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Chronic Forced Swim Stress Produces Subsensitivity to Nicotine

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PECK, J. A., S. C. DILSAVER AND M. McGEE. *Chronic forced swim stress produces subsensitivity to nicotine.* PHARMACOL BIOCHEM BEHAV **38**(3) 501–504, 1991.—Twice daily injections of saline reduce the thermic response to nicotine in the rat. The authors hypothesized that this was due to the stress of twice-daily handling and injection. However, the injection of saline is not a classic stressor. The hypothesis that stress blunts thermic responsiveness to nicotine was, therefore, tested using a classic form of chronic inescapable stress. Rats (n = 12) were subjected to a 14-day, twice daily course of inescapable cold water swim stress using a repeated measures design. Thermic responsiveness of nicotine was measured at baseline and every 7 days thereafter for 49 days. The mean response to nicotine (1.0 mg/kg IP) differed significantly across time, $F(7,88) = 10.6$, $p<0.0001$. Mean thermic responsiveness (±SEM) decreased from -0.75 ± 0.09 at baseline to -0.41 ± 0.18°C (54.7% of baseline) following 14 days of forced swim stress. This change was not significant. However, the thermic response to nicotine was -0.14 ± 0.13°C ($p<0.05$), $+0.55 \pm 0.12$°C ($p<0.05$), and $+0.04 \pm 0.11$°C ($p<0.05$) 7, 14, and 21 days following the discontinuation of forced swim stress. The mean response did not differ from baseline 28 days following the last session of forced swim stress. The data suggest that in the recovery phase the animals ceased to be sensitive to nicotine. These findings support the hypothesis that a chronic stressor can produce subsensitivity to nicotine.

Acetylcholine Cholinergic Nicotine Receptors Stress

FLEMMER and Dilsaver (16) reported that twice daily injections of normal saline (1 ml/kg) for 7–14 days produces subsensitivity to nicotine. Nicotinic mechanisms regulate the release of amines (1, 6, 7, 18, 23) and induce the synthesis of tyrosine hydroxylase (21). These points render the effects of stress on nicotinic mechanisms a curiosity to investigators interested in the biology of the disorders of mood. We now report that chronic forced swim stress (a method of producing learned helplessness) also blunts the thermic response to nicotine (19).

METHOD

Dependent Variable

The dependent variable in this study is mean change in thermic response over a 60-minute period following the intraperitoneal injection of nicotine (base), 1.0 mg/kg. Principles governing the use of this dependent variable in psychopharmacological research were recently reviewed (11).

Measurement of Core Temperature

Core temperature was telemetrically measured using the Model VM Mini-Mitter (Mini-Mitter Corp., Sun River, OR). These devices are hearing-aid-battery powered radio transmitters which emit amplitude modulated (AM) waves at a rate directly proportional to temperature. The Mini-Mitter provides reliable and valid measurements of core temperature. Two independent investigators can obtain statistically indistinguishable regression equations

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in the process of calibrating the Mini-Mitter and identical experimental results when independently applying it in the same psychopharmacological study (14).

The Mini-Mitters are calibrated by measuring the rate at which they emit pulses (of AM waves) at three temperatures overlapping with the physiological range in a temperature-controlled water bath (Precision Instruments Model 50).

Nicotine Challenges

All nicotine challenges started at 10:00 a.m. The first step in each of these challenges was the measurement of core (i.e., baseline) body temperature. Core temperature was then measured every 10 minutes for 60 minutes following the intraperitoneal injection of 1.0 mg/kg of nicotine (base). The mean thermic response was the average of the 6 deviations from baseline.

The animals were challenged with nicotine before first being subjected to forced swim stress and then every 7 days thereafter for 42 days. The second challenge with nicotine preceded the first session of forced swim on the eighth day of the 14-day course of swim stress; on this occasion the first session of forced swim stress was delayed to about 11:00 a.m. The second session of forced swim stress occurred at its usual time. The third challenge with nicotine occurred the day following the last session of forced swim. Dilsaver et al. (13) previously reported that the intraperitoneal injection of nicotine at a dose of 1.0 mg/kg at seven-day intervals does not produce carry-over effects (e.g., blunting of the thermic response to nicotine).

Inescapable Forced Swim Stress

The animals were subjected to forced swim at 12°C for 8 continuous minutes for 14 consecutive days at 7:00 a.m. and 5:00 p.m. The depth of the water was adjusted so that the animals could not balance themselves on their tails.

Environmental Conditions

The sessions of forced swim stress and challenges with nicotine were conducted in the same room in which the animals were housed. Conditions in this room and care for the animals were governed by the Ohio State University. This intensity of ambient lighting, the duration of the light/dark cycle (12 hours on/12 hours off), timing of the light/dark cycle, and temperature (21.1 to 22.2°C) were constant throughout the study.

Pharmaceutical Agents

Nicotine (base) was purchased from Sigma Chemical Co. (St. Louis, MO).

Animals

Adult, male Sprague-Dawley Rats were purchased from Harlan Laboratories (Indianapolis, IN).

Statistical Analysis

All data were subjected to a one-way analysis of variance (ANOVA) for repeated measures (20) followed by application of a *t*-test for the least significant difference (LSD) using SAS. The LSD performs pairwise *t*-tests in the case of equal cell sizes for all main effect means in the MEANS statement. The least significant difference was 0.37°C. The critical value of α was set for $p<0.05$ for both the ANOVA and LSD. All measures of variance in the test refer to the standard error of the mean (SEM).

RESULTS

The mean mass of the 12 rats used in this study was 286.7 ± 2.9 g. The mean core temperatures of the sample of undisturbed animals just prior to the injection of nicotine for the first through the last of eight challenges with this agonist were 36.7 ± 0.3°C, 36.9 ± 0.3°C, 36.9 ± 0.3°C, 36.9 ± 0.3°C, 36.0 ± 0.3°C, 36.6 ± 0.3°C, 37.2 ± 0.3°C, and 37.5 ± 0.3°C, respectively. These means are in the physiological range and are commonly observed in the Sprague-Dawley rat when core temperature is measured during the photoperiod using the noninvasive method of telemetry. However, these mean baseline temperatures differ, $F(7,88) = 2.31$, $p=0.033$. Mean core temperatures prior to challenges 5 and 6 were significantly lower (but not outside the range expected) than those preceding the other 6 mean measurements of baseline core temperature [see (11) for a review of studies in which baseline temperature is measured under the conditions prevailing in this study].

The mean thermic response of the sample prior to starting a course of forced swim stress was −0.75 ± 0.09°C. The 14-day course of forced swim stress started the morning following the measurement of baseline thermic responsiveness to nicotine. The sample exhibited blunting of the thermic response to nicotine after the first and second weeks of forced swim stress. However, this change was not significantly different, $F(2,33) = 1.41$, $p=0.25$. The second nicotine challenge occurred 18 hours (at 9:00 a.m.) after the seventh day of forced swim stress. The mean thermic response at this point was −0.56 ± 0.12°C (n.s.). The response to nicotine was next measured 18 hours after the last session of forced swim stress. This mean response (−0.41 ± 0.18°C) did not differ significantly from baseline.

In contrast, the mean thermic response across the entire time course of the study was highly significant, $F(7,88) = 10.62$, $p<0.0001$. The thermic responses to nicotine were significantly less than at baseline 7 (−0.14 ± 0.13°C), 14 (+0.55 ± 0.12°C), and 21 (+0.04 ± 0.11°C) days following the conclusion of the 14-day course of swim stress. The response did not differ from baseline 28 (−0.47 ± 0.12°C) or 35 (−0.70 ± 0.13°C) days following the last session of forced swim. Thus chronic inescapable cold water swim stress produced delayed but prolonged blunting of the thermic response to nicotine.

Table 1 summarizes the results of the study. Figure 1 illustrates the mean thermic response of the sample at baseline, after seven and fourteen days of inescapable swim stress, and each week thereafter for five weeks.

The occurrence of hyperthermia 7 and 14 days following the conclusion of the course of swim stress is characteristic of manipulations of the nicotinic cholinergic system. We have observed it in the process of recovery or reequilibration following a chronic treatment with amitriptyline (13) and in the course of treatment with bright light (9).

DISCUSSION

Chronic inescapable swim stress blunted the thermic response to nicotine in the rat. The response to nicotine continued to decrease following the conclusion of the 14-day course of swim stress. Thermic responsiveness significantly differed from baseline at 7, 14, and 21 days following the last day of forced swim. The sample exhibited a hyperthermic response following the injection 14 and 21 days following the conclusion of the course of forced swim stress.

We previously studied the effect of substituting normal saline for agonists producing hypothermia. The mean thermic response of 46 rats whose core temperature was measured every 10 min-

TABLE 1

Challenge No.	Condition	Baseline Core Temp. °C ± SEM	Mean Thermic Response °C ± SEM
1	Baseline	36.7 ± 0.3	−0.75 ± 0.09
2	Swim Stress for 7 Days	36.9 ± 0.3	−0.56 ± 0.12
3	Swim Stress for 14 Days	36.9 ± 0.3	−0.41 ± 0.18
4*	Poststress 7 Days	36.9 ± 0.3	-0.14 ± 0.13
5*	Poststress 14 Days	36.0 ± 0.3	+0.55 ± 0.12
6*	Poststress 21 Days	36.6 ± 0.3	+0.04 ± 0.11
7	Poststress 28 Days	37.2 ± 0.6	−0.47 ± 0.12
8	Poststress 35 Days	37.5 ± 0.3	−0.70 ± 0.13

Twelve (12) adult, male Sprague-Dawley rats with a mean weight ± SEM of 286.7 ± 2.9 g received an intraperitoneal injection of 1.0 mg of nicotine (base) before, and after 7 and 14 days of twice daily forced swim stress. The subjects were then challenged with the same dose of nicotine weekly for 5 weeks after the last session of forced swim stress. The change in thermic response across time was highly significant [$F(7,88) = 10.62$, $p<0.0001$, one-way ANOVA for repeated measures]. *Indicates that the thermic response differed from baseline at $p<0.05$.

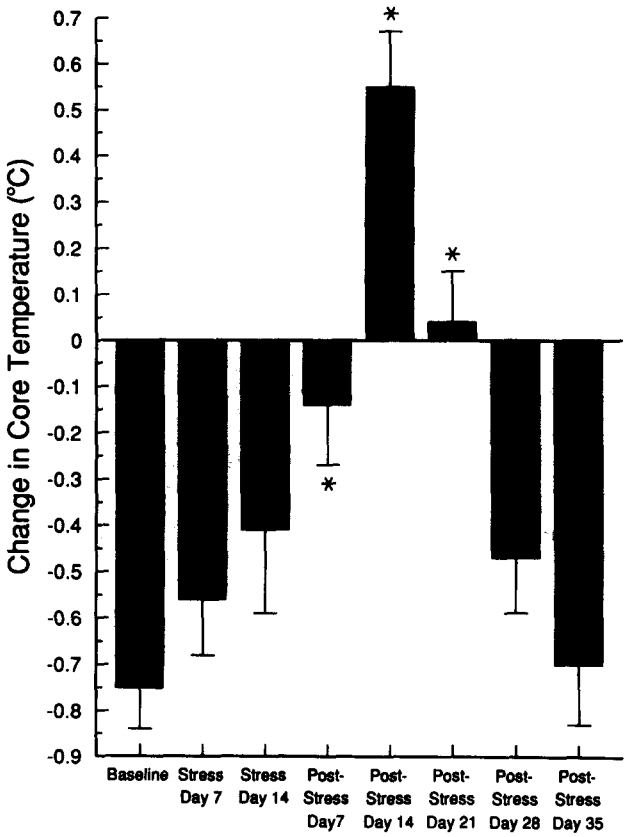

FIG. 1. The reduction in thermic responsiveness was not significant at the conclusion of a two-week course of twice daily inescapable cold water swim stress. The response was, however, significantly different from the preswim stress baseline one, two, and three weeks following the last swim stress session.

utes for 120 minutes following the injection of saline was $+0.55 \pm 0.07$°C, $t(45) = 7.84$, $p<0.000001$, Student's paired t-test (12). The injection of saline typically produces a mean thermic response of +0.2°C to +0.8°C over a 120-minute period following its injection (12). The occurrence of a mean hyperthermic response following the injection of nicotine suggests that the average animal became insensitive to the thermic effects of this agonist during the poststress (recovery) phase.

Our group previously reported that twice daily injections of saline affect a reduction in the thermic response of the rat to nicotine (16). We hypothesized that this phenomenon was due to the chronic stress of twice daily handling and injection. The results presented in this article are consistent with this hypothesis.

Chronic forced swim stress reportedly depletes the rat brain of biogenic amines (22). Forced swim stress (8, 9, 11, 15) and chronic inescapable footshock (10) also supersensitize a central muscarinic mechanism involved in the regulation of core temperature. Our studies assessing the effect of a forced stressor on a muscarinic cholinergic system also involved the measurement of muscarinic agonist induced change in core temperature. This is apparently due to an effect of the agonist on a hypothalamic mechanism (11). The findings reported here can be reconciled with the previous studies indicating that chronic stressors affect both aminergic (23) and muscarinic mechanisms (9, 10, 12, 17).

Nicotinic and muscarinic mechanisms increase and decrease the release of norepinephrine in select regions of the hypothalamus, respectively (23). A muscarinic mechanism similarly inhibits the release of norepinephrine in the myocardium (18). Nicotine causes the release of dopamine in both the nigrostriatal and mesolimbic tracts (1). Augmentation of nicotinic neurotransmission increases the activity of the enzyme governing the rate limiting step in the synthesis of catecholamines in the periphery (21). Thus nicotinic mechanisms are associated with the synthesis and release of amines and muscarinic cholinergic receptor activation with inhibition of the release of catecholamines.

A chronic stressor may affect aminergic mechanisms through an effect on nicotinic mechanisms or vice versa. Blunting of responsiveness to nicotine may functionally have an effect similar to the effect of depleting brain biogenic amines or of rendering aminergic systems ineffective. It is also possible that a chronic stressor alters muscarinic mechanisms consequent to an effect on an aminergic system. Dysfunction of the latter may tend to activate the former. Effects of inescapable stress on a muscarinic mechanism could also be due to a primary effect of the stressor on a nicotinic mechanism. Dysfunction of a nicotinic mechanism may allow the activity or sensitivity of a muscarinic mechanism to increase. The interaction of aminergic and muscarinic cholinergic mechanisms (7,8) and the effects of stress on muscarinic cholinergic mechanisms in man and animals were recently reviewed (8).

This report isolates an interesting phenomenon but does not provide evidence of an underlying mechanism. Thermoregulation is a complex phenomenon. Endogenous substances may either increase or decrease an animal's temperature when exogenously administered. The effect depends on the species studied, dose, mode and site of administration. Clarke and Lipton (2–4) have extensively reviewed this literature. The release of many endogenous substances such as peptides (3), amines (1), acetylcholine (2) and opioids (5,17) is affected by nicotine.

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