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Constant Exposure to Darkness Produces Supersensitivity to Nicotine[1]

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FLEMMER, D. D. AND S. C. DILSAVER. *Constant exposure to darkness produces supersensitivity to nicotine.* PHARMACOL BIOCHEM BEHAV **35**(3) 523–526, 1990.—Treatment with full-spectrum bright artificial light produces subsensitivity to the hypothermic effect of nicotine in the rat. The authors hypothesized that prolonged exposure to darkness would produce the opposite effect. The thermic responsiveness of 11 rats to nicotine (base), 0.25 mg/kg IP, was telemetrically measured at baseline, after 7 days of exposure to constant darkness, and 2, 5, and 12 days after being returned to standard vivarium conditions. Exposure to constant darkness enhanced the hypothermic response to nicotine. The sample exhibited a hyperthermic response to nicotine 2 and 5 days after being returned to the standard vivarium conditions with a 12-hour-light/12-hour-dark cycle. The magnitude of the hyperthermia observed is characteristic of the response to the injection of saline. Twelve days after return to standard vivarium conditions the thermic response of the sample was at baseline.

Acetylcholine Bright light Cholinergic Nicotine Telemetry Thermoregulation

IN 1972 Janowsky and associates published an important article setting forth the hypothesis that hyperfunction of central muscarinic cholinergic mechanisms is involved in the pathophysiology of depression (24). Since then a rich literature pertaining to this hypothesis has evolved [see (2, 8, 23, 29) for reviews]. Interesting twists were recently added to the classic version of the cholinergic hypothesis. First, Janowsky and Risch (22) proposed that stressors may activate muscarinic mechanisms in man. Clinical research suggests that some of the effects of stressors might indeed be mediated by muscarinic mechanisms (4, 25, 26, 27). Preclinical studies subsequently demonstrated that chronic forced stressors can activate a central muscarinic mechanism involved in the regulation of core temperature in the rat (4). Second, recent reports that six treatments for affective disorders—amitriptyline (17), desipramine (16), fluoxetine (9), phenelzine (12), lithium (5,11), and bright artificial light (3,11)—affect a nicotinic mechanism suggest that it is theoretically possible that the pathophysiology of the mood disorders or the mechanism of action of their treatments may also involve nicotinic cholinergic systems.

Treatment with full-spectrum bright artificial light consistently produces the remission of winter depression (28, 31, 32, 34). This treatment produces subsensitivity to the hypothermic effect of nicotine (3,13) in the rat. We hypothesized that chronic exposure to darkness for 7 days would produce an effect on the thermic response to nicotine opposite that produced by treatment with bright artificial light. The study reported here tests this hypothesis.

METHOD

Dependent Measure

The dependent variable in this study is change in core temperature in response to the intraperitoneal injection of nicotine in 11 adult, male Sprague-Dawley rats (mean weight ± SEM = 312.7 ± 7.4). Core temperature is measured immediately before the injection of nicotine (i.e., at baseline) and every 10 min thereafter for 120 min. The hypothermic response to nicotine is at a maximum about 20 min after the injection of nicotine. The mean hypothermic response at this time point is presented for descriptive purposes only. The mean thermic response for the entire 120-min period is the average of the 12 deviations from baseline. This measurement is subject to less influence by random factors than a single measurement or the mean maximum thermic response. Principles governing the use of measurement of core temperature in psychopharmacological research are presented in detail elsewhere (7). The reliability and validity of the method is established (15).

Selection of Dose of Nicotine

The procedures employed in this study are mildly stressful and produce a hyperthermic response (9, 14, 16, 18–20). These procedures include the act of telemetrically measuring core temperature every 10 min for 120 min (this requires that a small AM receiver be placed into the cage of individually housed animals), manual handling, and the injection of nicotine. We previously studied the effect of substituting normal saline (1 ml/kg) for nicotine. The mean thermic response (as calculated in this article) of 46 rats subjected to the injection of saline was +0.55 ± 0.07°C [$t(45) = 7.84$, $p < 0.000001$, Student's paired t-test]. Forty-three (43) of the 46 animals exhibited a mean increase in core temperature, two demonstrated no change, and one a slight fall ($p < 0.000001$, Wilcoxon Sign Rank Test for Matched Pairs) (14). The injection of saline typically produces an average hyperthermic

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response of +0.2 to +0.8°C over the following 120-min period (9, 16, 21).

A dose of nicotine which produces a minimal hypothermic response in naive animals was intentionally selected. The response to this dose may appear to be inconsequential. However, a definite drug effect can be appreciated by contrasting it with the hyperthermic response produced by injecting an equal volume of saline. Selection of a dose of nicotine producing minimal hypothermia prior to exposure to constant darkness is intended to avoid a "floor effect."

Animals

Adult, male Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN). The animals were housed in a vivarium with a 12/12 light-dark cycle when not in constant darkness.

Temperature Measurements

Core temperature was measured using an intraperitoneally implanted thermosensor, the Model VM Mini-Mitter (Mini-Mitter Corp., Sun River, OR). These devices emit radio waves at a rate directly proportional to temperature. The waves are detectable with a standard AM receiver attached to an automatic counter (Universal Global Specialties Model 5001, New Haven, CT). The Mini-Mitter can detect a 0.1°C change in temperature (33).

Pharmaceuticals

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

Nicotine Challenges

The baseline core temperature of each rat was determined. Nicotine, 0.25 mg/kg IP, was then administered and the core temperature of each rat was measured every 10 min for 120 min.

All challenges started at about 4:00 p.m. The first nicotine challenge occurred on Day 1 of the study, 7 days after the implantation of the Mini-Mitters. The animals were placed in a constantly dark environment for 6 days and 14 hours at the conclusion of the first nicotine challenge. They were removed from this environment at 7:00 a.m. on Day 7 of the study. The second nicotine challenge started 9 hours later. The third, fourth, and fifth nicotine challenges were on Days 9, 12, and 19 of the study (i.e., 2, 5, and 12 days after being removed from the constantly dark environment).

Statistical Analysis

The significance of the difference in mean response across time (challenges 1–5) was determined using a one-way ANOVA for repeated measures. Significance of this analysis was followed by post hoc testing using Student's paired *t*-test to determine the significance of the difference in the mean thermic responses of the rats for challenges 2–5 relative to challenge 1.

All measures of variance in the text refer to the standard error of the mean (SEM).

RESULTS

The mean core temperature of the sample prior to nicotine challenges 1–5 was 37.5 ± 0.07°C, 37.5 ± 0.16°C, 36.9 ± 0.07°C, 37.0 ± 0.12°C, and 37.3 ± 0.07°C, respectively. The mean thermic response 20 min after the injection of nicotine was −0.38 ± 0.09°C, −0.65 ± 0.07°C, +0.46 ± 0.07°C, +0.52 ± 0.07°C, and 0.0 ± 0.05°C for challenges 1–5. The mean thermic responses for the entirety of these challenges were −0.06 ± 0.06°C, −0.33 ± 0.05°C, +0.56 ± 0.07°C, +0.47 ± 0.07°C, and +0.06 ± 0.03°C. These means are significantly different, $F(4,55) = 38.66$, $p < 0.0001$. The mean thermic response of the sample 9 hours after being returned to standard vivarium conditions was significantly different from Challenge 1 in a negative direction, $t(10) = -4.75$, $p < 0.0008$. The thermic responses 2, $t(10) = 7.56$, $p < 0.00002$, and 5, $t(10) = 5.46$, $p < 0.0003$, days after removal from darkness were significantly different from Challenge 1 in a positive direction (as noted above the sample exhibited hyperthermic responses at these points in time). The mean thermic response to nicotine 12 (Challenge 5) days after removal from constant darkness was not significantly different from Challenge 1. However, the difference did constitute a trend, $t(10) = +2.89$, $p < 0.07$.

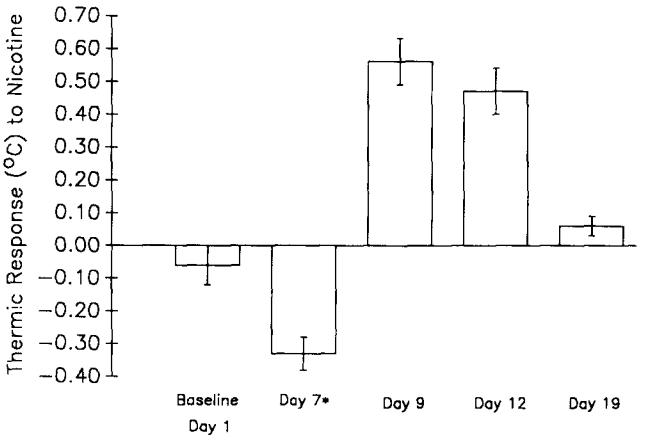

FIG. 1. (A) Nine hours after removal from constant darkness; (B) 2 days after removal from constant darkness; (C) 5 days after removal from constant darkness; (D) 12 days after removal from constant darkness. This figure illustrates the changes in thermic response to nicotine after chronic exposure to darkness (7 days) and various points in time after return to standard vivarium conditions. Baseline indicates the mean thermic response (± SEM) while maintained under standard vivarium conditions. Constant exposure to darkness produces an enhanced hypothermic response to nicotine. The sample exhibited rebound hyperthermia. The magnitude of the hyperthermic response measured 2 and 5 days after removal from constant darkness is characteristic of that produced by the intraperitoneal injection of a placebo (saline). Twelve days after removal from the constantly dark environment the mean hypothermic response did not differ from baseline. *Day 7's nicotine challenge occurred 9 hours after removal from darkness.

Figure 1 illustrates the results of this study.

DISCUSSION

Exposure to constant darkness apparently enhanced the hypothermic response to nicotine, but this was followed by a "rebound" hyperthermic response two and five days later. We previously showed that rats almost uniformly exhibit a hyperthermic response to the stress of an intraperitoneal injection of saline (14). The magnitude of the hyperthermic response on Days 9 and 12 (2 and 5 days after being removed from the dark) is similar to that produced by such an injection. We suggest that the animals became insensitive to the dose of nicotine used and responded to its injection as if it were saline. The hyperthermic response to the injection of nicotine on Days 9 and 12 is intriguing. This response suggests that returning the animals to a 12-hour-light/12-hour-dark cycle resulted in a rebound subsensitivity of the nicotinic mechanism(s) which regulates core temperature.

We previously reported that chronic treatment with amitripty-

line produced supersensitivity to the hypothermic effect of nicotine (1 mg/kg) and that the discontinuation of this tricyclic resulted in rebound subsensitivity to nicotine (17). Rebound subsensitivity may indicate that, in the process of recovering from a perturbation producing supersensitivity to nicotine, the rat "overshoots" the ideal degree of sensitivity.

Enhanced sensitivity to the hypothermic effect of nicotine could be the consequence of chronic stress produced by exposure to prolonged darkness. However, this effect is the opposite of that produced by forced swim stress (30) or the relatively minor stress of injecting animals twice daily with saline (21). These stressors produce subsensitivity to nicotine.

Full-spectrum bright artificial light affects subsensitivity of both (6) nicotinic (3,13) and muscarinic (10,18) mechanisms involved in the regulation of core temperature. Placement of rats in a constantly dark environment for 7 days (6 days and 14 hours) results in enhanced sensitivity to the thermic effects of both nicotine and a muscarinic agonist (3, 4, 6).

The effect of six somatic treatments (please see introduction) (3, 5, 6, 9, 11–13, 16, 17, 20) for depression on the thermic response of rats to nicotine are known. All of these treatments either markedly increase or decrease the hypothermic response to nicotine. Nicotine promotes the release of norepinephrine in the hypothalamus (35) and of dopamine within the mesolimbic and nigrostriatal tracts (1). A somatic treatment for depression which enhances the activity of aminergic neurotransmitter systems might induce a compensatory subsensitivity of those nicotinic mechanisms with which they interact. Subsensitization is not integral to the mechanism of action of these treatments. It is a compensatory response to those effects essential to their effectiveness as treatments for depression. Full-spectrum bright light could be such a treatment. Alternately, somatic treatments which supersensitize a nicotinic mechanism could mobilize aminergic systems and thereby have antidepressant properties. It is quite possible that the effects of somatic treatments for depression which subsensitize and supersensitize nicotinic mechanisms are essential or related to events critical to their mechanism of action.

CONCLUSION

Full-spectrum bright artificial light is an effective treatment for winter depression. This treatment produces profound subsensitivity to the hypothermic effects of nicotine in the rat. Placing this animal into a constantly dark environment has the opposite effect. The mechanism accounting for this is not yet known.

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