Pharmacology Biochemistry & Behavior, Vol. 40, pp. 53–59. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Conditioned Tolerance to the Anorectic and Corticosterone-Elevating Effects of Nicotine

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Received 26 September 1990

CAGGIULA, A. R., L. H. EPSTEIN, S. M. ANTELMAN, S. S. SAYLOR, K. A. PERKINS, S. KNOPF AND R. STILLER. *Conditioned tolerance to the anorectic and corticosterone-elevating effects of nicotine.* PHARMACOL BIOCHEM BEHAV **40**(1) 53–59, 1991.—We have shown that tolerance to the behavioral effects of nicotine is partially dependent on conditioned environmental cues that predict drug delivery. The present research extends this finding to physiological effects of nicotine by assessing both the appetite-suppressing and adrenocortical-activating effects of nicotine, as measured by plasma corticosterone (CORT). In the first study, male rats on a 22-h food deprivation schedule were injected daily with 0.33 or 0.66 mg/kg (free base) of nicotine bitartrate or saline in a distinctive environment and tested for milk intake. Nicotine initially suppressed milk intake and tolerance developed over 10 days. Changing cues associated with drug administration partially reversed tolerance since injection of nicotine in a new environment reduced milk intake of tolerant animals. Similarly, animals who repeatedly received nicotine in one environment exhibited CORT levels lower than rats injected for the first time, and this tolerance also was partially reversed when administration occurred in the new environment. The second experiment indicated that the increased CORT of Experiment 1 was not a stress response associated with injecting animals in a different environment. These results indicate that tolerance to both behavioral and neuroendocrine effects of nicotine is influenced by conditioning.

Nicotine Conditioned tolerance Corticosterone Anorexia

TOLERANCE, as defined by a decrease in the effects of a drug after repeated administration, develops to a wide variety of addictive drugs and is believed to be an important factor in the persistence of drug-taking behavior (25,26). While it was once thought that tolerance depended solely on repeated drug exposure, it is now clear that learned associations with environmental cues signaling drug delivery also are important in both the development and maintenance of tolerance to a number of drugs, including morphine (42,43), amphetamine (36), alcohol (23) and nicotine (7,18). For example, we have shown that maintenance of tolerance to the analgesic (18) and anorectic (7) effects of nicotine in rats depends, at least in part, on repeated administration of the drug in the environment within which tolerance first develops. Changing environmental cues associated with drug delivery disrupts behavioral tolerance in both instances.

Early studies of conditioned drug tolerance focused on behavioral effects, but more recent research has demonstrated that physiological responses, such as the thermic (11, 14, 22, 29, 30, 44), and immunostimulatory effects of drugs (16,17) can also be brought under environmental control. One important physiological effect that many drugs of abuse have in common is activation of the hypothalamic-pituitary-adrenocortical (HPA) system; activity in this system is thought to be an important mediator of many behavioral and adverse health effects of such drugs (19, 34, 39). However, to our knowledge, no one has asked whether tolerance to HPA activation is also influenced by learning.

Nicotine has a variety of physiological effects (15, 19, 45), including activation of the HPA system in rats, as measured by drug-induced increases in ACTH (2, 13, 33, 41) and corticosterone [CORT; (2, 10, 13, 47)]. Tolerance develops to both the ACTH- and CORT-activating effects of acute nicotine or cigarette smoke after repeated exposure (1, 5, 6, 9, 41).

The present research determined whether the conditioning effects previously demonstrated for behavioral tolerance to nicotine generalize across behavioral and neuroendocrine response systems. Specifically, in Experiment 1 we asked whether changing the environmental cues associated with tolerance development, which has been shown to disrupt behavioral tolerance by reinstating the anorectic effects of nicotine, will also disrupt tolerance to the HPA-activating effects of nicotine, as indexed by plasma CORT in male rats. In Experiment 2, we asked whether the reinstatement of the CORT response to nicotine after environmental change, found in Experiment 1, could have been due to the stress-induced release of CORT due to the environmental change per se.

EXPERIMENT 1

Animals

The subjects were adult, male Sprague-Dawley SPF rats

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(Zivic-Miller Laboratories) that weighed 250–300 g at the start of the study. The rats were housed individually in a sound and temperature controlled (24 ± 1°C) colony room on a 12:12-h lighting cycle, and given free access to Purina Rodent Chow 5001 and water during an initial 12-day acclimation period. Testing was conducted during hours 3–6 of the light period.

Behavioral Testing and Drug Administration

Phase 1: Baseline. All animals were placed on a 22-h food deprivation schedule 4 days before the end of the acclimation period and for the remainder of the study. Beginning approximately 30 min before food availability on each day, each rat was removed from its home cage and weighed, hand carried to an adjacent testing room and placed into a cage, similar to the home cage, onto which was attached a graduated tube containing sweetened milk ("Eagle Brand" Bordens condensed milk diluted with water, 1:3 water:milk) and permitted to drink for 10 minutes. This procedure continued for 10 days. On the last two days of baseline each rat was removed from its cage, weighed, returned to its home cage for 9 minutes, then placed into a plastic bucket with sawdust flooring and transported to the testing room. It was then injected with saline (1 ml/kg SC in the scruff of the neck) and returned to the transporter for 2 min before being placed into the test cage for the 10 min milk intake test. Chow was then made available for 2 h in the home cage. Water was always available in the home cage.

Phase 2: Nicotine tolerance. The procedure used during the last two days of baseline was continued through the 11 days of Phase 2, except that rats in the nicotine (NIC) groups received SC injections of nicotine whereas the saline (S) groups continued to receive saline. During this phase, the order of running between groups and within groups (first 3 rats, second 3 and third 3) was systematically changed over days to minimize the development of an association between temporal cues related to time since the first animal was removed from its cage each day and drug delivery. Thus environmental and procedural cues unique to drug delivery (and 2 days of saline injections during baseline) included the return to the home cage for 9 minutes after weighing and before removal to the testing room, transportation to the testing room in the plastic bucket with sawdust flooring, injection in the testing room and return to the bucket for 2 minutes after injection and before testing. These cues were changed in phase 3.

Phase 3: Environmental change. On the day following the last tolerance day (Change day), environmental and procedural cues associated with drug delivery were changed for the change (CHG) groups. The injection-test interval remained at 2 min. However, instead of 9 min between initial removal from their home cage, weighing and transportation to the testing room before injection, animals were injected immediately after removal from their home cage in the colony room, returned to their home cage for the 2 min interval, then hand carried to the testing room for measurement of milk intake.

Corticosterone Determination

Immediately after the measurement of milk intake (12 minutes after nicotine injection; a 2-min drug-test interval plus a 10-min milk intake test) animals were decapitated. Home cage controls that were not subject to any of the above procedures were sacrificed immediately after removal from their home cage and were interspersed among the experimental rats. Upon sacrifice, 10 ml of trunk blood was collected in heparinized beakers coated with 10,000 units/ml. The blood was centrifuged at 2000 rpm for 10 min and 0.5 ml of plasma saved and frozen at −70°C for later measurement of plasma corticosterone by competitive protein binding radioassay (35). This assay requires only 25 μl of plasma and is sensitive to 0.2 μg/dl.

Experimental Groups and Procedures

Forty-nine rats were randomly assigned to one of four groups. In the two NIC groups (N = 16 per group), after obtaining steady state baseline levels of milk intake (Phase 1), tolerance was established (Phase 2) by giving these animals nicotine injections before each of 11 daily tests of milk intake, using either 0.33 or 0.66 mg/kg of nicotine base (1 or 2 mg nicotine bitartrate/ml of saline; injection volume = 1 ml/kg). Half of each group (N = 8) was then given a single test after a nicotine injection in the changed context (CHG) and the other half was injected in the context within which tolerance originally developed (NCHG). Tolerance was reestablished on the next day by administering nicotine and testing all rats for one day in the old context, and then the conditions were reversed; the half of each group that was originally in the NCHG condition was now exposed to the new context and the half that was originally in the CHG condition was now injected using the old context and procedures. These groups were designed to assess the effects of dose on tolerance development, and the effects of conditioning on tolerance. The crossover design was used to demonstrate conditioning, reestablish tolerance, and then test the reliability of conditioning on a second group of animals. A third group received saline throughout the experiment and either 0.33 (N = 5) or 0.66 (N = 4) mg/kg of nicotine on the last test. This group (S-N) provided an estimate of nicotine's acute effects on milk intake and CORT. The fourth group (N = 8) was a home cage control (CTRL) that was not subjected to any manipulation and provided baseline CORT levels.

Data Analysis

The analyses were designed to compare the effects of nicotine on milk intake and corticosterone levels after an initial exposure to the drug, after tolerance had been induced by 11 exposures in the same context, and after the environmental context signaling drug delivery had been changed. For milk intake, a preliminary analysis established that the effect of environmental change was the same over the two replications in the crossover design (i.e., the order of context change in the crossover design did not influence its effectiveness in disrupting tolerance). When just the crossover was considered, there was no significant main effect or interaction involving this factor [all F's<1.0, $p>0.10$]. An overall ANOVA was then run which included two between factors: Condition (3 levels: tolerant rats tested in the new environment on the first change day, tolerant rats tested in the new environment on the second change day and rats that received saline throughout the experiment and given nicotine for the first time on the second change day); and Dose (0.33 or 0.66 mg/kg), and one within factor, Trials, with 4 repeated measures (last trial of baseline intake, first trial of nicotine, last trial of nicotine before the context change, i.e. tolerance, and the trial on which the context was changed). Planned comparisons were subsequently made using the within subjects error terms. For CORT, a one way ANOVA followed by linear contrasts were used to determine differences among groups.

RESULTS

Milk Intake

Baseline milk intake stabilized over 10 days of testing with no differences observed between groups (Fig. 1). Significant ef-

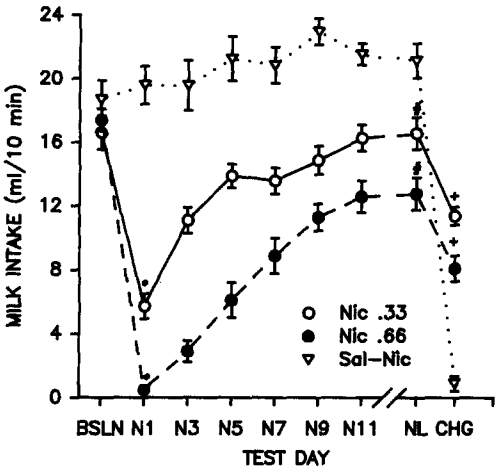

FIG. 1. Milk intake of animals in the two nicotine groups (NIC 0.33 and NIC 0.66) and in the third group (Sal-Nic) that received saline throughout tolerance testing and then nicotine (0.33 or 0.66 mg/kg) on the last change (CHG) day. Since there were no differences in the Sal-Nic animals that received the 0.33 of 0.66 doses on the last day, their data are combined here. In a crossover design, some rats were subjected to the new environment (Change #1) and others tested as usual (old environment), then, after all were again tested in the old environment to reestablish tolerance, the order of groups was reversed and the environmental change was repeated (Change #2). Since statistical analyses (see the Results section) indicated that the effects of environmental change were replicated over the two days, the results in this figure are collapsed over the two replications (CHG). Baseline (BSLN) milk intake was initially suppressed by both doses of nicotine (N1), with the decrease greater for the higher dose. Tolerance to these anorectic effects developed over the 11 days of nicotine administration (N1–11). Changing environmental cues partially disrupted tolerance, since milk intake decreased for both doses from the last day of tolerance (NL) to the change day (CHG), but was not as low as that of rats given nicotine for the first time (Sal-Nic group on the change day). *$p<0.001$, compared to BSLN; #$p<0.001$, compared to N1; +$p<0.001$, compared to NL.

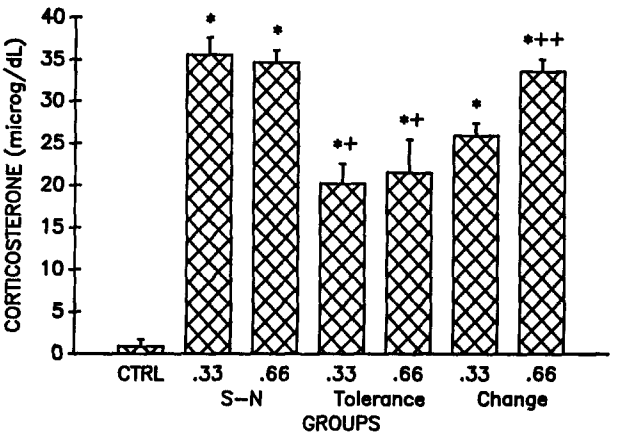

FIG. 2. Corticosterone levels were elevated by administering either 0.33 or 0.66 mg/kg of nicotine for the first time (S-N), when compared to animals undisturbed in their home cage (CTRL). Ten daily injections of nicotine in the same environment induced partial tolerance (Tolerance), but when environmental cues were changed, the tolerance was reversed (Change), and the reversal was complete for the 0.66 mg/kg group (see the Results section for statistical analyses). *$p<0.001$, compared to CTRL; +$p<0.005$, compared to S-N; + +$p<0.02$, compared to Tolerance. Mean ± S.E.

fects on milk intake were obtained for Condition, $F(2,35) = 11.4$, $p<0.001$, Dose, $F(2,35) = 9.92$, $p<0.01$, Trials, $F(3,105) = 153$, $p<0.001$, and the Condition × Trials interaction, $F(6,105) = 52.15$, $p<0.001$. Individual comparisons indicated that nicotine significantly depressed milk intake ($p<0.001$) on the first day of administration and that the decrease was greater ($p<0.001$) for 0.66 (from 17.4 ± 0.7 ml to 0.5 ± 0.2 ml; mean ± S.E.) than for 0.33 mg/kg (16.7 ± 1.1 ml to 5.7 ± 0.8 ml). Significant tolerance developed over the 11 days of injections, since milk intake was higher for all nicotine groups on the last day of nicotine (NicL), before the first change day, when compared to the first nicotine day (Nicl; $p<0.001$). Regarding the completeness of tolerance, if compared to the original baseline, rats given the 0.33 dose showed complete tolerance (16.6 ± 1.1 ml vs. 16.5 ± 0.6 ml, $p>0.10$), whereas rats in the 0.66 groups did not (17.4 ± 0.7 ml vs. 11.9 ± 0.7 ml; $p< 0.01$). Rats given just saline (S-N) showed a significant increase from baseline milk intake to the NicL test (18.7 ± 1.2 ml to 21.4 ± 0.6 ml; $p<0.05$) and if compared to intake of this S-N group on the last tolerance day, neither the 0.33 nor the 0.66 groups exhibited complete tolerance ($p<0.001$).

Changing the context of nicotine administration disrupted the manifestation of tolerance. Milk intake was significantly lower when animals were tested in the new context than when last tested in the old context within which tolerance was established. For the 0.33 mg/kg dose, intake decreased from 16.5 ± 0.6 ml to 11.4 ± 0.6 ml, $F(1,84) = 29.8$, $p<0.001$, and for 0.66, the values were 11.9 ± 0.7 ml to 8.1 ± 0.8 ml, $F(1,84) = 24.4$, $p<0.001$. While changing context significantly disrupted tolerance, the disruption was not complete, since the above values on the change days were still higher than milk intake of the S-N animals given nicotine for the first time (0.9 ± 0.5 ml, $p<0.001$, see Fig. 1).

An analysis of the effectiveness of the context change on just the last day of the experiment was undertaken, since only these animals contributed to the CORT values presented below. Changing the context of drug delivery significantly decreased milk intake for both the 0.33 dose (16.0 ± 1.8 ml to 11.6 ± 1.1 ml; $p<0.001$) and 0.66 dose (10.6 ± 1.3 ml to 7.8 ± 0.8; $p<0.05$).

Plasma Corticosterone

The stimulatory effects of nicotine on plasma CORT exhibited partial tolerance with repeated administration, and changing the environmental cues associated with drug delivery disrupted tolerance (Fig. 2). Linear contrasts, based on the overall ANOVA, $F(6,42) = 27.2$, $p<0.001$, indicated that nicotine increased CORT levels from the home cage control group (0.9 ± 0.9 μg/dl) but that there was no difference between the 0.33 (35.6 ± 2.1) and 0.66 mg/kg (34.7 ± 1.5) doses, $F(1,42) = 0.05$, $p>0.10$. Significant tolerance developed to repeated administrations of both doses, since the 0.33 and 0.66 mg/kg groups injected in the old context on the last test exhibited lower CORT levels (20.2 ± 2.4 and 21.6 ± 4.0 respectively) than those injected for the first time [35.6 ± 2.1 and 34.7 ± 1.5; F's(1,42) = 24.2 and 9.1 for 0.33 and 0.66 respectively, $p<0.005$]. However, these animals continued to show higher levels than home cage controls, indicating that tolerance to the nicotine and/or injection procedure was not complete.

Overall, changing drug-related cues reduced tolerance, since rats subjected to the new context had higher CORT levels than did those in the old context, $F(1,42) = 4.2$, $p<0.05$. This effect was largely due to the higher dose, since animals receiving 0.66

mg/kg in the new context were higher (33.7 ± 1.5) than those in the usual context [21.6 ± 4.0; $F(1,42) = 6.1$, $p < 0.02$] and did not differ from rats receiving nicotine for the first time [34.7 ± 1.5; $F(1,42) = 0.2$, $p > 0.10$]. A similar trend for the 0.33 mg/kg dose in the new vs. old context was not significant [26 ± 1.5 vs. 20.2 ± 2.4; $F(1,42) = 0.2$, $p > 0.10$].

EXPERIMENT 2

In the first study, changing environmental cues from those consistently associated with drug administration (test environment) to those never associated with drug administration (home environment) partially disrupted tolerance by reinstating the CORT response to nicotine. However, it could be argued that the change itself was a stressor that stimulated CORT release. Experiment 2, which was originally conducted for another purpose, addresses this issue of whether an environmental change per se increases CORT, by giving animals a series of saline injections in the test environment and then injecting them with either saline or nicotine in the home environment.

Experimental Groups and Procedures

Thirty-six male rats were assigned to one of 4 groups (N = 9). Daily milk intake testing was conducted as in Experiment 1. The one difference was that these rats were maintained on a reversed, 12:12 light cycle and testing was conducted during hours 3–6 of the dark period. All other procedural details were identical to those of Experiment 1. During 10 baseline tests, all rats were weighed, returned to their home cage for 9 minutes, then hand carried to the testing room, injected with saline and placed into a holding cage for 2 min before being tested for milk intake. Saline injections and testing of milk intake continued throughout an additional 11 day period corresponding to Phase 2 (tolerance) in Experiment 1. On the next day, two groups received either saline (1 ml/kg SC) or nicotine bitartrate (0.33 mg/kg, free base) injections in the testing room according to the above procedure (NCHG). The other two groups (CHG) received saline or nicotine in the colony room immediately after removal from their home cage. They were returned to their home cage for the two min drug-test interval before being carried to the testing room. This change procedure was identical to that used in Experiment 1.

RESULTS

Milk Intake

As in Experiment 1, rats drank between 17 and 20 ml of sweetened milk in the 10 minute tests (Table 1) and nicotine (0.33 mg/kg), when given for the first time, reduced intake by approximately 67% (compared to 65% in Experiment 1). However, switching the injection site from the testing room to the colony room did not affect intake of either the saline (NCHG = 18.6 ± 1.3 vs. CHG = 17.4 ± 1.1) or nicotine rats (NCHG = 5.2 ± 1.0 vs. CHG = 6.6 ± 1.2).

The overall ANOVA included two between factors and one within factor. The between factors were Condition, (change or no change) and Treatment, (nicotine or saline). The within factor, Trials, included 2 repeated measures; the next to the last test day (corresponding to the end of the tolerance period, Phase 2 of Experiment 1) and the last test day, on which rats were given either saline or nicotine in the usual or new environment. Post hoc comparisons (Tukey) were subsequently made using the within subjects error term. The results indicated significant effects of Trials, $F(1,32) = 63.1$, $p < 0.001$, Treatment, $F(1,32) = 16.3$, $p < 0.001$, and Trials × Treatment, $F(1,32) = 49.4$, $p < 0.001$, but not of Condition, $F(1,32) = 2.9$, $p = 0.10$. Individual comparisons indicated that intake did not differ between tests in which rats were injected in the testing or colony room for either saline ($p > 0.05$) or nicotine ($p > 0.05$) treatments.

TABLE 1

MILK INTAKE OF EXPERIMENT 2

Group	Last Day Before Change	Change Day
Sal-Sal-CHG	20.3 ± 1.0	18.6 ± 1.3
Sal-Sal-NCHG	17.6 ± 1.0	17.4 ± 1.1
Sal-Nic-CHG	19.3 ± 1.0	6.6 ± 1.2
Sal-Nic-NCHG	19.4 ± 3.5	5.2 ± 1.0

Values = Mean ml/10 min ± S.E.

Sal-Sal = Rats received saline throughout experiment including last day (Change Day).

Sal-Nic = Rats received saline throughout experiment but nicotine (0.33 mg/kg) on last day.

CHG, NCHG = Environmental change and no change, respectively.

Plasma Corticosterone

As can be seen from Fig. 3, 0.33 mg/kg of nicotine increased plasma CORT levels above saline-injected controls, but changing the location of injection did not further alter CORT levels for either group. An overall one-way ANOVA yielded a significant between groups effect, $F(3,32) = 57.1$, $p < 0.001$. However, post hoc comparisons (Newman-Keuls) indicated no effect of changing the location of injection for either the saline ($p > 0.10$) or nicotine ($p > 0.10$) treatments.

DISCUSSION

The results demonstrate that when nicotine administration is repeatedly paired with distinctive environmental and procedural cues, behavioral and neuroendocrine tolerance develops, and changing those cues partially disrupts the tolerance, as reflected by a reinstatement of a substantial part of both the anorectic and

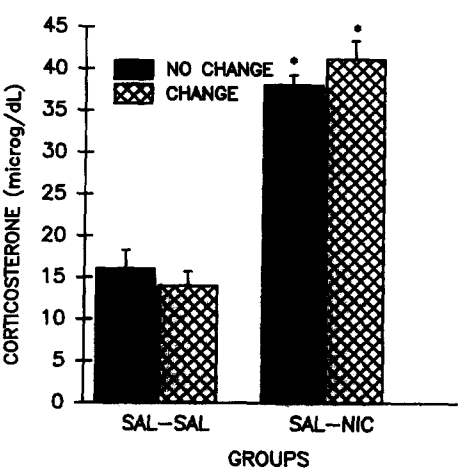

FIG. 3. Corticosterone levels were elevated in saline-treated rats injected with nicotine (0.33 mg/kg) for the first time (Sal-Nic) compared to rats given an equal volume of saline (Sal-Sal), but changing the injection environment did not affect CORT levels. *$p < 0.01$ vs. Sal-Sal.

CORT-activating effects of the drug. These findings not only confirm our original reports that tolerance to the behavioral effects of nicotine contains an important associative component (7,18), but also extend them by suggesting that a similar conditioning process may contribute to the development of tolerance to nicotine's neuroendocrine effects. This study also confirms the results of others using different drugs and measures in demonstrating that conditioned tolerance extends to physiological as well as behavioral effects of drugs (11, 14, 16, 17, 22, 29, 30, 44).

The initial (acute) effects of nicotine were to decrease food intake and increase plasma CORT levels. The decreased food intake cannot be entirely explained by motoric impairment or decreased activity since: 1) the lower dose results in little if any motor impairment; 2) locomotor activation, not depression, is produced by comparable doses of nicotine after the second through the fifth daily injection [unpublished data; see also (28)], whereas the decreased intake persisted, albeit with some tolerance, over the same injection schedule; 3) others have reported that the anorexia produced by nicotine in male rats is specific to sweetened, high-caloric foods and does not generalize to the intake of other foods [see (7) for further discussion].

The anorectic effects were dose dependent, but the CORT effects were not; the elevations in CORT were similar for the 0.33 and 0.66 mg/kg doses. It is likely that this discrepancy between the effects of dose on the two measures is the result of differences between the time course and sensitivity of behavioral and adrenocortical effects of nicotine (5,6). Differential effects of these doses on plasma CORT levels may only be manifest at or after the peak CORT response to nicotine, which is not reached until at least 20 minutes after administration (8), rather than the 12 minutes used here. This is supported by our recent observation that when measured 60 minutes after injection, plasma CORT levels produced by 0.66 mg/kg are three times those of 0.33 mg/kg (unpublished data).

The near total tolerance which developed to the anorectic effects of nicotine after 10 daily administrations is consistent with our previous work in which a minimum of 10 days was required for intake to approach baseline values when nicotine was given daily [(7) and unpublished data]. Tolerance to the CORT-stimulating effects of nicotine injections may take longer since significant but incomplete tolerance developed after 10 injections in Experiment 1, and others have found that up to 30 daily injections are required for total tolerance of this system when compared to saline-injected controls (9). However, the injection procedure itself may have contributed to the apparent incompleteness of tolerance to nicotine (relative to untreated controls) since the CORT levels of rats repeatedly injected with saline in Experiment 2 also appeared elevated, and others have reported difficulty in obtaining complete habituation to the CORT response to physical stressors (37). The possibility that the difference in CORT levels between the rats repeatedly injected with nicotine and untreated controls may have been due to the incompletely habituated response to the injection procedure will have to be tested in a future experiment.

Changing cues associated with drug delivery prevented the full expression of tolerance. The disruption of tolerance was complete for the CORT-elevating effects of 0.66 mg/kg, since CORT levels of animals that received the thirteenth injection in the new environment were the same as values obtained from rats injected with nicotine for the first time. Significant but incomplete disruption of tolerance was obtained for milk intake at both doses and for the CORT effects of the lower, 0.33 mg/kg dose; in none of these cases did the effects of the thirteenth injection equal those of the first administration. A number of factors can contribute to the proportion of associative and nonassociative tolerance that develops to repeated drug administration, and thus the degree to which changing cues will disrupt tolerance, including dose and interdrug interval (4,46). In addition, we have previously suggested that changing only a subset of cues that normally accompany and predict drug delivery may be expected to incompletely reverse associative tolerance (7). Thus the incompleteness of the reversal seen in the present study may reflect a mix of tolerance due to pharmacodynamic factors and residual associative tolerance surviving the environmental manipulation. Moreover, it would be premature to suggest that greater associative tolerance was generated for the CORT effects of the higher dose, since the degree of tolerance and its reversal were assessed in this study by using a single test dose identical to the conditioning dose, and a thorough determination of the completeness of tolerance and its reversal can only be made by measuring shifts in comparable dose-response curves (4,46).

Both peripheral and central mechanisms may mediate nicotine's action on the HPA axis, although central effects are likely to dominate (19,33). Centrally, nicotine acts on nicotinic cholinergic receptors in the hypothalamus to either directly release CRF and trigger the ACTH-CORT cascade or stimulate CRF indirectly via other neurotransmitter systems, such as norepinephrine (19). Rapid desensitization of nicotinic cholinergic receptors with repeated treatment has been proposed as a mechanism for acute tolerance to nicotine's ACTH effects (19,41). Receptor changes also have been suggested for chronic tolerance to some of nicotine's effects, and the reported increase in the number of nicotine binding sites in the brain after chronic nicotine (28, 31, 40) could reflect neural compensations that render drugs less effective pharmacologically [(31,40) but see (38)]. However more recent studies reporting dissociations between the pattern and duration of behavioral tolerance on the one hand, and increases in nicotine binding on the other hand (12,32), suggest that additional explanations for chronic tolerance must be sought. One possible reason for discrepancies between the development of tolerance and the development of receptor changes to repeated administration is that if, in most instances, tolerance is made up of associative and nonassociative components, receptor changes may only be important for the nonassociative component. The mechanisms for associative tolerance are likely to be more complex in order to account for the suddenness of tolerance reversal when environmental cues are changed and the observation that tolerance to very different responses, i.e., analgesia (18), anorexia and HPA activation [(7) and present paper], appear to be dependent on the same conditioning process.

One possible explanation for the reversal of tolerance obtained in this study is that changing environmental cues during testing induced nonspecific stress which was responsible for the decreased milk intake and/or increased CORT in the CHG condition. This hypothesis is deemed unlikely for several reasons. First, it has been investigated and found inadequate in explaining other instances of environmentally dependent tolerance (20, 27, 43). Second, while the increase in CORT after environmental change is consistent with a stress hypothesis, the decrease in milk intake is not, since mild stress tends to increase, not decrease consummatory responses (3). Manipulations effective in inducing severe stress in rats, which could disrupt consummatory behavior, normally consist of aversive stimuli or novel, and presumably threatening, environments (24), but in this study, the environmental change was produced by switching the injection location from a test room to the animal's home environment. Finally, in Experiment 2, changing injection location from the testing room to the home environment did not increase CORT levels produced by repeated saline injections or by a first nicotine injection, even when CORT levels in saline-injected rats were similar to the nicotine tolerant rats of Experiment 1. These

results suggest that the environmental change used in Experiment 1 was not sufficiently stressful to account for the observed increase in CORT.

In summary, the present results suggest that tolerance to the hypothalamic-pituitary-adrenocortical effects of nicotine may be subject to the same principles of conditioning and therefore, environmental control, as is tolerance to nicotine's behavioral effects. If these results apply to the effects of nicotine in humans, then recent reports that chronic smokers show little or no tolerance to the HPA-activating effects of smoking (15, 21, 48, 49) may reflect a disruption of conditioned tolerance produced by testing smokers in a unique experimental environment very different from that in which they normally smoke, rather than a total lack of tolerance. A more far-reaching implication of the present results is that smokers who have learned to disrupt tolerance to some of the effects of nicotine, i.e., those that are responsible for the reinforcing effects of the drug, by altering the psychological or physical conditions of smoking, may also be reinstating the HPA-activating effects of the drug, which have serious health implications for long term smokers since chronic adrenocortical hypersecretion can adversely affect immune, cardiovascular and behavioral systems (19, 34, 39, 45).

ACKNOWLEDGEMENTS

This research was supported by grants BNS-8909487, MH24114 and MH42530.

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