Pharmacology Biochemistry & Behavior, Vol. 34, pp. 261–263. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Chronic Injections of Saline Produce Subsensitivity to Nicotine[1]

DUANE D. FLEMMER

Psychopharmacology Program, Department of Psychiatry, The Ohio State University

AND

STEVEN C. DILSAVER[2]

Department of Psychiatry, The Ohio State University
473 West 12th Avenue, Columbus, OH 43210-1228

Received 21 February 1989

FLEMMER, D. D. AND S. C. DILSAVER. *Chronic injections of saline produce subsensitivity to nicotine.* PHARMACOL BIOCHEM BEHAV **34**(2) 261–263, 1989.—The routine handling of rats and the injection of saline is a stressor. The authors report that chronic twice daily injections of normal saline (1 ml/kg IP) for 14 days produced subsensitivity to the hypothermic effects of nicotine (1 ml/kg IP). The weekly injection of nicotine (1 mg/kg IP) does not produce this effect. The investigators propose that their findings reflect the effect of chronic stress on a nicotinic mechanism. Lithium, desipramine, fluoxetine, and amitriptyline also alter the thermic response to systemically injected nicotine. A nicotinic mechanism(s) may be involved in the neurobiology of chronic stress, actions of antidepressants, and *conceivably* the pathophysiology of depression.

Acetylcholine Cholinergic Nicotine Placebo Rats Receptors Stress Telemetry Thermoregulation

IN 1972 Janowsky and associates published an article advancing the hypothesis that hyperfunction of central muscarinic cholinergic mechanisms is involved in the pathophysiology of depression (16). The literature supporting this hypothesis was recently summarized (2,3). A recent twist to the classic version of the cholinergic hypothesis is provided by the finding that pharmacologic treatments for depression alter the sensitivity of rats to the thermic effects of nicotine (5, 8–11, 13). Chronic forced stressors, consistent with the muscarinic cholinergic hypothesis of depression, activate a central muscarinic cholinergic mechanism(s) in rats (4, 6, 15). Recent reports that six treatments for affective disorders—amitriptyline (13), desipramine (10), fluoxetine (8), phenelzine (11), lithium (5), and bright artificial light (9)—affect a nicotinic mechanism(s) suggest that the neurobiology of the mood disorders may also involve nicotinic mechanisms. This suggestion also raises questions about the behavioral, physiological, and biochemical effects of stress on nicotinic mechanisms.

The injection of saline (1 ml/kg IP) results in a robust hyperthermic response in rats (12). Placing rats in an open field also produces a hyperthermic response (19). This response is regarded as an effect of acute stress. Many of our protocols require multiple twice daily intraperitoneal injections of drugs (1 ml/kg). The effect of simply injecting an equal volume of saline (on a ml/kg basis) twice daily is, therefore, of methodologic relevance to our group and other investigators using similar protocols. The two experiments reported here were designed to determine whether twice daily injections of saline alter the thermic response to nicotine. We previously demonstrated that the injection of nicotine (1 mg/kg IP) does not produce the effect reported here (13).

METHOD

Dependent Variable

The dependent variable in this study is mean change in core temperature in response to the intraperitoneal (IP) injection of nicotine (base) 1 mg/kg in rats. Principles governing the use of this variable in psychopharmacological research were recently reviewed (7).

Measurement of Core Temperature

Core temperature was telemetrically measured using the Model

[1]Supported by Physician-Scientist Career Development Award Grant #MH00553-04 (Muscarinic Receptor Abnormalities in Affective Illness), National Institute of Mental Health.

[2]Requests for reprints should be addressed to Dr. Dilsaver.

VM Mini-Mitter (Mini-Mitter Corp, Sun River, OR). These devices are hearing-aid-battery-powered radio transmitters which emit AM radio waves at a rate proportional to temperature. The rate of emission is measured using a digital frequency counter. Core temperature was measured every 10 minutes for 120 minutes following the injection of nicotine (base), 1 mg/kg IP, prior to initiating twice daily injections of saline (1 ml/kg IP) for 14 days in two separate experiments involving 11 rats each. The reliability and validity of data obtained using the Mini-Mitter is established (14).

Pharmaceuticals

Nicotine (base) was purchased from Sigma Chemical Company (St. Louis, MO). This drug was diluted to a concentration of 1 mg/ml immediately prior to use.

Animals

Adult, male Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN). The animals were housed in a vivarium maintained by The Ohio State University with a 12-hour light/dark cycle (lights on at 6:00 a.m. and off at 6:00 p.m.).

Experimental Design and Statistical Analysis

This study involved two experiments based on a repeated measures design. The two groups of rats (n = 11) were challenged with nicotine (1 mg/kg IP) at 9:00 a.m. prior to beginning twice daily injections of saline (1 ml/kg IP). The injections of saline started the day following the first nicotine challenge. Saline was injected at 8:00 a.m. and 4:00 p.m. in Experiment 1 and at 9:00 a.m. and 5:00 p.m. in Experiment 2. The animals were rechallenged with nicotine at 7-day intervals. Each group of animals was challenged with nicotine three times.

The average thermic response of each rat was calculated by adding the 12 deviations from core temperature immediately prior to the injection of nicotine and dividing by 12. These averages (n = 11) were subjected to a one-way ANOVA for repeated measures followed by paired Student's *t*-tests to determine the significance of the change in thermic response between specific nicotine challenges. Measures of variance refer to the SEM.

RESULTS

Experiment 1

The mean mass of the rats used in this experiment was 302.8 ± 7.4 g. The mean core temperature of the animals at rest (at 9:00 a.m.) prior to the first injection of nicotine was 37.9 ± 0.44°C.

The sample exhibited mean thermic responses to nicotine of −0.47 ± 0.08°C, −0.19 ± 0.06°C, and −0.18 ± 0.07°C prior to and after one and two weeks of treatment with saline, respectively. These means differed, $F(2,20) = 6.5$, $p<0.01$. The thermic response to nicotine was blunted after one, $t(10) = 2.31$, $p<0.05$, and two, $t(10) = 2.48$, $p<0.05$, weeks of treatment with twice daily injections of saline relative to baseline.

Experiment 2

The mean mass of the rats used in this experiment was 314.5 ± 5.5 g. The mean core temperature of the rats at rest (at 9:00 a.m.) prior to the first challenge with nicotine was 37.2 ± 0.22°C.

The sample exhibited mean thermic response to nicotine of −0.63 ± 0.26, −0.47 ± 0.30, and −0.19 ± 0.06°C prior to and after one and two weeks of treatment with saline, respectively. These means differed, $F(2,21) = 22.86$, $p<0.0001$. The thermic response to nicotine was not blunted after one, $t(10) = 1.27$, $p>0.10$, week of twice daily injections with saline. However, the response was diminished after two weeks of treatment with saline, $t(10) = 3.17$, $p<0.015$. The response was also blunted following two weeks of saline injections relative to the end of the first week of this treatment, $t(10) = 3.30$, $p<0.009$.

Figure 1 illustrates the results of Experiments 1 and 2.

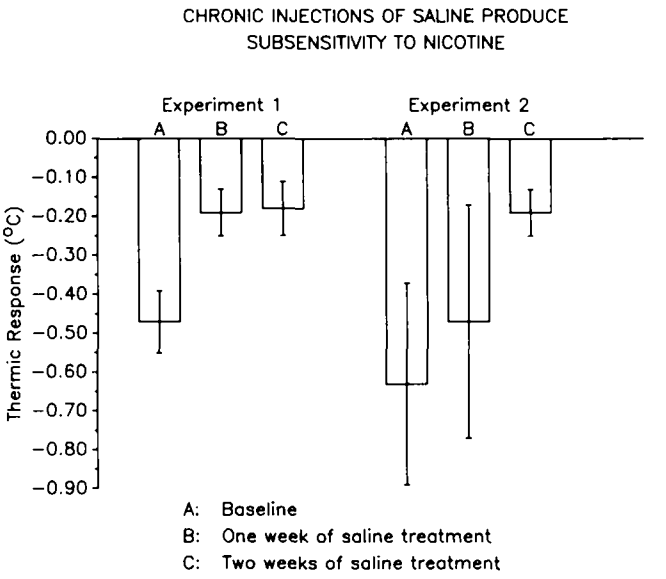

FIG. 1. This illustrates the progressive decline in thermic response to three challenges with nicotine (1 mg/kg) in Experiments 1 and 2. In Experiment 1 the blunting was significant after one week of twice daily injections with saline (1 ml/kg). The decrease was significant in Experiment 2 after two weeks of bi-daily injections of saline.

DISCUSSION

These data suggest that twice daily injections of saline reduce the thermic response to nicotine. It is critical to note that the administration of 1 mg/kg of nicotine (base) at an interval of 7 days for 28 days (i.e., 5 injections) does not produce a carryover effect. That is, the injection of nicotine (base) at the dose used in the generation of the experimental results presented here does not in the least decrease the hypothermic response to subsequent injections (13). It is, therefore, highly unlikely that the multiple injections account for our results. Chronic forced swim stress also produces prolonged subsensitization of a nicotinic mechanism involved in the regulation of core body temperature in the rat (18). We hypothesize that the documented diminution in the hypothermic response to nicotine is due to the effect of the chronic stress posed by twice dialy handling and injection of saline.

Chronic forced swim stress is reported to deplete the rat brain of biogenic amines (21). Forced swim stress (15) and chronic inescapable footshock (6) 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 involved the measurement of a dependent variable contingent on a hypothalamic mechanism (7). The findings reported here can be reconciled with the previous studies indicating that chronic stressors affect both aminergic and muscarinic mechanisms.

Nicotinic and muscarinic mechanisms increase and decrease the release of norepinephrine in the hypothalamus, respectively (22). A muscarinic mechanism similarly inhibits the release of

norepinephrine in the myocardium (17). 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 (20). 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. It is also possible that a chronic stressor alters muscarinic mechanisms secondary to an effect on an aminergic system consequent to a primary effect on a nicotinic mechanism. For now these are points of speculation, albeit speculations with a basis in the literature. The interaction of aminergic and muscarinic cholinergic mechanisms (2) and the effects of stress on muscarinic cholinergic mechanisms in man and animals were recently reviewed (4).

The immediate and most practical point of this report is that multiple injections of saline are not inert. This intervention alters the response of rats to nicotine. All studies requiring multiple injections of a drug in order to *assess* the effect of the drug on a behavioral, physiological, or biochemical parameter in rats to nicotine must control for this factor.

REFERENCES

1. Andersonn, K.; Fuxe, K.; Agneti, L. E. Effects of single injections of nicotine in the ascending dopamine pathways in the rat: Evidence for increases of dopamine turnover in neostriatal and mesolimbic dopamine neurons. Acta Physiol. Scand. 112:345–347; 1981.
2. Dilsaver, S. C. Cholinergic-monoaminergic interaction in the pathophysiology of the affective disorders. Int. Clin. Psychopharmacol. 1:181–198; 1986.
3. Dilsaver, S. C. Cholinergic mechanisms in depression. Brain Res. Rev. 11:285–316; 1986.
4. Dilsaver, S. C. Effects of stress on muscarinic mechanisms. Neurosci. Biobehav. Rev. 12:23–28; 1988.
5. Dilsaver, S. C. Lithium produces supersensitivity to nicotine. Biol. Psychiatry 25:795–798; 1989.
6. Dilsaver, S. C.; Alessi, N. E. Chronic inescapable footshock produces cholinergic system supersensitivity. Biol. Psychiatry 22:914–918; 1987.
7. Dilsaver, S. C.; Alessi, N. E. Temperature as a dependent variable in the study of cholinergic mechanisms. Prog. Neuropsychopharmacol. Biol. Psychiatry 12:1–32; 1988.
8. Dilsaver, S. C.; Davidson, R. K. Fluoxetine subsensitizes a nicotinic mechanism involved in the regulation of core temperature. Life Sci. 41:1165–1169; 1987.
9. Dilsaver, S. C.; Hariharan, M. Nicotinic effects of antidepressants. In: Gershon, S.; Lever, B., eds. New directions in affective disorders. New York: Springer-Verlag; 1989:109–112.
10. Dilsaver, S. C.; Hariharan, M.; Davidson, R. K. Desipramine subsensitizes nicotinic mechanism involved in regulating core temperature. Psychiatry Res. 25:105–108; 1988.
11. Dilsaver, S. C.; Hariharan, M.; Davidson, R. K. Phenelzine produces subsensitivity to nicotine. Prog. Neuropsychopharmacol. Biol. Psychiatry; in press.
12. Dilsaver, S. C.; Majchrzak, M. J. Effects of placebo (saline) injections on core temperature in the rat. Prog. Neuropsychopharmacol. Biol. Psychiatry; in press.
13. Dilsaver, S. C.; Majchrzak, M. J.; Alessi, N. E. Chronic treatment with amitriptyline produces supersensitivity to nicotine. Biol. Psychiatry 23:169–175; 1988.
14. Dilsaver, S. C.; Majchrzak, M. J.; Alessi, N. E. Telemetric measurement of core temperature in psychobiological research: Reliability and validation. Prog. Neuropsychopharmacol. Biol. Psychiatry; in press.
15. Dilsaver, S. C.; Snider, R. M.; Alessi, N. E. Stress induces supersensitivity of a cholinergic system in rats. Biol. Psychiatry 21:1093–1096; 1986.
16. Janowsky, D. S.; Davis, J. M.; El-Yousef, M. K.; Sererke, H. S. A cholinergic adrenergic hypothesis of mania and depression. Lancet 2:632–635; 1972.
17. Muscholl, E. Regulation of catecholamine release. The muscarinic inhibitory mechanism. In: Usdin, E.; Snyder, S. H., eds. Frontiers in catecholamine research. New York: Pergamon Press; 1973:537–549.
18. Peck, J.; McGee, M.; Jaeckle, R. S.; Dilsaver, S. C. Chronic stress produces delayed and prolonged subsensitization of a nicotinic mechanism. Biol. Psychiatry 25(7A):35A; 1989.
19. Singer, R.; Harker, C. T.; Vander, A. J.; Kluger, M. J. Hyperthermia induced by open-field stress is blocked by salicylate. Physiol. Behav. 36:1179–1182; 1986.
20. Thoenen, H.; Otten, O.; Oesch, E. Transsynaptic regulation of tyrosine hydroxylase. In: Usdin, E.; Snyder, S. H., eds. Frontiers in catecholamine research. New York: Pergamon Press; 1973.
21. Weiss, J. M.; Goodman, P. A.; Losito, B. G.; Corrigan, S.; Charry, J. M.; Bailey, W. H. Behavioral depression produced by an uncontrollable stressor: Relationship to norepinephrine, dopamine and serotonin levels in various regions of rat brain. Brain Res. Rev. 3:167–205; 1981.
22. Westfall, T. C. Effect of acetylcholine on the release of [^{3}H]norepinephrine by nicotine and potassium chloride from rat brain slices. In: Usdin, E.; Snyder, S. H., eds. Frontiers in catecholamine research. New York: Pergamon Press; 1973:617–668.