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Influence of Genotype on Nicotine-Induced Increases of Plasma Corticosterone in Mice as a Result of Acute Nicotine Pretreatment

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MARTIN, B. J. AND J. M. WEHNER. *Influence of genotype on nicotine-induced increases of plasma corticosterone in mice as a result of acute nicotine pretreatment.* PHARMACOL BIOCHEM BEHAV **30**(4) 1065–1070, 1988.—Acute exposure to nicotine produces an elevation of plasma corticosterone levels in rodents. The consequences of repeated exposure to nicotine administered intraperitoneally (IP) were examined in three inbred strains of mice, DBA/2Ibg, C3H/2Ibg and A/J. These strains of mice have been shown previously to differ in a variety of behavioral and physiological responses to acute nicotine exposure. Mice were administered saline or 1.00 mg/kg nicotine IP, followed 30 min later by a range of nicotine doses (0.25–1.00 mg/kg). Strain differences were observed for the dose-response to the second injection; however, no effect of acute nicotine pretreatment was demonstrated. The observed lack of desensitization was consistent across genotype. An intragastric administration of a pretreatment dose of nicotine (4.00 mg/kg) also failed to produce desensitization to a subsequent IP nicotine injection in any strain. Increasing the time interval between injections to 45 min did not alter the CCS response. However, at 90 min between injections, a supersensitive CCS response was measured in all strains.

Nicotine Corticosterone release Genetic differences Inbred mouse strains

THE acute administration of nicotine by intraperitoneal (IP) injection [6–8], subcutaneous (SC) injection [4], or cigarette smoke inhalation [1] induces dose-related increases in levels of plasma corticosterone (CCS) in rodents. In general, glucocorticoid synthesis is mediated via the hypothalamic-pituitary adrenal axis (HPA) and is a homeostatic response to stressful events. Exposure to stress causes the release of adrenocorticotropin (ACTH) from the anterior pituitary, which in turn promotes the synthesis of corticosterone in the adrenal cortex [2]. Conte-Devolx *et al.* [8] and Cam *et al.* [6] have demonstrated that this relationship between ACTH release and glucocorticoid synthesis is retained when nicotine is the agent precipitating the rise in plasma CCS. Therefore, the corticosterone response is not a direct result of nicotine interaction at the level of the adrenal gland.

An association between nicotine and stress has been implied from anecdotal references by smokers to the "calming effects" of nicotine and from the observation that smokers appear to smoke more when confronted with a stressful situation [15,27]. Rats trained in a shock avoidance paradigm under the influence of nicotine become dependent on nicotine for a successful performance, contingent on the degree of stress to which the animal has been exposed [15].

The use of nicotine as a coping mechanism in managing stressful situations may be explained in the context of tolerance. If tolerance develops to the effects of nicotine on the release of CCS, it is possible that cross tolerance to stress-induced release of the steroid could develop, thereby creating a hormonal environment less responsive to environmentally-induced stress.

It has been established that behavioral and physiological tolerance to nicotine occurs after chronic treatment. Marks *et al.* [22] have demonstrated that mice chronically infused for 8–10 days (0.2–5.0 mg/kg/hr) exhibit significant tolerance to the effects of nicotine for both body temperature and rotarod performance when challenged with an acute dose of nicotine (2.0 mg/kg). Tolerance to the stimulatory effects of nicotine on CCS release also occurs after chronic nicotine treatment. Rats receiving daily IP injections (0.4 mg/kg) of nicotine exhibit rapid tolerance to nicotine's effects; by the

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fifth day of treatment no increase in plasma CCS above baseline was observed [5]. In addition, chronic oral administration of nicotine induces rapid tolerance to the CCS response when the animal is challenged with a SC injection of nicotine after 24 hours of chronic intragastric (IG) administration [3].

The rapid development of tolerance after chronic treatment suggests that tolerance might be observed after acute pretreatment as well. Balfour [3] has shown that in rats an acute oral dose of nicotine blocks the CCS response to a SC injection of nicotine. More recently, Sharp and Beyer [30] have demonstrated rapid desensitization to the stimulatory effects of nicotine on the HPA response in rats. Their results indicated that nicotine-induced ACTH secretion is significantly attenuated after a single IP injection of nicotine.

Inasmuch as nicotine-induced elevations in plasma CCS in inbred strains of mice have been determined to be genotype-dependent [13], genetic influences on nicotine-induced adrenocortical responses to a second injection of nicotine after a single pretreatment dose were investigated. The present study was conducted using three inbred strains of mice (DBA/2Ibg, C3H/Ibg and A/J) previously shown to differ in their degree of nicotine-induced increases in CCS [13] as well as acute [16] and chronic [23] behavioral responses to nicotine.

METHOD

Animals

Male and female mice of the inbred strains DBA/2Ibg, C3H/2Ibg and A/J were used in this study. All mice were weaned at 25 days of age and housed with 1–5 like-sex littermates. Animals were maintained on a 12-hour light/dark cycle (lights on at 0700 to 1900) and were permitted free access to food (Wayne Lab Blox) and water. All testing was done when the mice were 60–90 days old.

Chemicals

Nicotine was purchased from Sigma Chemical Company, St. Louis, MO and was redistilled periodically. [^{3}H]-Corticosterone was obtained from Amersham, Arlington Heights, IL (specific activity=50 Ci/mmole). Corticosterone antiserum was purchased from Dr. Gordon Niswender, Department of Physiology and Biophysics, Colorado State University, Fort Collins, CO.

Procedure

Animals were weighed and placed in the testing room overnight before testing in order to minimize potential stress effects due to relocation to a novel environment. All testing was done between 0800 and 1200 hr. Previous investigations from this lab (unpublished results) concerning the effects of prehandling on subsequent nicotine-induced increases of CCS indicated that the response is genotype-dependent; C3H mice were unaffected by prehandling; however, DBA mice were made more sensitive. Therefore, naive mice were used in this study to eliminate the confounding effects of prehandling.

To determine whether a single pretreatment injection of nicotine affects the CCS response to a second dose, groups of 6 mice from each strain received either a 1.00 mg/kg intraperitoneal (IP) injection of nicotine or saline (0.01 ml/g body weight) followed 30 min later by saline or a range of nicotine doses from 0.25–1.00 mg/kg. This time interval between injections was selected based on a study by Miner [25] in which desensitization to nicotine-induced seizures in mice was observed to be optimal at 30 min. Time course data [13] measured after a single injection of nicotine indicated that the peak CCS response occurs at 30 min. Therefore, blood was collected into heparanized micro-hematocrit tubes via retro-orbital sinus punctures 30 min after the second injection.

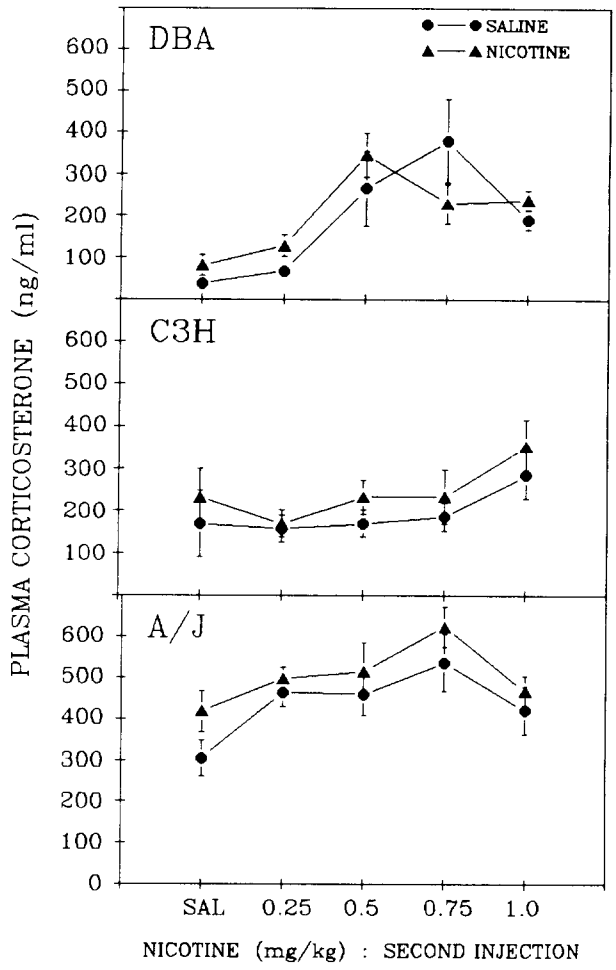

FIG. 1. Dose-response relationships for nicotine-induced elevations of CCS in three inbred strains of mice receiving either saline or nicotine preinjection. Mice received either a saline or 1.00 mg/kg nicotine IP injection followed 30 min later by 1.00 mg/kg nicotine (IP). Blood was sampled 30 min after the second injection. Data are the mean±SEM for 6 mice at each dose.

In order to evaluate whether the injection interval influenced the CCS response, groups of 6–12 mice were preinjected with 1.00 mg/kg nicotine or saline followed 45 or 90 min later by a 1.00 mg/kg nicotine or saline injection. Blood was collected 30 min after the second injection.

To assess whether the response was dependent on the route of administration of the pretreatment dose of nicotine, mice were pretreated with an acute oral dose of nicotine. Initially, the effects of a single IG dose (4.00 mg/kg nicotine or saline) were measured in two groups of 6 mice from each strain. Blood was taken 30 min later. Acute tolerance to a second dose of nicotine after oral pretreatment was evaluated in groups of 6 mice that received either 4.00 mg/kg

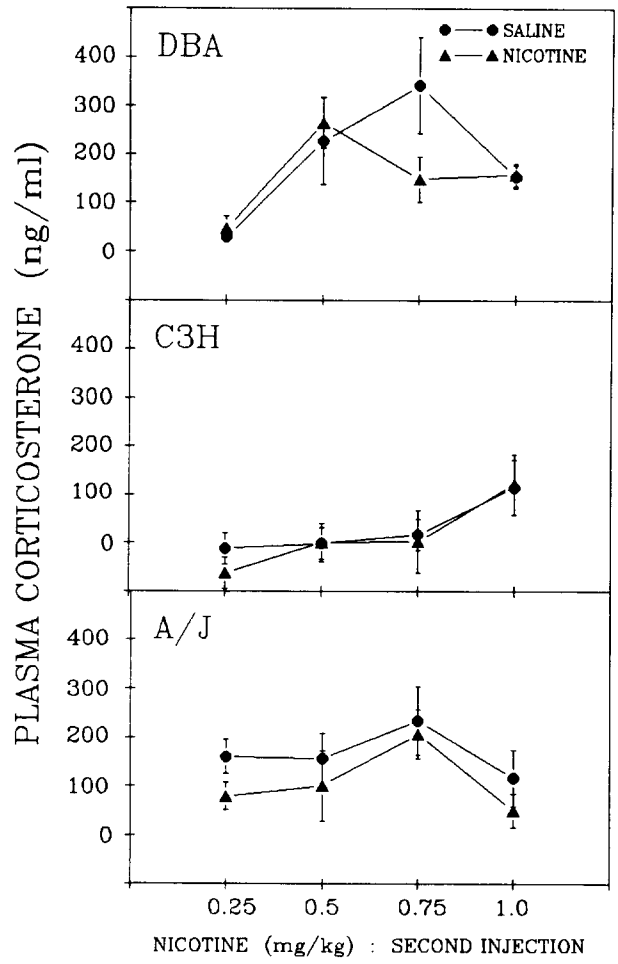

FIG. 2. Dose-response relationships for the net-effect of nicotine-induced elevations of CCS following a pretreatment dose of nicotine (1.00 mg/kg) or saline. Mean CCS values from control groups (saline or nicotine pretreatment followed by saline IP injections) were subtracted from individual CCS values within the appropriate pretreatment group at each dose. Data were derived from those in Fig. 1.

nicotine or saline administered IG followed 30 min later by a 1.00 mg/kg nicotine or saline IP injection. Blood was collected 30 min after the IP injection.

RIA

Levels of plasma CCS were determined using a modification of a radioimmunoassay developed by Gwosden-Cohen *et al.* [14]. Blood was centrifuged in a tabletop International Clinical Centrifuge at setting 4 for 4.5 minutes; 10 μl plasma was extracted with absolute ethanol. Proteins were pelleted by centrifugation at 2100×g for 15 minutes; 50 μl of the supernatant was used for the assay. Phosphate-buffered saline with gelatin (PBSG), pH 7.2, was prepared with 0.08 M $Na_2HPO_4 \cdot 7H_2O$, 0.02 M $NaH_2PO_4 \cdot H_2O$, 0.85% NaCl, and 0.10% gelatin. After evaporation of the solvent, samples were resuspended in PBSG buffer and incubated at 4°C with antibody diluted to give 40% binding of [^{3}H]-CCS. Ninety minutes later [^{3}H]-CCS (10,000 cpm) in PBSG buffer was added to each tube. After incubation overnight, a dextran charcoal suspension was added to tubes (at 4°C) to remove unbound CCS. After centrifugation (2100×g for 10 min at 4°C), 300 μl of supernatant was transferred to scintillation vials containing, 4.0 ml of scintillation fluid and counted for 1 min. Plasma levels of CCS were quantified using a standard curve generated with each RIA performed.

Data Analysis

The dose-response data were intially assessed using three-way analysis of variance (ANOVA) followed by two-way ANOVAs within each strain to evaluate the effects of dose and pretreatment. For those analyses in which significant effects were observed, the results were subjected to post hoc tests to determine rank-order. Single point comparisons between groups receiving two injections within the same time interval as well as comparisons between two means following IG pretreatment were made using unpaired *t*-tests. One-way ANOVAs were conducted to assess single-factor effects within treatment groups.

RESULTS

Plasma CCS levels following a second injection of nicotine after a single pretreatment of either saline or nicotine (1.00 mg/kg) are presented in Fig. 1. Strain differences in CCS were observed for the dose-responses to the second nicotine injection, $F(2,203)=94.4$, $p<0.001$, as well as the strain by dose interaction, $F(8,203)=3.9$, $p<0.001$. Post hoc analysis gave the following rank order of strain responses: A/J > DBA = C3H. Two-way ANOVAs within each strain indicated significant dose effects for all strains [$F(4,71)=15.1$, $p<0.001$; $F(4,59)=2.5$, $p<0.05$; $F(4,71)=5.5$, $p<0.001$; DBA, C3H, A/J, respectively]. The evaluation of desensitization, i.e., the main effect of pretreatment, indicated that for the DBA and C3H strains CCS responses were not significantly attenuated due to a single acute nicotine preinjection. The effect of pretreatment was significant within the A/J strain, $F(1,71)=8.1$, $p<0.01$. However, the direction of the response demonstrated an increased sensitivity among the nicotine-pretreated animals rather than a desensitized response.

Because the dose by pretreatment interaction term was not significant in the analysis of the A/J strain data, the differences observed between saline- and nicotine-pretreated animals may be due to baseline differences represented by the two groups receiving saline as the second injection. A *t*-test comparing these two groups did not reveal a significant difference, however.

Strain differences were observed within control groups for both pretreatment schedules (saline pretreatment followed by a saline injection and nicotine pretreatment followed by a saline injection) [$F(2,33)=13.9$, $p<0.001$; $F(2,17)=11.1$, $p<0.001$; saline and nicotine preinjections, respectively]. Post hoc analysis gave the following rank order within the saline-pretreated groups: A/J > C3H > DBA; and within the nicotine pretreated groups: A/J > C3H = DBA.

These variations in control CCS responses to double injections may have masked possible strain differences in the nicotine-induced CCS response, as well as the magnitude of the responses over and above control values. Therefore, it was necessary to subtract mean control group CCS values (saline second injection) from individual nicotine CCS values at each dose. This calculation of the net effect of nicotine should allow the detection of desensitization as evidenced by shifts of the nicotine dose-response curves for the second

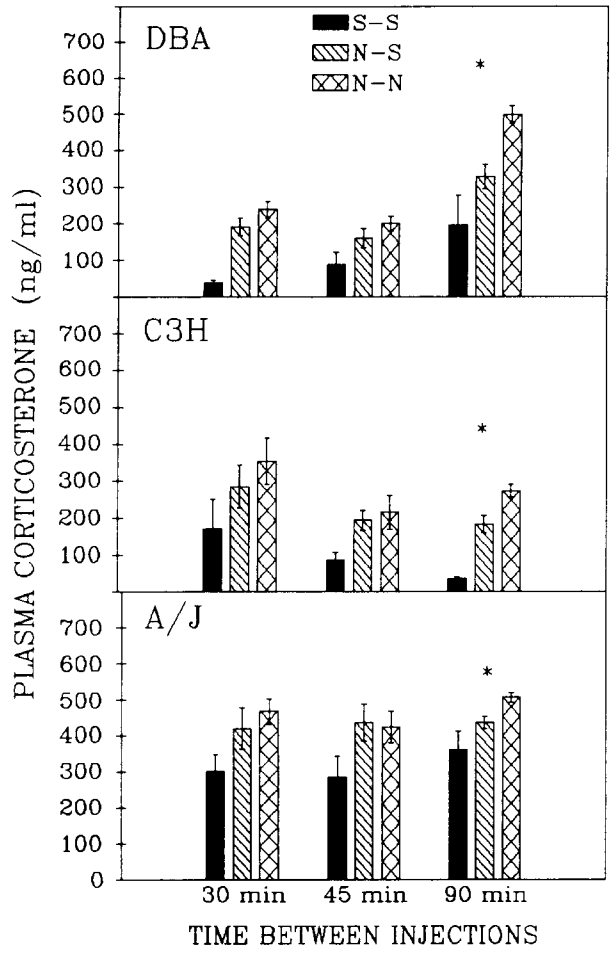

FIG. 3. Effect of time between two IP injections of nicotine on the plasma CCS response. Mice received double saline injections (S-S), saline preinjections followed by 1.00 mg/kg nicotine injections (S-N), or double-nicotine injections (N-N). Comparisons were made between saline and nicotine pretreatment groups at each interval using unpaired t-tests. *$p<0.01$. Data are means±SEM for 6–12 mice.

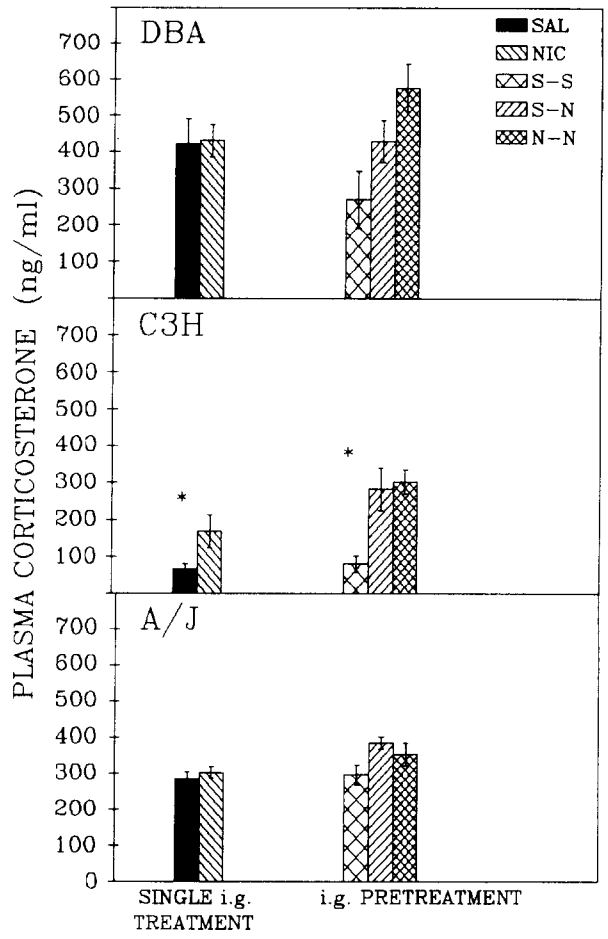

FIG. 4. The effects of a single IG dose of nicotine on the plasma CCS response are shown on the left of the graphs. Mice received an IG dose of saline (solid bars) or 4.00 mg/kg nicotine (diagonal bars). The effects of an acute IG pretreatment with nicotine (4.00 mg/kg) on a subsequent IP injection (1.00 mg/kg) administered 30 min apart are shown on the right of the graphs. Single point comparisons between pretreatment groups were made using unpaired t-tests. *$p<0.05$. Data are means±SEM for 6 mice at each treatment.

injection. The results of these analyses are shown in Fig. 2. Significant dose effects were indicated for the DBA and C3H strains, $F(3,51)=6.7$, $p<0.001$; $F(3,47)=4.2$, $p<0.01$; but no differences were observed between saline- and nicotine-pretreated animals. Although the main effect for dose approached significance within the A/J strain ($p<0.06$), neither dose nor pretreatment differed between the two groups.

In order to determine whether longer intervals between injections could result in a desensitized CCS response, two additional injection schedules were tested (Fig. 3). Groups of mice receiving a saline pretreatment followed by a saline injection were included at each time point as matched double-injection controls. t-Tests conducted between saline- and nicotine-pretreated animals at each time interval resulted in no significant differences in plasma CCS levels at the 30 and 45 min time intervals for any strain. However, for those animals receiving injections 90 min apart, nicotine-pretreated animals exhibited an increase in plasma CCS over those animals preinjected with saline [$t(20)=19.4$, $p<0.01$; $t(21)=19.1$, $p<0.01$; $t(10)=9.4$, $p<0.01$; DBA, C3H, A/J, respectively].

The CCS responses of the double-injected saline animals varied with the injection interval. One-way ANOVAs within each strain indicated that DBA mice receiving double saline injections showed significant increases in plasma CCS levels as the time between injections increased, $F(2,27)=4.9$, $p<0.05$. Post hoc analysis gave the following rank order of time intervals: 30 min = 45 min < 90 min between injections. Neither the C3H nor the A/J mice exhibited any significant change in CCS levels due to changing time intervals between injections.

To evaluate whether the route of administration was a determinant of the CCS response in mice, oral pretreatment with 4.00 mg/kg nicotine was examined. A single oral dose of nicotine did not elevate CCS levels above the saline response for either the DBA or A/J strain. In contrast, C3H mice did display an increase in plasma CCS levels in response to an acute oral dose of nicotine, $t(9)=-2.43$, $p<0.05$ (Fig. 4). Intragastric pretreatment with nicotine failed to produce a desensitized CCS response to a challenge IP injection of nicotine (1.00 mg/kg) given 30 min later in any strain. How-

ever, saline-pretreated (IG) mice from the A/J and C3H strains did display a significant increase in plasma CCS when challenged with an IP injection of nicotine [$t(10)=-2.8$, $p<0.05$; $t(10)=3.4$, $p<0.01$; A/J and C3H, respectively].

DISCUSSION

Nicotine-induced increases in plasma CCS do not undergo desensitization in three inbred strains of mice after a single acute pretreatment of nicotine. These results are somewhat unexpected in light of recent investigations by Sharp and Beyer [30] and Balfour [3] in which adrenocortical responses to nicotine did desensitize in the rat after a single pretreatment of nicotine. These contradictory results may be due to a species difference in nicotine elimination and/or the adrenocortical product measured. The pharmacokinetics of nicotine disposition has been evaluated in both rat and mouse, indicating a 10-fold greater rate of elimination in mouse than in rat. In rat, the half life of nicotine is 60–90 min [24,29], whereas in mice, the half-life is approximately 6 min, and the drug is completely eliminated by 30 min [28].

The lack of acute desensitization observed in this study may be of some biological advantage to the organism in maintaining a consistent CCS response to nicotine administration. Recent investigations have demonstrated that certain endogenous adrenal steroids, including CCS, are capable of potentiating the inhibitory actions of GABA in brain [17, 20, 21], resulting in anxiolytic actions that modulate stress-related behaviors [9,12]. Therefore, a constant, rather than attenuated level of plasma CCS may represent an adaptive response to the second nicotine injection. In a recent review of glucocorticoids' actions, McEwen [19] states that glucocorticoids are capable of modulating neurotransmitters released in response to stress and may function in the adaptation to stress.

When the intervals between injections were increased to 45 min, desensitization was not observed in any strain. However, at 90 min between injections plasma CCS responses to a second nicotine injection were significantly increased over saline-pretreated controls in all strains. Inasmuch as CCS levels are back to baseline by 90 min [13], the observed increase is not due to an additive effect of circulating plasma CCS remaining from the first injection. The mechanisms involved in this supersensitive response are not clear, but may also reflect a generalized increased adrenocortical response regulating an organism's reaction to the second nicotine injection. All three strains responded in a like manner suggesting that a common factor may be responsible for the effect.

Several baseline differences were observed among the strains demonstrated by the variation in plasma CCS among the saline-treated groups. For those animals receiving double-saline injections 30 min apart, DBA animals displayed the lowest plasma CCS levels, whereas A/J mice exhibited the highest. A similar pattern occurred within the groups receiving a nicotine pretreatment (1.00 mg/kg) followed by a saline injection; DBA and C3H mice had equal but significantly lower levels than the A/J animals. An IP injection might be expected to induce a stress response, and since genetic factors have been shown to regulate plasma CCS levels [10, 11, 13, 26], these strain differences within the saline-treated animals were not unexpected. The higher levels exhibited by the A/J strain are consistent with earlier investigations that reported higher plasma CCS levels in A/J mice than in DBA/2 mice after novelty stress or electric shock [18]. These results also agree with dose-response data measured after single IP injections of nicotine [13]. Both A/J and C57BL mice had significantly higher levels of CCS than did DBA and C3H mice after a single IP injection of saline (control values).

Analyses to determine whether the saline response is dependent on the time interval between injections also revealed strain differences. Among the three strains, only the DBA mice demonstrated any differences due to time between injections. Plasma CCS levels increase as the interval increases, suggesting that for the DBA strain, a second IP injection becomes a greater stressor the longer the interval between injections. In addition, qualitative comparisons of responses to acute IG administration of saline and double injections of saline for each strain suggest that DBA mice respond to a greater degree to a single IG treatment than to the double IP injections (cf., S-S groups in Fig. 3 to Sal group in Fig. 4). In contrast, C3H mice respond to a lesser degree to the oral treatment, and the A/J strain does not appear to respond differently to the two routes of administration. These results indicate that control and/or baseline values are dependent on several factors including genotype, route of administration and time between injections.

Because the control values varied among the strains, the dose-response data were reanalyzed after mean saline values had been subtracted from individual CCS values at each dose. The results of the reanalyses were not different from those initially done for the DBA or C3H strains; however, the significant effect of dose observed for the A/J animals in the original data was not apparent after the baseline responses had been taken into account. This observation that A/J mice do not demonstrate a response to nicotine within this dose range suggests that for this schedule of drug treatment, the animals' initial responses to the double injections raise plasma CCS levels to an extent that may mask any possible increase due to nicotine. A similar analysis of adjusted dose-response data measured after a single nicotine injection indicated that A/J mice do respond in a dose-related manner over and above their initial saline responses [13]. It appears that the double-injection procedure in some way abolishes the dose-response to nicotine. This suggests that a form of desensitization may be occurring for this strain as a result of the double-injection procedure itself.

As was previously mentioned, nicotine elimination rates are 10-fold greater in mice than in rats. Therefore, to approximate the slower elimination rate in rats, mice received a high nicotine dose (4.00 mg/kg IG) 30 min before receiving a second nicotine dose (1.00 mg/kg) IP, in order to ensure an effective dose of nicotine after the first pass effect. Again, no strain exhibited an attenuated CCS response. These results are not consistent with those reported by Balfour [3] in which acute IG pretreatment with nicotine resulted in a significantly reduced CCS response to a second SC injection of nicotine in rat. In mice, it does not appear that different routes of administration of two nicotine treatments alter adrenocortical responses to the second dose. In addition, these three strains do not differ in their responses, which suggests a common genetic influence.

A single oral dose of nicotine had no effect on plasma CCS levels above that indicated by saline administration for either the DBA or A/J strains. This is consistent with Balfour's [3] report of no effect in rat. However, C3H mice did display elevated plasma CCS as a result of an oral dose of nicotine. Therefore, nicotine-induced increases in ad-

renocortical activity are dependent on both genotype and route of administration.

In summary, nicotine-induced increases of plasma CCS levels in DBA, C3H and A/J inbred strains of mice do not desensitize in response to an acute pretreatment dose of nicotine, regardless of the route of administration or time between injections. The maintenance of a consistent level of CCS as well as the supersensitive response exhibited at the 90 min interval between injections may reflect a biological advantage for the organism's response to stress. These results may provide some insight into stress and anxiety related smoking behaviors among humans.

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