listed previously (six to 10 animals per treatment group). The ANOVA failed to detect significant effects of time on response to nicotine in the control animals; as a result, the data obtained with control animals were combined for days 1–7. Animals were tested only once.

Figure 2 presents the results of the startle response test. Data are expressed as nicotine-induced increase in startle response (nicotine-injected startle score minus the saline-injected average). One-way ANOVA indicated a significant

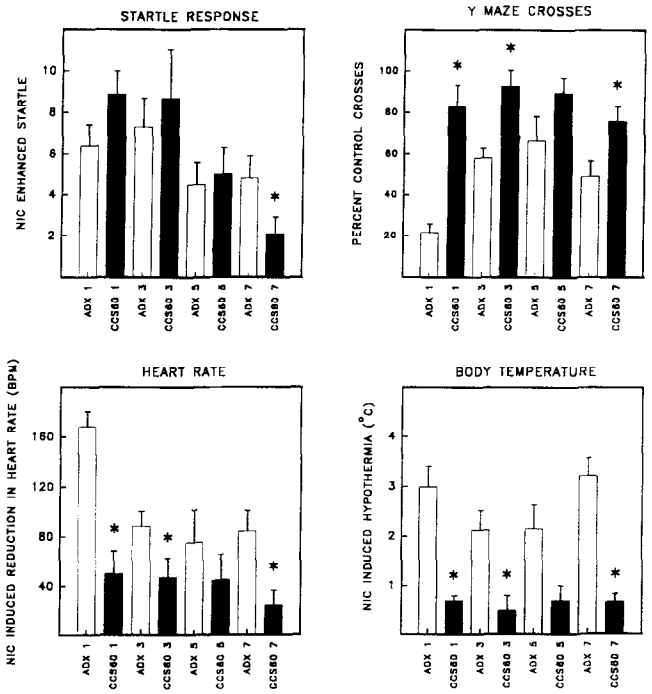

FIG. 2. Effects of nicotine in adrenalectomized (ADX) and chronic CCS-treated C3H mice. C3H mice were adrenalectomized and implanted with either a cholesterol- (control) or 60% CCS-containing pellet. Animals were challenged with saline or nicotine (1 mg/kg) and responses were measured as described in Methods 1, 3, 5, or 7 days after initiation of treatment. Control values obtained following saline injection were (mean ± SE): 5.4 ± 0.8 (startle), 76.7 ± 4.3 (Y-maze crosses), 755.6 ± 12.9 (heart rate), and 37.4 ± 0.2 (body temperature). Each bar represents the mean ± SE of six to nine animals. *$p < 0.05$.

effect of days on the response to nicotine [$F(7, 81) = 3.55$, $p < 0.01$]. Nicotine-induced increases in startle decreased with time after adrenalectomy. CCS treatment did not alter response to nicotine at 1 [$F(1, 11) = 2.74$], 3 [$F(1, 15) = 0.43$], or 5 [$F(1, 13) = 0.09$] days of treatment. However, after 7 days nicotine-enhanced startle was significantly [$F(1, 42) = 4.13$, $p < 0.05$] reduced by chronic CCS treatment.

Y-maze data (line crosses) are also presented in Fig. 2. In this case, response to nicotine is expressed as the percent change from control (activity following saline injection was scored as 100%). A significant effect of test day on nicotine sensitivity was detected [$F(3, 94) = 4.50$, $p < 0.01$]. In adrenalectomized control animals, a 1.0-mg/kg nicotine challenge decreased the number of Y-maze crosses by nearly 80% on day 1, whereas adrenalectomized animals that were implanted with CCS pellets showed a <20% decrease in activity following nicotine [$F(1, 14) = 30.06$, $p < 0.01$]. The differences between CCS-treated and control animals were less 3, 5, and 7 days after treatment started, but significant tolerance to nicotine persisted. Similar data were obtained for Y-maze rearing activity (data not shown). A significant overall effect of day following adrenalectomy was detected for the Y-maze rearing-reducing effects of nicotine [$F(3, 94) = 3.33$, $p < 0.01$] and significant ($p < 0.05$) decrease in sensitivity to nicotine's effects were elicited by CCS treatment when measured 1 [$F(1, 14) = 21.42$], 3 [$F(1, 17) = 4.95$], and 7 [$F(1, 44) = 4.69$] days after treatment started; the 5-day treated group did not show significant effects of CCS treatment.

Nicotine challenge produced a decrease in heart rate (Fig. 2) and, as was the case with the other measures, this effect varied with time after adrenalectomy [$F(3, 94) = 6.50$, $p < 0.01$]. CCS treatment reduced the response to nicotine at 1 [$F(1, 14) = 28.87$], 3 [$F(1, 17) = 28.19$], and 7 [$F(1, 44) = 7.67$] days after treatment started. Once again, the 5-day group did not show a significant reduction in sensitivity, but the trend was in the same direction as was seen at the other time points.

Injection with nicotine produced a decrease in body temperature (Fig. 2) that decreased somewhat with time following adrenalectomy [$F(3, 94) = 9.54$]. A significant ($p < 0.05$) decrease in this effect was produced by CCS treatment at all times tested except for day 5 (F values = 36.50, 11.99, 4.17, and 30.89 for the 1-, 3-, 5-, and 7-day time points, respectively).

Figure 3 depicts the responses of the adrenalectomized and CCS-treated groups 1 and 3 days after pellet removal. The controls used in this experiment were animals that had been adrenalectomized and implanted with cholesterol pellets for 7 days. At this point pellets were removed and animals were assigned to the 1- and 3-day withdrawal groups. Both control and CCS-treated animals failed to show nicotine-induced increases in startle 1 day after pellet removal, perhaps as a consequence of surgery. By 3 days after pellet removal, all evi-

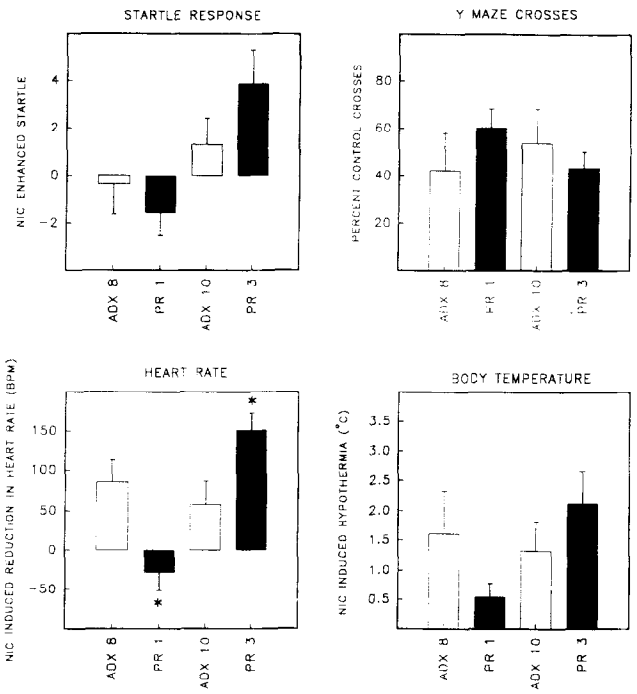

FIG. 3. Effects of nicotine following removal of control and CCS-containing pellets. Mice were adrenalectomized (ADX) and treated chronically with cholesterol- or 60% CCS-containing pellets for 7 days. The pellets were removed and the animals were challenged with saline or nicotine (1 mg/kg) 1 or 3 days after pellet removal. Responses were measured as described in Methods. Each bar represents the mean ± SE of seven animals for the 1-day data and eight to nine animals for the 3-day data. Control values obtained following saline injection were (mean ± SE): 5.9 ± 0.7 (startle), 80.1 ± 6.4 (Y-maze crosses), 742.0 ± 14.8 (heart rate), and 37.0 ± 0.3 (body temperature). *$p < 0.05$.

dence for altered sensitivity to nicotine had been lost in the chronic CCS-treated mice [$F(1, 15) = 1.98$]. Figure 3 also shows that chronic CCS-treated mice did not differ from controls at either 1 or 3 days after pellet removal for the Y-maze crossing test. Similar data were obtained for the Y-maze rearing test (data not shown). Altered sensitivity to nicotine persisted for 1 day after pellet removal for the heart rate test [$F(1, 12) = 7.58, p < 0.05$] but was gone by 3 days. Reduced sensitivity to nicotine-induced hypothermia was lost within the 1st day after pellet removal.

Effects of CCS Administration and Withdrawal on α-[^{125}I]-BTX Receptor Binding

The time courses of CCS-induced reductions in brain α-BTX binding and the normalization of binding after removal of the pellets are shown in Figs. 4 and 5 for eight brain regions.

The two-way ANOVA detected significant overall effects of CCS treatment in cortex [$F(1, 85) = 20.36, p < 0.001$]. An overall effect of day was not detected, but a significant Day × Treatment interaction was observed [$F(3, 85) = 3.72$, $p < 0.05$], presumably because α-BTX binding continued to decline as the time of treatment increased. Similarly, significant effects of CCS treatment were detected in hippocampus [$F(1, 85) = 84.04$], striatum [$F(1, 85) = 70.08$], hypothalamus [$F(1, 85) = 36.91$], cerebellum [$F(1, 85) = 7.49$], midbrain [$F(1, 85) = 20.93$], hindbrain [$F(1, 85) = 13.19$], and colliculli [$F(1, 85) = 14.10$]. However, only striatum [$F(3, 85) = 2.98$] and colliculli [$F(3, 85) = 2.39$] showed significant Treatment × Time interactions, which indicates that in most brain regions maximal, or near maximal, changes in α-BTX binding occurred within 24 h of pellet implantation.

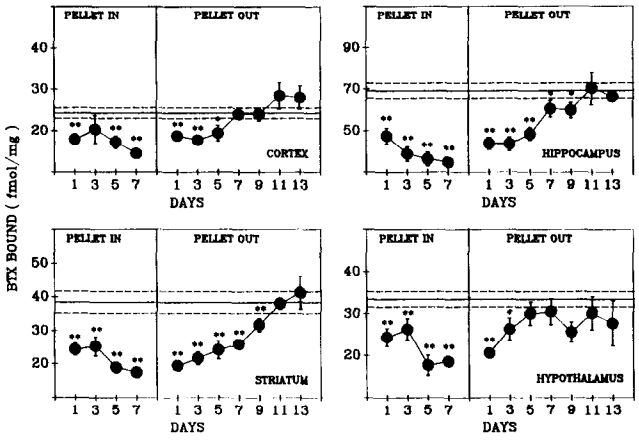

FIG. 4. Effects of chronic CCS treatment on brain α-[^{125}I]-BTX binding in four brain regions. Mice were adrenalectomized and treated chronically with cholesterol- or 60% CCS-containing pellets for 1-7 days. Regional brain α-BTX binding was measured as described in Methods. Other animals were treated for 7 days, the pellets were removed, and α-BTX binding measured 1-13 days after pellet removal. Each point represents the mean ± SE of nine to 10 animals for days 1-5 and 21 animals for day 7 of pellets in, and 13 for each day after pellets were removed. The ANOVA indicated no significant change in binding with time in the control group. Therefore, the control data were combined ($n = 43$) into the overall value. The horizontal solid line represents the mean control binding value, and the horizontal dashed line represents the limits of the SE. *$p < 0.05$; **$p < 0.01$; $n = 43$ for the control values.

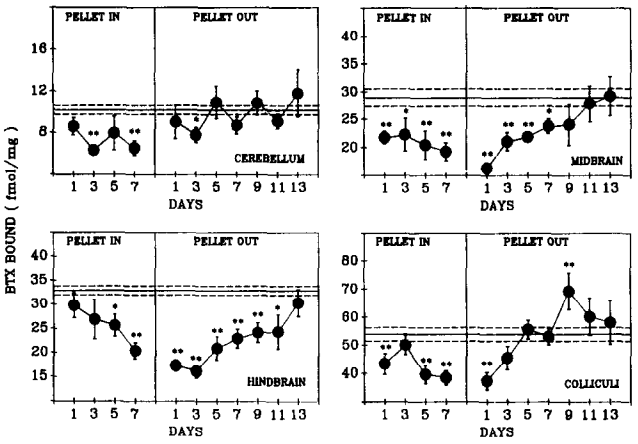

FIG. 5. Effects of chronic CCS treatment on brain α-[^{125}I]-BTX binding in four brain regions. See Fig. 4 for more details.

The CCS-induced decreases in α-BTX binding were reversible in every brain region; the ANOVA detected significant overall effects ($F = 5, 85$) in cortex ($F = 5.00$), hippocampus ($F = 11.32$), striatum ($F = 12.94$), hypothalamus ($F = 5.09$), cerebellum ($F = 2.56$), midbrain ($F = 3.84$), hindbrain ($F = 7.90$), and colliculli ($F = 6.19$). Control levels were regained in all of the brain regions but some variance was seen in time of recovery. In cerebellum control levels of binding were regained within 5 days after CCS treatment was stopped. Binding in the hindbrain, which decreased slowly after pellet implantation, was slowest to recover: 13 days were required to recover from chronic CCS-induced decreases in binding.

The results of a saturation analysis of CCS effects on cortical α-BTX binding are presented in Fig. 6. The affinity of cortical α-BTX binding proteins for α-BTX was not altered by chronic corticosterone administration as the K_d for binding did not differ between days ($F = 0.73$, not significant). The B_{max} for α-BTX binding was significantly affected by CCS chronic treatment. Significant overall effects of treatment [$F(1, 141) = 27.58$] and day [$F(4, 141) = 4.02$] were detected, and a significant Treatment × Day [$F(4, 141) = 5.99$] inter-

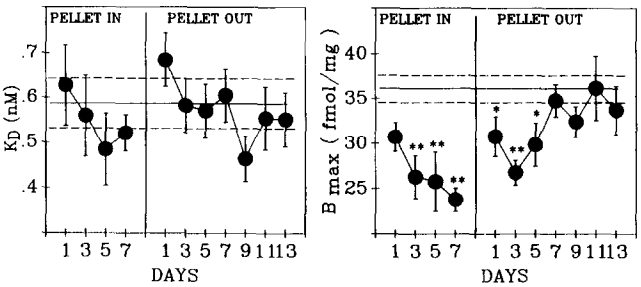

FIG. 6. Saturation analysis of the effects of chronic CCS treatment on cortical α-[^{125}I]-BTX binding. Animals were adrenalectomized and treated chronically with cholesterol- or 60% CCS-containing pellets for 1-7 days. Pellets were removed from 7-day animals. Saturation analyses of α-BTX binding were done using cortical tissue. Maximal binding (B_{max}) and affinity (K_d) were calculated as described in Methods. The horizontal solid line surrounded by two dashed lines presents the mean ± SE of control binding data. Each point represents the mean ± SE of 11-13 determinations. *$p < 0.05$; **$p < 0.01$.

action was detected. Significant reductions in maximum α-BTX binding were measured on days 3, 5, and 7 following pellet implantation on days 1, 3, and 5 after pellet removal. This result is virtually identical to the data presented in Fig. 4 (cortical α-BTX binding measured at a single ligand concentration).

Effects of CCS Administration on [^{3}H]-Nicotine Binding

Previous studies from our laboratory have demonstrated that after 7 days of chronic CCS treatment, the number of brain [^{3}H]-nicotine binding sites is relatively unaltered (26,27). In the present experiment we measured nicotine binding on each test day to detect possible changes that occur in the early phases of CCS administration. The effects of CCS administration on nicotine binding in four brain regions (cortex, hippocampus, striatum, and hypothalamus) are shown in Fig. 7. The ANOVA indicated that there were no significant effects of CCS administration on nicotine binding in any brain region. Similar results were obtained with cerebellum, midbrain, hindbrain, and colliculli (data not shown). Kinetic analysis of binding in cortex confirmed this observation as neither the K_d nor the B_{max} for nicotine binding varied with days of CCS treatment (data not shown).

DISCUSSION

The results reported here confirm earlier studies (27,29) demonstrating that chronic CCS treatment results in decreased sensitivity to several behavioral and physiologic actions of nicotine. This is accompanied by an extensive loss of brain α-BTX binding sites. These findings suggest that the reduced sensitivity to nicotine might arise as a consequence of a down-regulation of the α-BTX binding site. However, the studies reported here indicate that pellet removal resulted in rapid loss of the reduced sensitivity to nicotine. Control levels of sensitivity to nicotine were regained 1–3 days after pellet removal, whereas control levels of α-BTX binding were not regained (except in cerebellum) until a minimum of 5 days after pellet removal. Therefore, unless changes in specific nuclei are dramatically different from those detected in dissected tissue, it seems most probable that the downregulation of the α-BTX binding site is not the cause of the reduced response to nicotine. It is more likely that the elevated CCS levels somehow decrease sensitivity to nicotine. Further evidence supporting the latter argument comes from the observation that chronic nicotine injection results in tolerance to nicotine and increases in preinjection plasma CCS levels (28). Because chronic nicotine injection-induced tolerance is quickly reversed by adrenalectomy, it seems likely that the CCS plays a role in chronic injection-induced tolerance to nicotine.

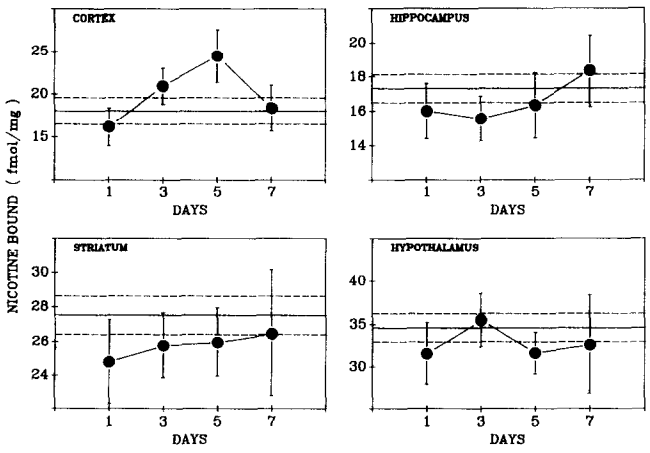

FIG. 7. Effects of chronic CCS treatment on brain L-[^{3}H]-nicotine binding. Animals were adrenalectomized and treated chronically with cholesterol- or 60% CCS-containing pellets for 1–7 days. L-[^{3}H]-Nicotine binding was measured in regionally dissected tissue as described in Methods. The horizontal line surrounded by two dashed lines represents the mean ± SE of control data (n = 43). Each point represents the mean ± SE of nine to 21 animals.

Not all of the responses to nicotine seem to be affected by CCS treatment. Nicotine-induced increases in acoustic startle, in particular, did not seem to be readily affected by chronic CCS treatment. Significant effects were not observed until 7 days after pellet implantation. The reduced sensitivity to nicotine was reversed quickly following pellet removal. Indeed, by 3 days after CCS withdrawal the CCS-treated mice were more sensitive than controls to nicotine's startle enhancing effects.

Chronic CCS treatment resulted in a reversible decrease in brain α-BTX binding in all of the brain regions, whereas [^{3}H]-nicotine binding was not affected in any region. However, these results should not be interpreted to indicate that the [^{3}H]-nicotine binding site is totally unaffected by chronic CCS treatment. In a recent autoradiographic analysis (26), we detected effects of CCS treatment on nicotine binding in seven of 83 regions measured (α-BTX binding was decreased in 77 of 115 regions). Thus, it seems likely that the CCS effect is not specific to the α-BTX binding site but some degree of selectivity clearly exists.

Prolonged CCS exposure and stress have been shown to accelerate the loss of hippocampal neurons that occurs during aging and also to increase the neurotoxicity of metabolic insults (32,33,37). These findings suggest that the loss of receptors that occurred following chronic CCS treatment was the result of neuronal loss. Because total, or near total, recovery of receptor numbers was seen in all brain regions, it does not seem likely that neurotoxic actions of CCS mediate the loss of α-BTX receptors.

The observation that chronic CCS-induced changes in α-BTX binding do not seem to underlie changes in sensitivity to nicotine does not mean that the α-BTX binding site is not involved in regulating responses to nicotine. Previous studies from our laboratory have demonstrated that there is a significant correlation between the number of hippocampal α-BTX receptors and sensitivity to nicotine-induced seizures in nicotine-naive mice. This correlation was based on the analysis of α-BTX binding and seizure sensitivity in 19 inbred strains of mice (20), as well as a study that performed a classical genetic analysis (21,22). Perhaps chronic CCS-induced changes in α-BTX binding would correlate closely with changes in sensitivity to nicotine-induced seizures.

The present study confirms our previous findings that chronic high-dose CCS treatment evokes reduced response to nicotine and a widespread downregulation of α-BTX binding (26,27). No changes in L-[^{3}H]-nicotine binding sites were observed. A reduced sensitivity to nicotine was seen without changes in L-[^{3}H]-nicotine binding and was readily dissociated from changes in α-BTX binding. Therefore, it does not seem likely that changes in receptor number underlie the altered sensitivity to nicotine unless changes in specific brain nuclei are especially important in regulating sensitivity to nicotine. This seems possible given that, even in grossly dissected tissue,

differences in return to control levels of α-BTX binding were seen. However, the best explanation for the data seems to be that nicotine sensitivity is not regulated by the number of α-BTX binding sites. It seems more likely that sensitivity is regulated by plasma CCS levels.

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