Pharmacology Biochemistry & Behavior, Vol. 30, pp. 1059–1064. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Genetic Differences in Plasma Corticosterone Levels in Response to Nicotine Injection

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Received 8 October 1987

FREUND, R. K., B. J. MARTIN, D. A. JUNGSCHAFFER, E. A. ULLMAN AND A. C. COLLINS. *Genetic differences in plasma corticosterone levels in response to nicotine injection.* PHARMACOL BIOCHEM BEHAV **30**(4) 1059–1064, 1988.—Changes in plasma corticosterone (CCS) levels following intraperitoneal injections of nicotine were measured in four inbred mouse strains: DBA/2Ibg, C57BL/6Ibg, C3H/2Ibg, and A/J. In all four strains, nicotine produced a dose-dependent (0.5–2.0 mg/kg nicotine) increase in plasma CCS levels which peaked 10–30 min after injection. Saline increased plasma CCS levels in C57BL, A, and C3H, but not in DBA mice. After correcting for plasma CCS levels produced by saline injection, the nicotine-induced rise in plasma CCS was significantly lower for the C57BL strain than for the other three strains tested. These mouse strains also varied in their responses to saline injection with the rank order: C57BL > A = C3H > DBA. However, the two most divergent strains (C57BL and DBA) did not differ in the effects of a cold water stress. The response to nicotine was completely inhibited by mecamylamine in two strains tested (C3H and C57BL) whereas the response to saline injection was unaffected, suggesting that only the response to nicotine was mediated by nicotinic receptors. It is clear that elevations in plasma CCS induced either by saline injection or by nicotine are influenced by genetic factors.

Nicotine Corticosterone Genotype Inbred strains Mouse Stress

MANY smokers identify the reduction of negative affect or the production of a relaxed state as motives for smoking [41]. These effects may be due to nicotine because smoking nicotine-containing cigarettes reduces anxiety whereas smoking zero-nicotine cigarettes does not [33]. Smokers also appear to smoke more when confronted with a stressful situation [17,31]. Thus, it may be that nicotine facilitates the release of an endogenous substance(s) that is useful in coping with a stressful environment.

Several studies in animals have demonstrated that the acute administration of nicotine by intraperitoneal (IP) injection [4, 8–10], subcutaneous injection [5,38], or cigarette smoke inhalation [1,21] induces increases in corticosterone (CCS). This increase in CCS levels is mediated by activation of central nervous system nicotinic receptors since it has been demonstrated that stress-induced release and basal release of CCS are regulated by nicotinic receptors in the rat [7]. Tolerance to nicotine-induced release occurs in rats [4,5].

It is unknown whether CCS plays a role in the behavioral and physiological effects elicited by nicotine. However, several studies have demonstrated that CCS or its metabolites decrease the excitability of the central nervous system [18], in particular the hippocampus [35, 36, 42] and recent evidence suggests that corticosterone and derivatives modulate the activity of the GABA/benzodiazepine receptor complex [18, 23, 24]. In addition, preliminary (unpublished) studies from our laboratory indicate that adrenalectomized mice are much more sensitive to the behavioral and physiological effects of nicotine than are sham-operated mice. Thus, it seems possible that some of the behavioral or physiological effects of nicotine may be mediated by CCS.

Genetic factors may influence whether humans smoke [34,40]. Whether these genetic factors relate to nicotine is unknown. However, it is well documented that genetic factors regulate the response of laboratory animals to nicotine. Inbred mouse strains differ with respect to the effects of nicotine on locomotor activity as measured in the Y-maze [26] and in the open-field arena [3,25]. Rat strains also differ with respect to the effects of nicotine on locomotor activity [6, 14, 15, 37]. Mouse strains differ with respect to the effects of nicotine on respiration rate, acoustic startle re-

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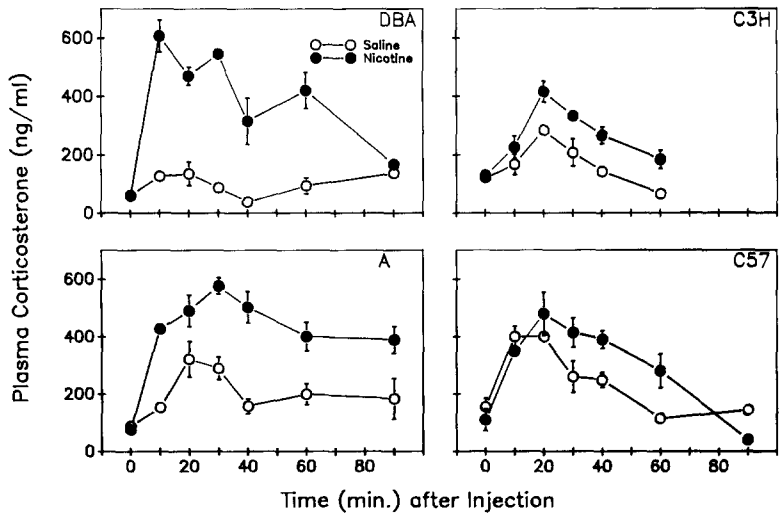

FIG. 1. Time course for changes in plasma CCS levels following IP injection of saline or nicotine (2.0 mg/kg) for four inbred mouse strains. Each data point represents the mean±S.E.M. for 5–16 animals at each point. Each animal was tested only once.

sponse, heart rate, and body temperature [25,27], and strain differences have been detected for nicotine-induced seizures [28, 29, 39] and nicotine-induced excitation of hippocampal neurons [13]. The mouse strain differences in behavioral and physiological responses to nicotine do not appear to be due to differences in the rate of metabolism of nicotine [32] or to differences in the number or affinity of brain nicotinic receptors [25] with the possible exception of nicotine-induced seizures [28,29]. Thus, strain differences in response to nicotine are well documented, but an explanation for these differences is not apparent. One possible explanation may involve nicotine-induced CCS release. Mouse strains could differ in response to nicotine, at least in part, because of differences in the amount of CCS released by nicotine. The data presented here comprise an initial test of this hypothesis.

METHOD

Compounds

L-Nicotine, mecamylamine hydrochloride, and corticosterone were obtained from Sigma Chemical Co. (St. Louis, MO). ^{3}H-Corticosterone (specific activity: 50 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). Highly specific [16] anti-corticosterone antisera was obtained from Dr. Gordon Niswender (Dept. of Physiology, Colorado State University, Fort Collins, CO).

Animals

Mice from the inbred strains DBA/2Ibg, C57BL/6Ibg, C3H/2Ibg, and A/J were tested. These strains were used because previous studies from our laboratory have demonstrated that these strains differ in behavioral and physiological responses to nicotine [25,26]. These mice were weaned at 25 days and housed in groups of like-sex littermates and were tested at 60–90 days of age. Since in preliminary studies there was no consistent sex difference in CCS levels following nicotine injection, animals from both sexes were tested. A 12-hr light/12-hr dark cycle (lights on at 7 a.m. until 7 p.m.) was maintained, and animals were allowed free access to food (Wayne Lab Blox) and water.

Blood Sampling

Animals to be tested were placed in the testing room and weighed at least 2 hr before testing which was done between 0900 and 1200 hr. For determination of the time course of CCS responses, nicotine (2 mg/kg) or saline was injected in a volume of 0.01 ml/g. Blood samples (40–60 μl) were obtained from the retro-orbital sinus using heparinized microhematocrit tubes at various times after injection. Each mouse was sampled only once in order to minimize the potential effects of sampling-induced stress.

Comparisons of nicotine-induced CCS release were made by assessing the time course of the CCS response and constructing dose-response curves in each of the four mouse strains. The time course analyses involved injecting the animals with a 2 mg/kg dose of nicotine or with saline (controls). Blood samples (40 μl) were taken immediately after injection (0 time) or 10, 20, 30, 40, 60, and 90 min later. Dose-response curves were constructed by injecting mice with nicotine (0.5–2.0 mg/kg) or saline in a volume of 0.01 ml/g. Blood samples were taken 25 min after injection. This time was chosen because maximal, or near maximal, elevations in CCS were seen at this time in the time course study.

The effects of mecamylamine pretreatment on nicotine-induced elevations in plasma CCS levels were determined in two of the strains, the C3H and C57BL. In the first study, mice were injected with mecamylamine (0, 0.1, 1, 2, or 4 mg/kg for C3H mice and 0, 0.1, 1, 2, 4, or 6 mg/kg for C57BL mice) 10 min before injection with a 2 mg/kg nicotine dose. Blood samples were taken 25 min after the nicotine injection. In the second experiment C3H and C57BL mice were injected with either saline or 4 mg/kg mecamylamine 10 min before injection with saline or nicotine (2 mg/kg). Four treatment groups were developed: saline-saline, saline-nicotine, mecamylamine-saline and mecamylamine-nicotine. This procedure assessed the effects of two injections and the

effects of mecamylamine on both stress-induced and nicotine-induced increases in plasma CCS.

Strain differences in nicotine-induced increases in plasma CCS were detected. In order to assess whether these differences are specific to nicotine, animals from the DBA and C57BL strains were subjected to a cold water stress. These two strains were used because they differed maximally in both nicotine- and saline-induced increases in plasma CCS. Animals were placed in cold water (5–7°C) for 3 min. Blood samples were taken for CCS assay immediately upon removal from the water and 10, 20, 30, 40, 60, or 90 min later.

Corticosterone Determination

Plasma CCS was determined by a modification of the radioimmunoassay (RIA) described by Gwosdow-Cohen *et al.* [16]. Plasma was separated by centrifugation and plasma proteins were precipitated with absolute ethanol. After removing proteins by centrifugation (1400×g for 15 min), a known quantity of supernatant was evaporated to dryness, resuspended in phosphosaline buffer with gelatin (PBSG), and incubated at room temperature for 30 min. The efficiency of this steroid extraction procedure was determined and taken into account for subsequent calculation of CCS levels. The composition of the buffer is 0.08 M Na_2HPO_4, 0.02 M NaH_2PO_4, 0.15 M NaCl, and 0.10% gelatin (pH 7.2). Corticosterone antiserum was added to the reconstituted samples and the mixture was incubated for 90 min at 4°C. ^{3}H-Corticosterone was included and allowed to equilibrate overnight at 4°C. Unbound CCS was precipitated by addition of a charcoal suspension followed by centrifugation (1400×g for 10 min). Known quantities of supernatant were transferred to scintillation vials, and scintillation cocktail (Safety-Solve, Research Products International) was added for determination of radioactivity.

Standard curves were performed for each RIA using varying quantities of unlabelled CCS (10–1100 ng/ml). Nonspecific binding was determined in the absence of antiserum, and maximum binding was determined in the absence of unlabelled CCS. The interassay coefficient of variation, calculated for 20 representative assays, was 15.2%. The average intraassay coefficient of variation, which was based on triplicate measurements from the same 20 assays, was 5.5%.

Data Analysis

Analyses of strain and dose effects were performed using two-way analysis of variance techniques. Significance of strain rank-orders was determined using Newman-Keuls post hoc analysis. Where indicated, single point comparisons were made using Student's *t*-test.

RESULTS

The time courses for the CCS response following injection of either saline or nicotine (2.0 mg/kg) are depicted in Fig. 1. Nicotine injection resulted in an increase in plasma CCS levels with a peak response 10–30 min after injection. For most strains tested, this increase exceeded that induced by saline injection alone; C57BL mice were the exception, exhibiting similar peak CCS levels after saline and nicotine injection ($p>0.10$, *t*-test).

Following nicotine injection, there were significant overall effects of strain, $F(3,180)=18.6$, $p<0.001$, and time $F(5,180)=43.8$, $p<0.001$, for the time course of changes in plasma CCS levels. Because the significant strain effect may only reflect differences in baseline CCS levels, it was necessary to examine the significance of the strain by time interaction term. This parameter was significant, $F(15,180)=3.8$, $p<0.001$, indicating true strain differences to the CCS-elevating effects of nicotine injection. Post hoc analysis gave the following rank order for elevation of CCS levels after nicotine (2.0 mg/kg) injection: A = DBA > C57BL = C3H ($p<0.01$).

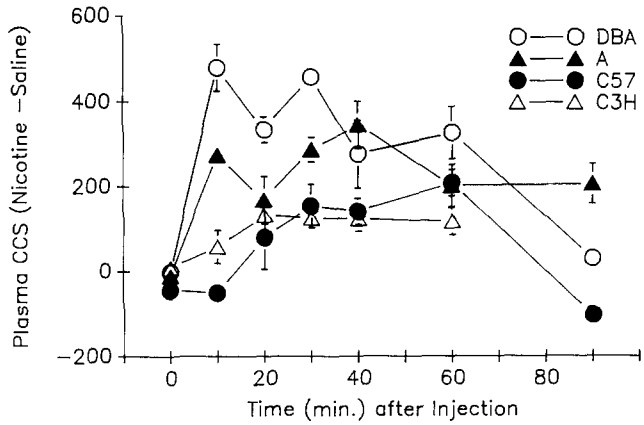

FIG. 2. Net effect of nicotine (2.0 mg/kg) on changes in plasma CCS levels. Mean levels of CCS following saline injection were subtracted from individual CCS values for each time point following nicotine injection to give the net effect of nicotine above that of saline. Data were derived from those in Fig. 1.

There were also significant overall effects of strain, $F(3,157)=25.1$, $p<0.001$, time, $F(5,157)=20.3$, $p<0.001$, and strain by time interaction, $F(15,157)=3.1$, $p<0.005$, for the time course of changes in plasma CCS following saline injection. Post hoc analysis revealed the following rank order: C57BL > A = C3H > DBA ($p<0.05$).

The mean saline CCS value was subtracted from each nicotine CCS value for each time point in order to test for possible strain differences in the net effect of nicotine (Fig. 2). The results indicated a significant effect of strain, $F(3,180)=47.1$, $p<0.001$, time, $F(5,180)=17.3$, $p<0.001$, and a significant strain by time interaction, $F(15,180)=4.0$, $p<0.001$. Post hoc analysis gave a rank order for nicotine-induced CCS levels of DBA > A > C3H = C57BL ($p<0.01$).

Dose-response data for the nicotine-induced rise in plasma CCS are presented in Fig. 3. Since there were significant differences in CCS levels after saline injection (A = C57BL > DBA = C3H, $p<0.05$, *t*-test; see 0 mg/kg nicotine in Fig. 3A), mean saline CCS values were subtracted from individual nicotine CCS values for statistical analysis of the nicotine effect (Fig. 3B). We observed a significant effect of both nicotine dose, $F(2,136)=12.9$, $p<0.001$, and strain, $F(3,136)=5.1$, $p<0.005$. Post hoc analysis indicated the following rank order for degree of nicotine-induced increase in CCS: DBA = C3H = A > C57BL ($p<0.05$).

Mecamylamine pretreatment resulted in a dose-dependent antagonism of the nicotine response in both mouse strains (C3H and C57BL) tested, $F(4,58)=15.1$, $p<0.001$ (see Fig. 4). The ED_{50} values for antagonism by mecamylamine of nicotine-induced increases in CCS levels were less than 1 mg/kg for both strains. When mecamylamine (4.0 mg/kg, IP) pretreatment was followed by either saline or nicotine (2.0 mg/kg, IP), CCS levels were

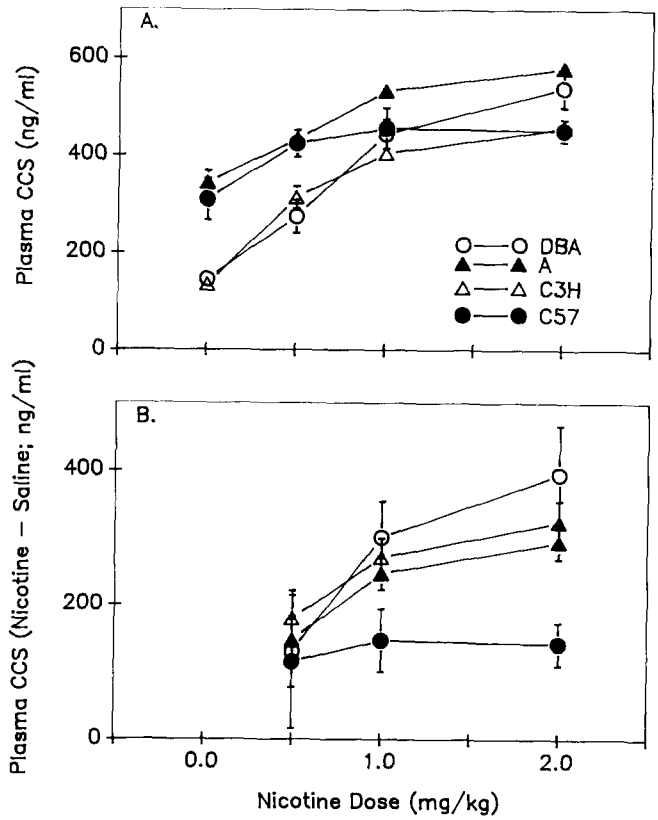

FIG. 3. (A) Dose-response relationships for the nicotine-induced elevation in plasma CCS. Mice were injected with either saline (0 mg/kg nicotine) or the indicated doses of nicotine, and blood was sampled 25 min after injection for determination of CCS. Data are the mean±S.E.M. for 5–23 animals at each point. (B) Dose-response relationships for the net nicotine effect on changes in plasma CCS. Mean saline CCS levels (0 mg/kg nicotine) were subtracted from each nicotine CCS value in A.

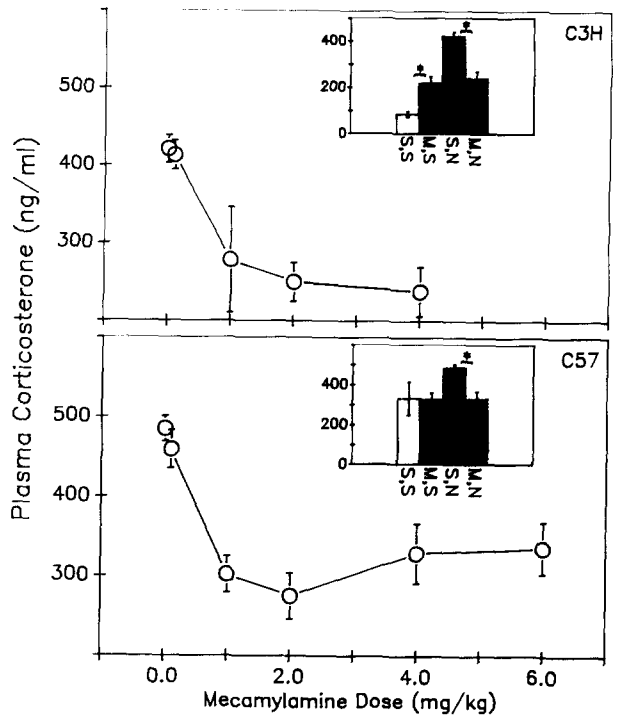

FIG. 4. Dose-response curves for antagonism by mecamylamine of the CCS-elevating effects of nicotine (2.0 mg/kg) for C3H and C57BL mice. Data represent the mean±S.E.M. from 5–6 animals at each point. Mecamylamine or saline was injected IP 10 min prior to nicotine (2.0 mg/kg, IP). Blood was sampled 25 min following nicotine injection. Insets represent data for experiments in which saline (S) or mecamylamine (M; 4.0 mg/kg) was injected IP 10 min prior to injection of either saline (S) or nicotine (N; 2.0 mg/kg). *$p<0.005$, t-test.

similar and low compared to that after saline followed by nicotine for both strains ($p<0.005$, t-test; see Fig. 4 insets).

Mice from the DBA and C57BL strains were tested for differences in CCS levels at various intervals following cold water stress (Fig. 5). No differences were found between these two strains in the CCS response produced by cold water stress, $F(1,114)=0.3$, $p>0.1$.

DISCUSSION

While increases in plasma CCS levels following nicotine administration have been observed previously [4, 5, 8–10], the results reported here indicate that the degree of nicotine-induced rise in plasma CCS is dependent on genotype. Intraperitoneal injection of nicotine increased levels of plasma CCS in all four inbred mouse strains tested, but the degree of CCS elevation was both strain- and dose-dependent.

For both time course and dose-response experiments, DBA mice exhibited the greatest nicotine-induced increase in plasma CCS while C57BL mice exhibited the least. Responses to saline injection also varied with strain. Saline injection resulted in the highest CCS level for the C57BL strain and in the lowest for the DBA strain. Since intraperitoneal injection might be expected to evoke a stress response, and since reactions to stress may differ among strains, the saline response difference is not unexpected. Indeed, these data are consistent with previous reports that plasma CCS levels are greater for C57BL as compared to DBA mice after saline injection [11] or electric shock [22], lending support to the notion that C57BL mice are more responsive to some generalized stressors than are DBA mice. These strains did not differ, however, in the CCS response to cold water stress (Fig. 5), indicating that DBA mice are not inherently deficient in their ability to produce and release CCS. Furthermore, regarding the nicotine response, it may be that for strains with the greater saline injection response (e.g., C57BL), it is more difficult to observe a nicotine effect than in strains with a lesser response to injection stress (e.g., DBA). Additional experiments using a nicotine administration technique that is relatively nonstressful, e.g., intravenous infusion with implanted cannulae, would be necessary to determine whether the response to nicotine is partially masked by the response to injection stress in those strains which respond most to IP saline injection.

For both a high nicotine-responding strain (C3H) and a low nicotine-responding strain (C57BL), the elevation in plasma CCS following nicotine injection could be blocked by preinjection with the nicotinic antagonist mecamylamine, indicating that the nicotine-induced increase in plasma CCS is mediated by nicotinic receptors. The rise in CCS levels after

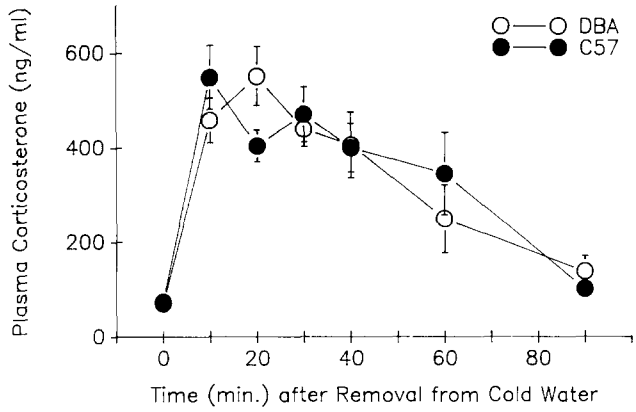

FIG. 5. Time course of changes in plasma CCS following 3 min immersion in cold (5–7°C) water bath. Symbols are the mean± S.E.M. for 5–11 animals at each point.

saline injection, however, did not have a detectable nicotinic component, since mecamylamine pretreatment did not reduce CCS levels following saline injection relative to those which were obtained after two saline injections.

Three of these mouse strains have been analyzed previously for differences in binding of the nicotinic ligands nicotine and alpha-bungarotoxin [25]. Among the DBA, C57BL, and C3H inbred strains, there was no difference in brain nicotine binding. In DBA mice, however, binding of alpha-bungarotoxin (BTX) was significantly lower in hippocampus and midbrain, compared with the other two strains. It is known that the hippocampus exerts an inhibitory influence on the stress response [35,36], and this effect may be mediated by nicotinic cholinergic receptors [7], but it is not known whether the number of these receptors in the hippocampus determines the level of corticosterone released following nicotine administration. While other explanations are possible, the present data support the possibility that the number of nicotinic receptors regulates the amount of CCS released since the strain with the smallest number of hippocampal nicotinic receptors (DBA), as determined by BTX binding, also had the highest levels of circulating CCS following nicotine injection. The degree of CCS responsiveness cannot be explained entirely by the differences in hippocampal BTX binding, however, since C3H and C57BL mice have similar numbers of hippocampal BTX binding sites while they differ in CCS response to nicotine. Part of the explanation for these apparent discrepancies may involve differential nicotine-induced release of adrenocorticotropic hormone (ACTH) [2,10] or differential ACTH-induced release of CCS.

Others have observed differential CCS responses to stress which suggest a genetic basis. For example, mouse strain differences in plasma CCS levels have been identified following stress due to saline injection [11] or to electric shock [22]. Isolated adrenal glands from C57BL/10 mice were found to secrete greater amounts of CCS than those from the DBA/2J strain [11]. A recombinant-inbred strain analysis using seven such strains derived from C57BL/6By and BALB/cBy mouse strains suggested that baseline plasma CCS levels are controlled by two genetic loci [12]. Differences have also been observed between long-sleep and short-sleep mouse lines, selectively bred for differences in ethanol-induced sleep time, for baseline levels of plasma CCS and for CCS levels following acute ethanol administration [19,43]. Various inbred mouse strains also differ in CCS levels following acute ethanol treatment [20]. Recently, Overstreet *et al.* [30] reported different levels of plasma CCS in two rat lines after injection of the muscarinic agent arecoline. To the best of our knowledge, however, the present data represent the first report of genetic variation of the nicotine effect on circulating CCS levels.

In summary, the nicotine-induced increase in plasma CCS varied with genotype among four inbred mouse strains. Strain differences were also observed for baseline CCS levels and for CCS levels following saline injection. These data may provide at least a partial explanation for the variability in behavioral response to nicotine in these mouse strains and may also relate to differences in the use of tobacco products by humans.

ACKNOWLEDGEMENTS

This research was supported by a grant from R. J. Reynolds Tobacco Company. R.K.F. was supported, in part, by training grant DA-07043 and B.J.M. was supported by training grant MH-16880.

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