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Strain Differences in Adrenalectomy-Induced Alterations in Nicotine Sensitivity in the Mouse

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PAULY, J. R., E. A. ULLMAN AND A. C. COLLINS. *Strain differences in adrenalectomy-induced alterations in nicotine sensitivity in the mouse.* PHARMACOL BIOCHEM BEHAV **35**(1) 171–179, 1990.—Adult mice of four inbred strains (A, BUB, C57BL, DBA) and two selectively bred lines [Long-Sleep (LS) and Short-Sleep (SS)] were tested for differences in glucocorticoid regulation of nicotine sensitivity. One week following adrenalectomy (ADX), animals were tested for nicotine sensitivity in a battery of tests that included acoustic startle response, Y-maze activity (line crosses and rearings), heart rate and body temperature. Although each type of animal tested had increased nicotine sensitivity in at least one of the test battery measurements, there was clear evidence for a genetic influence on the scope of ADX-induced changes in sensitivity. LS animals had the largest increase in sensitivity with altered responses in four of five tests following ADX. The sensitivity of DBA animals was increased in two tests while for A, BUB, C57BL and SS animals, only one test was affected. ADX-induced alterations in nicotine sensitivity could not be explained on the basis of changes in nicotinic receptor number since changes were consistent across strains. The mechanism by which ADX causes increased nicotine sensitivity is not known. However, these data support the hypothesis that nicotine sensitivity is modulated by adrenal glucocorticoid secretion and also suggest that this phenomenon is under strict genetic control.

Nicotine Adrenalectomy Cholinergic receptors Strain differences

GENETIC factors have been shown to influence the use of tobacco by humans. Obviously not all humans use tobacco, and among those who do, the dynamics of smoking behavior differ extensively. Fischer (5) has reported a higher degree of concordance for tobacco use in monozygotic (identical) twins than in dizygotic (fraternal) twins. Shields (31) extended these findings to twin sets raised together or separately and found similar patterns of smoking in monozygotic twins, regardless of rearing conditions. Our laboratory has been investigating genetic regulation of nicotine action (e.g., acute sensitivity and the development of tolerance) using inbred strains of mice. To date, we have identified stocks of mice that differ extensively in sensitivity to nicotine; however, the biochemical substrates that underlie these differences in sensitivity have been difficult to uncover. Nicotine metabolism does not differ substantially between mouse strains (10,27) and studies investigating the distribution and/or number of central nervous system (CNS) nicotinic receptors have provided only partial explanation for the large differences in strain sensitivity (18,20). For example, recent studies in our laboratory have analyzed 19 inbred strains of mice for nicotine sensitivity and CNS nicotinic receptors (21,22). Approximately 36% of the variance in strain differences in nicotine sensitivity (measured in a battery of behavioral and physiological tests) could be accounted for on the basis of the number of CNS nicotinic receptors.

Recently, we have begun to investigate interactions between nicotine and the primary glucocorticoid produced by the mouse adrenal cortex, corticosterone (CCS). Nicotine, administered via injection or smoke inhalation, has been shown to induce a dose-dependent increase in plasma CCS (1, 2, 6, 12, 23, 34). This increase is not due to a direct activation of the adrenal gland, rather it is mediated through CNS nicotinic receptors since CCS release is blocked by centrally administered mecamylamine (a CNS nicotinic receptor antagonist) (6) or antibodies directed against nicotinic receptors purified from myasthenia gravis patients (1,33).

We have previously reported that the CCS released in response to nicotine administration modulates some of the behavioral and physiological actions of this drug. Adrenalectomized (ADX) animals (C3H strain) were significantly more sensitive to acute nicotine administration than were sham-operated controls as measured in a battery of tests (25). When chronic CCS was administered in the drinking solution of ADX animals, nicotine sensitivity was normalized. Interestingly, naive (unoperated) animals were subsensitive to nicotine when CCS was administered chronically, suggesting an antinicotine action of CCS. The present study was performed to test for genetic differences in nicotine/corticosterone interactions within various inbred strains and selectively bred lines

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of mice. Profound strain differences in the effects of adrenalectomy on nicotine sensitivity were determined, indicating that genetic factors are important determinants of nicotine/corticosterone interactions in mouse strains.

METHOD

Animals and Surgery

Animals of four inbred strains (A/J, BUB/J, C57BL/6Ibg and DBA/2Ibg) and two selectively bred lines (LS and SS) of mice were utilized in these studies. LS and SS mice have been selectively bred for differences in ethanol-induced sleep time, but also differ in sensitivity to nicotine (3). Animals were raised in the colony at the Institute for Behavioral Genetics and maintained on a 12-hour light/12-hour dark cycle with lights on at 0700. Food (Wayne Lab Blox) and water were provided ad lib. All surgical procedures and behavioral testing were performed between 0800 and 1300 on animals 60–90 days of age.

Animals were anesthetized with pentobarbital (54 mg/kg) and adrenalectomized using a dorsal approach. Surgery for sham-operated animals was identical to that for ADX animals except that the adrenals were not removed. Following surgery, animals were housed individually and provided with 0.9% saline as a drinking solution to insure ionic homeostasis in the absence of mineralocorticoid hormones. Behavioral testing was performed 7 days postsurgery.

Behavioral Testing

Nicotine was obtained from Sigma Chemical Co. (St. Louis, MO) and purified by distillation. For injections, nicotine was dissolved in physiological saline, neutralized with HCl and administered in a volume of 0.01 ml/g body weight. Doses were altered by changing the concentration of nicotine in the injection solution. Six–nine animals of each strain, surgical manipulation and nicotine dose were used for behavioral testing.

Nicotine sensitivity was measured in a multifactorial test battery that included acoustic startle response, Y-maze activity (crosses and rearings), heart rate and body temperature. Previous studies from our laboratory have demonstrated that no significant intertest interactions occur between these components of the test battery (20). The timing of these tests was determined from the results of a time course study on the effects of nicotine on several components of the test battery (18). Each test in the battery was conducted as follows.

Acoustic startle response. A Columbus Instruments Startle Reflex Monitor (Columbus Instruments, Columbus, OH) was used to quantitate the startle response elicited by a short auditory stimulus. Mice were placed in a Plexiglas cage inside a sound-attenuated box, and ten auditory stimuli were delivered (frequency, 6250 Hz; intensity, 120 dB; duration, 50 msec; interstimulus interval, 10 sec). For each stimulus, response amplitude and response latency were recorded. Startle scores were calculated based on the response amplitude for each stimulus (possible scores were 0 to 1,000). Each startle response from 0–20 units was scored as 0, 20–350 units as 1 and >350 units as 2, yielding a score per animal of 0–20. This test was initiated 2.5 min following nicotine injection.

Y-maze activity. The maze is a symmetrical Y-shaped runway with each arm being 26.0 cm long, 6.1 cm wide and 10.2 cm high. Each arm of the maze was divided into two sections and crosses from one section/arm to another were counted for 3 min. The number of rearings that occurred during the test period was also recorded. This test was conducted 4.5 min after nicotine administration.

Heart rate. After completion of the Y-maze test, mice were placed in a restrainer and needle electrodes were inserted through the skin (one behind the left foreleg and the other in front of the right hindleg). The electrodes were connected through a preamplifier to an E and M Physiograph (Narco Biosystems, Houston, TX). Heart rate was monitored for 6 sec beginning 8.5 min after nicotine injection.

Body temperature. Rectal temperature was measured using a Bailey Instruments (Saddlebrook, NJ) digital thermometer. The probe was lubricated with peanut oil before it was inserted 2.5 cm into the rectal cavity. Body temperature was measured 15 min following nicotine injection.

CCS Radioimmunoassay

In order to verify successful adrenalectomy, following behavioral testing, plasma CCS levels were determined using the radioimmunoassay described by Gwosdow-Cohen *et al.* (8) and modified for use in our laboratory as previously described (6,23). CCS antiserum was purchased from Dr. G. Niswender, Department of Physiology and Biophysics, Colorado State University, Fort Collins, CO.

Nicotinic Receptor Binding

The brains of some animals (C3H, C57BL, LS and SS) were assayed for CNS nicotinic cholinergic receptor binding to determine if ADX affected the number of these receptors. Only female LS and SS mice were used. Following the completion of behavioral testing, animals were decapitated and the brains were dissected into the following eight anatomical regions: cortex, striatum, midbrain, hypothalamus, hippocampus, colliculi, cerebellum and hindbrain. Tissue regions were placed in 10 volumes of HEPES-buffered Ringer's solution (NaCl, 118 mM; KCl, 4.8 mM; $CaCl_2$, 2.5 mM; $MgSO_4$, 1.2 mM; HEPES, 20 mM; pH adjusted to 7.5 with NaOH) and then frozen at −70°C. The particulate fraction from these samples was assayed for nicotinic receptor binding.

The binding of L-[^{3}H]-nicotine was measured using a modification of the procedure of Romano and Goldstein (28) as previously described (19). For C3H animals, K_D and B_{max} determinations were made using Scatchard Analysis; single point assays were performed using a single concentration of radiolabeled nicotine in all other strains. The binding of alpha-[^{125}I]-bungarotoxin (BTX) (New England Nuclear, Boston, MA) was performed as described by Marks and Collins (19); K_D, B_{max}, and single point assays were performed. Protein determinations were made using the method of Lowry *et al.* (15), using bovine serum albumin as a standard.

Data Analysis

Results from behavioral testing were analyzed by two-way analysis of variance (treatment × dose). ED_{50} values for nicotine dose-response curves were determined using linear regression. Differences in ED_{50} values between ADX and sham-operated animals were determined using a Student's *t*-test.

RESULTS

Nicotine sensitivity was determined in all animals one week following surgical manipulation. Sham-operated animals and unoperated animals were not significantly different in terms of nicotine response (unpublished observation). However, adrenalectomy resulted in increases in nicotine sensitivity that were dependent upon the strain/line of animal investigated. Table 1 presents a statistical summary of adrenalectomy effects on nicotine sensitivity in all strains of animals tested. Data for C3H animals are

TABLE 1

STRAIN COMPARISON OF ADRENALECTOMY EFFECTS ON NICOTINE SENSITIVITY IN A BATTERY OF TESTS

Strain	Startle Response	Y-Maze Rears	Y-Maze Crosses	Heart Rate	Body Temperature
A	n.s.	n.s.	n.s.	$F(1,24)=14.23$, $p<0.002$	n.s.
BUB	$F(1,32)=11.66$, $p<0.002$	n.s.	n.s.	n.s.	n.s.
C3H*	$F(1,36)=9.01$, $p<0.005$	$F(1,36)=10.59$, $p<0.003$	$F(1,36)=6.20$, $p<0.02$	$F(1,36)=9.79$, $p<0.004$	$F(1,36)=25.54$, $p<0.001$
C57	n.s.	n.s.	n.s.	$F(1,56)=6.46$, $p<0.02$	n.s.
DBA	n.s.	n.s.	n.s.	$F(1,55)=33.21$, $p<0.001$	$F(1,55)=19.08$, $p<0.001$
LSF	n.s.	$F(1,36)=5.30$, $p<0.03$	$F(1,36)=8.78$, $p<0.007$	$F(1,36)=9.17$, $p<0.006$	$F(1,36)=19.81$, $p<0.001$
LSM	n.s.	$F(1,37)=9.92$, $p<0.004$	$F(1,37)=7.68$, $p<0.01$	$F(1,37)=17.13$, $p<0.001$	$F(1,37)=15.81$, $p<0.001$
SSF	n.s.	n.s.	n.s.	n.s.	$F(1,31)=7.73$, $p<0.01$
SSM	n.s.	n.s.	n.s.	n.s.	$F(1,40)=4.21$, $p<0.05$

Two-way ANOVAs (treatment × dose) were used to test for differences between ADX and sham-operated dose response curves. Data for both male (M) and female (F) LS and SS animals are presented. *Behavioral data for C3H animals taken from Pauly *et al.* (25) and presented for comparative purposes. n.s. = not significant.

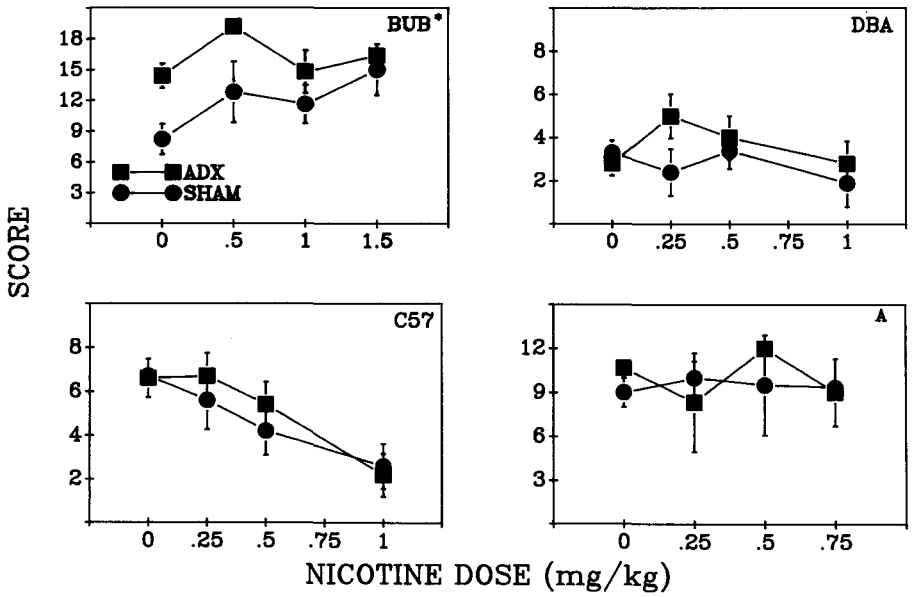

FIG. 1. Dose-response curves for nicotine effects on acoustic startle response for adrenalectomized (ADX) and sham-operated mice of four inbred strains. A significant increase in nicotine response was determined for the BUB strain (*$p<0.05$). However, this line difference is due to an increase in the baseline startle response (saline-injected) for these animals.

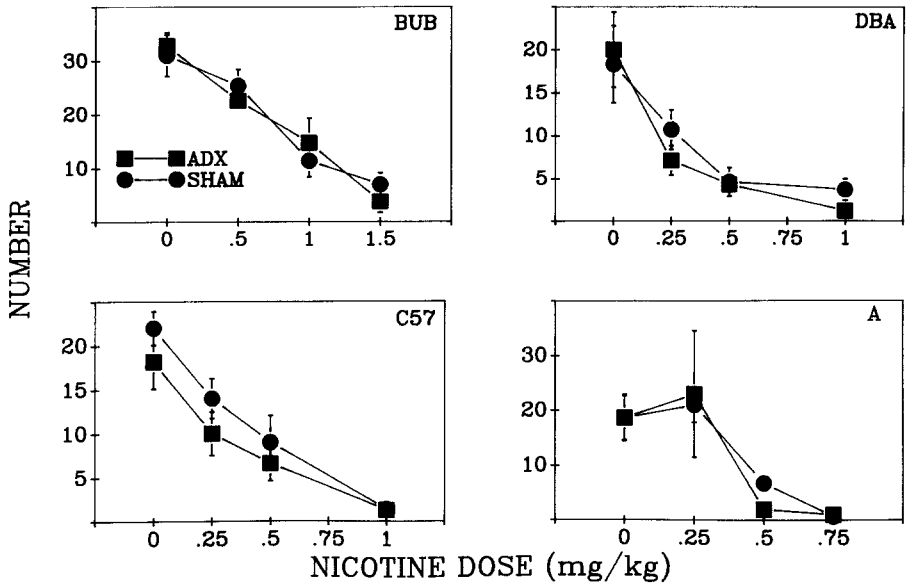

FIG. 2. Dose-response curves for nicotine effects on Y-maze rearing activity in ADX and sham-operated animals of four inbred strains. No significant effects of adrenalectomy on nicotine sensitivity were determined for this test.

taken from Pauly *et al.* (25) and are presented for comparative purposes. Each type of animal tested had increased nicotine sensitivity in at least one test battery parameter.

Startle response for four inbred strains of mice (A, BUB, C57BL, and DBA) is shown in Fig. 1. Nicotine has been shown to elicit an increase in acoustic startle response, but only in certain stocks of animals (21). Adrenalectomy resulted in a significant increase in startle sensitivity only for the BUB strain. However, the significant difference between the ADX and sham-operated BUB dose-response curves is secondary to an increase in the baseline startle (saline-injected) of ADX BUB animals, since the treatment by dose interaction for this group was not significant. In Fig. 2, Y-maze rearing activity is presented for sham-operated and ADX animals of the four inbred strains used in this study. Adrenalectomy did not alter nicotine sensitivity for this test in any of the strains tested.

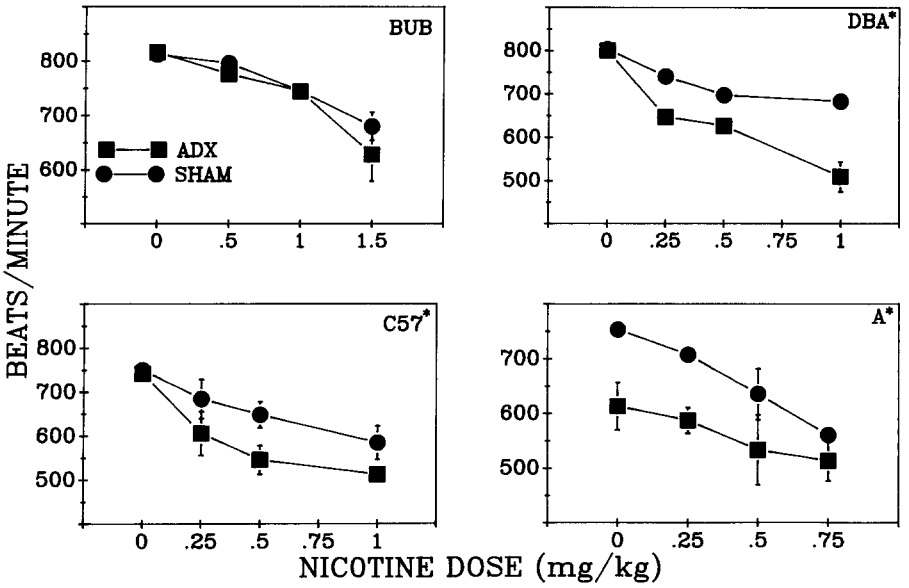

FIG. 3. Dose-response curves for nicotine effects on heart rate in ADX and sham-operated animals of four inbred strains. Lines were significantly different (*$p<0.05$) (ADX increased sensitivity to nicotine) for the C57BL, DBA and A strains. In the A strain, the significant difference between dose-response curves may be due to a decrease in baseline heart rate.

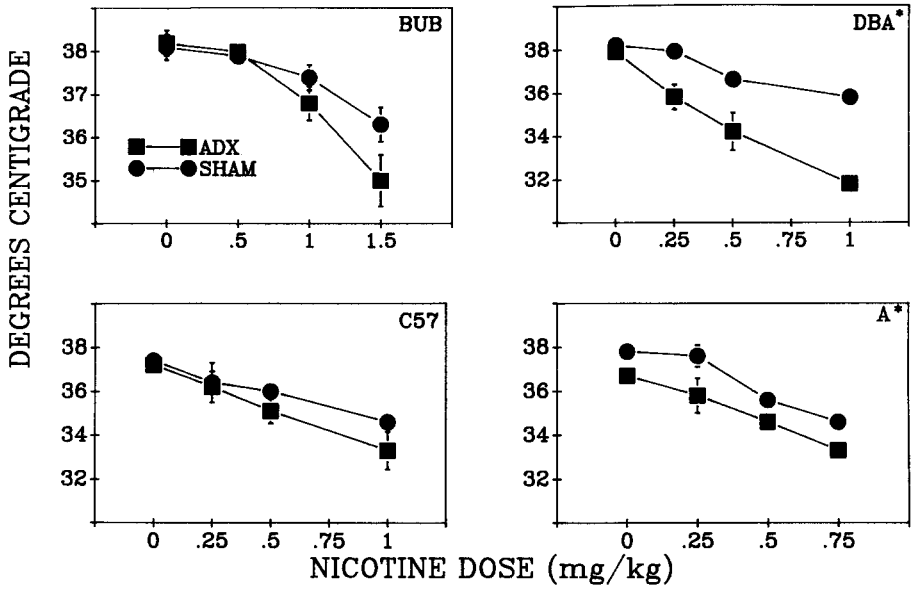

FIG. 4. Dose-response curves for nicotine effects on body temperature in ADX and sham-operated animals of four inbred strains. ADX increased sensitivity to the hypothermic action of nicotine in only the DBA and A strains (*$p<0.05$). The BUB and C57BL strains were not affected by ADX for this test battery parameter.

Three strains (A, C57BL and DBA) demonstrated significant increases in sensitivity to nicotine's effects on heart rates following adrenalectomy (Fig. 3), although in the A strain this difference may be due to a substantial decrease in the baseline heart rate. Only the DBA strain had an increase in nicotine sensitivity for the body temperature test (Fig. 4).

Test battery data (Y-maze crosses, Y-maze rears, heart rate and body temperature) for ADX and sham-operated LS and SS female mice are graphically presented in Figs. 5 and 6, respectively. For the LS line, nicotine sensitivity was increased in all test battery measurements except startle response (data not shown). In SS animals, only the body temperature parameter was significantly affected. Male animals were also tested, but the data are not presented as no significant effects of sex were determined (Table 1).

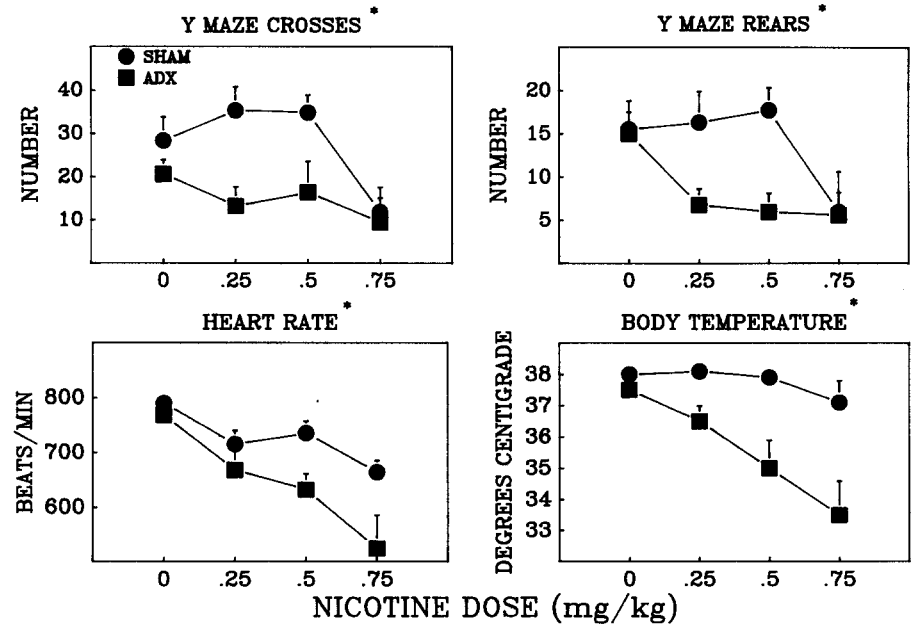

FIG. 5. Dose-response curves for nicotine effects for several test battery parameters for ADX and sham-operated LS female animals. ADX animals were significantly more sensitive to nicotine for each test battery parameter (*$p<0.05$).

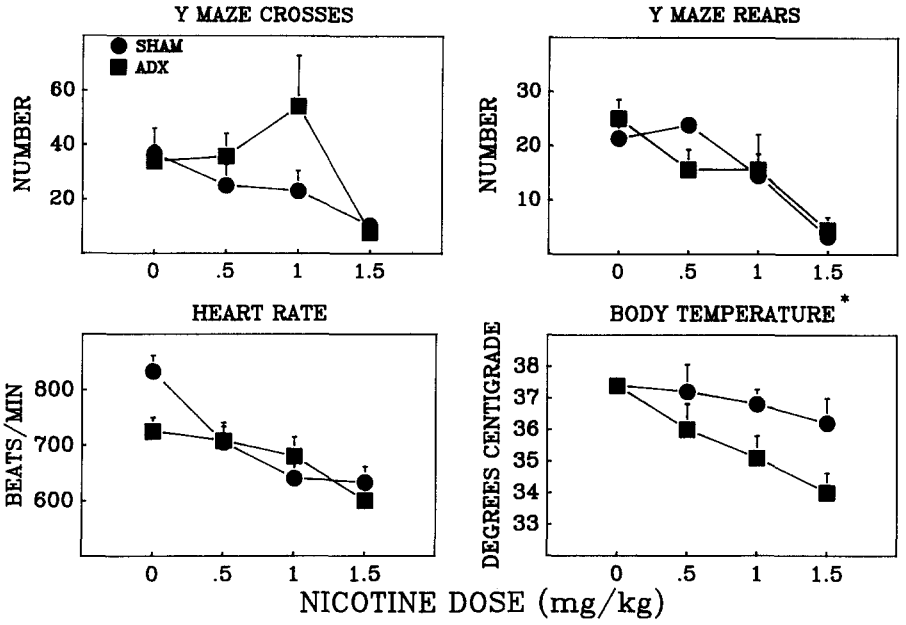

FIG. 6. Dose-response curves for nicotine effects for several test battery parameters in ADX and sham-operated SS female animals. ADX animals were more sensitive to nicotine for only the body temperature measurement (*$p<0.05$).

In Table 2, ED_{50} values for test battery parameters are presented for all animals tested. In general, the ED_{50} data support the findings of the two-way analysis of variance of dose-response curves. Adrenalectomy resulted in a decrease in ED_{50} values that was dependent on strain. The C3H strain had significant decreases in the ED_{50} values for Y-maze rears, heart rate and body temperature. There were no significant changes in ED_{50} values for BUB and SS male animals.

In order to verify successful adrenalectomy, following behavioral testing, plasma CCS levels were determined by radioimmunoassay. Postadrenalectomy CCS values for the strains/lines tested were: BUB (29.9 ± 5.7 ng/ml), C3H (35.2 ± 5.1), LS (37.8 ± 7.1), DBA (40.6 ± 6.4), C57BL (51.8 ± 6.3), A (52.6 ± 11.1), SS (60.0 ± 7.9). A one-way analysis of variance indicated that there were no significant differences in post-ADX levels of CCS between these strains.

Data for nicotinic receptor binding as measured by [^{3}H]-nicotine and alpha-[^{125}I]-bungarotoxin (in eight dissected brain regions) in ADX and sham-operated animals are shown in Table 3. Adrenalectomy did not consistently alter the number of [^{3}H]-nicotine binding sites in any brain region for any strain of animal tested. Alpha-[^{125}I]-bungarotoxin binding was significantly elevated in the hippocampal formation of all stocks of animals tested. This change in receptor binding was due to an alteration of the number of receptors and not to receptor affinity. K_D and B_{max} determinations were performed for C3H animals, a strain that was

TABLE 2

STRAIN COMPARISON OF ADRENALECTOMY EFFECTS ON ED_{50} VALUES FOR TEST BATTERY PARAMETERS

	Y-Maze Crosses		Y-Maze Rears		Heart Rate		Body Temperature	
Strain	SHAM	ADX	SHAM	ADX	SHAM	ADX	SHAM	ADX
A	0.50 ± 0.01	0.43 ± 0.22	0.47 ± 0.10	0.42 ± 0.26	0.72 ± 0.04	0.42 ± 0.09*	0.49 ± 0.12	0.48 ± 0.05
BUB	1.00 ± 0.40	0.77 ± 0.17	0.73 ± 0.19	0.76 ± 0.05	1.26 ± 0.24	0.96 ± 0.30	1.87 ± 0.43	1.20 ± 0.10
C3H	1.45 ± 0.53	0.77 ± 0.28	1.52 ± 0.47	0.30 ± 0.22*	0.89 ± 0.11	0.51 ± 0.14*	1.07 ± 0.02	0.42 ± 0.05*
C57	0.54 ± 0.06	0.50 ± 0.14	0.47 ± 0.11	0.43 ± 0.17	0.54 ± 0.11	0.25 ± 0.32	0.70 ± 0.08	0.50 ± 0.03*
DBA	0.51 ± 0.30	0.41 ± 0.22	0.46 ± 0.33	0.33 ± 0.28	0.68 ± 0.31	0.23 ± 0.20	0.80 ± 0.17	0.27 ± 0.08*
LSF	0.71 ± 0.32	0.37 ± 0.57	0.70 ± 0.03	0.45 ± 0.33	0.63 ± 0.27	0.31 ± 0.08	1.35 ± 0.37	0.40 ± 0.04*
LSM	0.56 ± 0.22	0.22 ± 0.10	0.52 ± 0.40	0.20 ± 0.17	0.39 ± 0.08	0.22 ± 0.04*	0.60 ± 0.10	0.17 ± 0.02*
SSF	1.02 ± 0.16	1.29 ± 0.13	1.09 ± 0.04	1.03 ± 0.32	0.46 ± 0.16	1.40 ± 0.38*	2.60 ± 0.36	0.35 ± 0.08*
SSM	0.80 ± 0.17	0.91 ± 0.21	0.62 ± 0.06	0.78 ± 0.72	1.11 ± 0.26	1.45 ± 0.17	0.76 ± 0.04	0.34 ± 0.25

ED_{50} values were calculated by linear regression of data from dose-response curves. All data are nicotine concentrations in mg/kg. The ED values calculated for the heart rate and body temperature were ED minus 100 beats per minute and ED minus 2 degrees Celsius, respectively. For Y-maze activity, the ED_{50} value represents that dose that decreases control activity (saline-injected) by 50%. Significant differences between treatment ED_{50} values (*$p<0.05$) were determined using a Student's t-test.

TABLE 3

[^{3}H]-NICOTINE AND ALPHA-[^{125}I]-BUNGAROTOXIN BINDING IN 8 BRAIN REGIONS OF ADX AND SHAM-OPERATED C57BL, LSF AND SSF ANIMALS

Strain	CX	CB	MB	HB	HP	ST	HY	CL
					[^{3}H]-Nicotine			
C3H SHAM (n=30)	14.4 ± 1.1	6.3 ± 0.7	32.0 ± 3.1	21.6 ± 2.6	19.2 ± 2.0	30.6 ± 2.3	29.9 ± 2.8	44.9 ± 5.0
C3H ADX (n=15)	17.3 ± 2.1	7.0 ± 0.9	35.7 ± 3.9	22.4 ± 3.5	19.0 ± 1.6	33.3 ± 4.6	30.3 ± 3.7	45.0 ± 6.0
C57 SHAM (n=8)	12.8 ± 0.9	6.4 ± 0.7	41.3 ± 3.2	23.6 ± 1.6	19.5 ± 2.1	30.0 ± 3.5	27.4 ± 2.7	51.9 ± 8.5
C57 ADX (n=8)	12.0 ± 2.1	4.9 ± 0.9	37.6 ± 3.4	16.1 ± 1.5*	16.4 ± 2.3	22.6 ± 1.9	21.7 ± 1.7	41.3 ± 16.4
LSF SHAM (n=8)	21.2 ± 4.0	8.0 ± 0.9	46.8 ± 7.4	28.1 ± 2.6	23.3 ± 2.9	30.9 ± 4.1	41.7 ± 4.1	63.4 ± 13.2
LSF ADX (n=9)	25.0 ± 3.1	7.1 ± 1.7	45.4 ± 9.2	26.1 ± 4.9	35.1 ± 4.8	41.7 ± 7.0	38.8 ± 8.4	67.0 ± 11.3
SSF SHAM (n=8)	18.1 ± 2.2	6.1 ± 1.3	35.5 ± 2.3	24.5 ± 1.2	21.4 ± 3.6	30.5 ± 2.0	30.3 ± 3.7	61.5 ± 6.1
SSF ADX (n=9)	23.5 ± 3.4	5.3 ± 1.2	41.9 ± 5.0	30.2 ± 4.7	28.4 ± 6.4	37.6 ± 2.9	25.0 ± 2.4	62.0 ± 4.9
					[^{125}I]-Bungarotoxin			
C3H SHAM	27.2 ± 1.2	12.3 ± 0.8	27.3 ± 1.5	31.0 ± 2.3	77.3 ± 5.1	48.9 ± 4.0	47.5 ± 3.8	93.3 ± 4.4
C3H ADX	26.4 ± 2.5	11.9 ± 0.8	27.3 ± 2.3	31.6 ± 1.9	97.4 ± 5.7*	54.6 ± 5.1	51.5 ± 4.8	88.1 ± 5.4
C57 SHAM	25.8 ± 1.0	11.5 ± 1.1	25.2 ± 1.5	30.8 ± 5.2	62.7 ± 2.2	39.3 ± 4.5	42.9 ± 2.3	90.0 ± 16.4
C57 ADX	29.6 ± 1.5	10.6 ± 1.8	28.6 ± 2.3	23.6 ± 1.2	72.8 ± 3.6*	41.5 ± 2.2	36.6 ± 2.6	83.4 ± 21.9
LSF SHAM	28.5 ± 3.7	10.4 ± 1.9	27.4 ± 3.0	33.4 ± 4.3	61.9 ± 5.4	40.9 ± 7.6	31.6 ± 5.7	74.9 ± 15.7
LSF ADX	33.1 ± 3.3	13.1 ± 1.3	20.1 ± 3.8	32.2 ± 5.4	93.8 ± 10.8*	38.3 ± 6.3	41.6 ± 6.5	96.1 ± 7.4
SSF SHAM	23.1 ± 2.2	11.3 ± 1.4	20.7 ± 2.2	29.0 ± 2.5	56.1 ± 4.0	29.4 ± 3.9	35.0 ± 5.6	77.1 ± 11.7
SSF ADX	27.5 ± 3.7	12.4 ± 1.9	20.2 ± 2.5	34.1 ± 4.7	83.0 ± 10.6*	39.1 ± 4.4	32.6 ± 3.9	67.3 ± 11.5

For each strain, treatment means were tested for significant differences (*$p<0.01$) using a Student's t-test. Although no consistent changes in [^{3}H]-nicotine binding were determined, alpha-[^{125}I]-bungarotoxin binding was significantly elevated in the hippocampus of all ADX mouse strains. Most binding values were determined using a single concentration of radiolabelled ligand and are presented in fmols/mg protein. For some animals of the C3H strain (n=7), K_D and B_{max} determinations were made using Scatchard Analysis. No change in K_D was detected (sham $K_D=0.640 \pm 0.03$ nM; ADX $K_D=0.681 \pm 0.02$ nM). CX, cortex; CB, cerebellum; MB, midbrain; HB, hindbrain; HP, hippocampus; ST, striatum; HY, hypothalamus; CL, colliculi.

maximally affected by ADX (see Table 3). BTX binding in other brain regions was not affected by adrenalectomy.

DISCUSSION

Previous studies in our laboratory have demonstrated that adrenalectomized C3H mice have increased sensitivity to nicotine as measured by a variety of physiological and behavioral tests (25), indicating that nicotine sensitivity may be regulated, in part, by plasma CCS levels. The results of the present study indicate that there are important genetic components to nicotine/corticosterone interactions in mice. All strains/lines investigated had adrenalectomy-induced increases in nicotine sensitivity for at least one test battery parameter, the most commonly affected being body temperature. The types of animals that had maximal increases in nicotine sensitivity following adrenalectomy were C3H [as reported by Pauly *et al.* (25)] and LS. The strains of animals least affected by adrenalectomy were A, BUB, C57BL and SS. No significant sex differences of adrenalectomy effects on nicotine sensitivity were present in LS and SS animals.

Previously, we determined that adrenalectomy does not alter nicotine metabolism. Blood and brain levels of nicotine were not significantly different between ADX and sham-operated C3H animals (25). Although we have not tested for ADX-induced alterations in nicotine metabolism in the strains of animals used in the present study, it is unlikely that changes in nicotine metabolism account for the large strain differences in effects of adrenalectomy on nicotine sensitivity. If adrenalectomy altered nicotine metabolism, it would be expected that nicotine sensitivity would be altered for all test battery parameters for all stocks of mice. Since this is not the case, and also because nicotine metabolism is not altered in a strain maximally affected by adrenalectomy (C3H), we conclude that strain-selective changes in nicotine metabolism probably do not explain genetic differences in adrenalectomy effects on nicotine sensitivity.

Because earlier studies from our laboratory have shown that the number of brain receptors that bind nicotine partially regulate sensitivity to nicotine (20), it seemed possible that adrenalectomy-induced changes in nicotine sensitivity were due to changes in the

number and/or functional status of central nervous system nicotinic receptors. Adrenalectomy did not significantly alter the number of CNS nicotinic sites labeled by [^{3}H]-nicotine in any brain region of the strains tested. The binding of alpha-[^{125}I]-bungarotoxin was affected by adrenalectomy, but not in a strain-dependent fashion; binding was elevated in the hippocampus of all strains tested. No change in receptor affinity (K_D) was detected. Thus, changes in receptor number cannot explain differential sensitivity to nicotine following adrenalectomy in two strains of mice that are affected maximally by this treatment (C3H, LS) and two strains that are affected minimally (C57BL, SS). The possibility that adrenalectomy causes changes in the functional status of these receptors that may be dependent on strain has not been eliminated. It may be that adrenalectomy affects the rate of nicotinic receptor desensitization (13) in some strains of mice, but not others.

Many studies have shown that, in rodents, the effects of nicotine are regulated by genetic factors (7, 9, 10, 21, 22, 24). Inbred strains of mice differ by a factor of 2–3 in nicotine sensitivity without any differences in nicotine metabolism (18,27). Initial sensitivity of these strains to nicotine does not seem to be of predictive value in estimating whether a strain will or will not become more sensitive to nicotine following adrenalectomy. The two strains of mice that were most affected by adrenalectomy (C3H and LS) are at opposite ends of the sensitivity spectrum with LS being very nicotine-sensitive and C3H being nicotine-insensitive (3,21).

A previous study from our laboratory has demonstrated genetic differences in the magnitude and duration of CCS release induced by nicotine (6). The rank order of net nicotine-induced CCS release (the net difference in CCS levels seen following nicotine and saline administration) for the strains used in this study was DBA > A > C3H = C57BL. Two strains of mice that are not significantly different in terms of nicotine-induced CCS release (C3H and C57BL) differ maximally in adrenalectomy-induced changes in nicotine sensitivity (C3H—increased nicotine sensitivity in five tests; C57BL—increased nicotine sensitivity in one test). Although LS and SS animals differ slightly in nicotine-induced CCS release (26), they differ maximally in change in nicotine sensitivity following adrenalectomy. Thus, it is not likely that initial sensitivity to nicotine-induced adrenocortical activation is a primary determinant of ADX-induced nicotine sensitivity.

Hummel (11) demonstrated the presence of histologically intact accessory adrenal cortical nodules in mouse strains. The nodules were unilaterally distributed (with a preponderance on the left side) and females generally had more than males. Hummel examined a total of 4040 mice (of nine inbred strains) and found accessory adrenal tissue in 50.4% of the animals. All strains examined demonstrated the presence of some accessory adrenal tissue; however, there were profound genetic influences on the incidence of the nodules. Of the nine strains examined by Hummel, four were utilized in the present study. These strains and the percentage incidence of accessory adrenal tissues are: C3H (41.3%); A (49.7%); DBA (52.9%); and C57 (54.3%). Interestingly, the C3H strain had the lowest incidence of nodules and was suggested by Hummel to be the most appropriate stock of mice for studying the effects of adrenalectomy (11). It is not known whether or not the amount of accessory adrenal tissue present in ADX animals is sufficient to produce enough CCS to maintain life and adjust to stress, but we were able to measure postadrenalectomy levels of CCS in our animals.

Although there were no overall effects of strain on the postadrenalectomy levels of CCS; the levels of CCS were the lowest in the BUB strain (29.9 ± 5.7 ng/ml) which was relatively unaffected by ADX and the C3H strain (35.2 ± 5.1 ng/ml) which was affected most. Thus, two strains that differed maximally in adrenalectomy effects were very similar in postadrenalectomy CCS levels. SS mice, on the other hand, had the highest postadrenalectomy CCS and were insensitive to the effects of this surgery. It should be noted that low plasma levels of CCS are difficult to measure using radioimmunoassay (our lowest standard concentration is 25 ng/ml and the reliability of the assay at this portion of the curve is decreased).

Changes in nicotine sensitivity subsequent to adrenalectomy could be due to the lack of adrenal cortical hormones or, alternatively, these changes could be secondary to the variety of other neurochemical aberrations incurred by this surgery (4, 16, 23, 30, 32). For example, basal and stress-induced levels of adrenocorticotrophic hormone (ACTH) and corticotrophin releasing hormone (CRH) are significantly elevated following adrenalectomy due to the lack of CCS negative feedback on these systems (17). We have shown previously that the effects of ADX are reversed by chronic CCS hormone therapy. Importantly, it was also demonstrated that chronic dexamethasone [a synthetic glucocorticoid that normalizes ACTH levels in brain but acts through neuronal receptors distinct from CCS binding sites (29)] does not reverse the effects of ADX except on nicotine-induced hypothermia (25). In the present study, the test battery measurement most commonly affected in ADX animals was body temperature. Possibly this parameter was the most often affected because all strains of animals probably have increased ACTH levels following removal of the adrenal glands and ACTH has been shown to have hypothermic actions in rodents (14).

Obviously, the differential effects of CCS and dexamethasone replacement need to be tested in all of the strains of animals utilized in the present study.

In conclusion, since mouse strains differ qualitatively and quantitatively in ADX-induced changes in nicotine sensitivity, it is inferred that they differ in response to some substance released by the adrenal gland, presumably CCS. Nicotine stimulates CCS secretion in all mouse strains that we have investigated; however, since ADX affects only some strains of mice and only in certain test battery parameters, it is apparent that genetic regulation of nicotine/CCS interactions occurs in mouse strains. Further investigation of such phenomena may elucidate biochemical factors that underlie the substantial strain differences in acute nicotine sensitivity.

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