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Bright Light Blocks the Capacity of Inescapable Swim Stress to Supersensitize a Central Muscarinic Mechanism

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FLEMMER, D. D., S. C. DILSAVER AND J. A. PECK. *Bright light blocks the capacity of inescapable swim stress to supersensitize a central muscarinic mechanism.* PHARMACOL BIOCHEM BEHAV **36**(4) 775–778, 1990.—Clinical and basic researchers have proposed that muscarinic cholinergic mechanisms mediate some effects of chronic stress. Chronic inescapable (forced) swim stress depletes brain biogenic amines and is used to produce learned helplessness in rats. Behavioral and biochemical characteristics of animals in the state of learned helplessness lead some investigators to believe this condition provides a useful animal model of depression. Inescapable swim stress also produces supersensitivity to the hypothermic effect of the muscarinic agonist oxotremorine in the rat. The authors previously demonstrated that bright light potently induces subsensitivity of a central muscarinic mechanism involved in the regulation of core temperature under a variety of circumstances. They now report using a repeated measures design that inescapable swim stress of five days duration produces supersensitivity to oxotremorine (increase in thermic response of 405%). This supersensitivity is reversed within five days by treatment with bright light, despite continuation of daily swim stress. Daily inescapable swim stress was continued beyond cessation of treatment with bright light. Five days later, supersensitivity to the hypothermic effect of oxotremorine was once again evident.

Acetylcholine Affective disorders Bright light Cholinergic Depression Muscarinic Oxotremorine Receptors Seasonal Affective Disorder Stress Thermoregulation

IN 1972 Janowsky *et al.* (19) published a seminal article setting forth the hypothesis that hyperfunction of central muscarinic cholinergic mechanisms is involved in the pathophysiology of depression. The literature on this topic is now extensive (1–7, 19–21, 23, 25, 26, 31, 32). Janowsky's group (20–22) later proposed that muscarinic mechanisms mediate some effects of stress in man. A recent review of the clinical and preclinical literature suggested the preponderance of evidence is consistent with the hypothesis that the pathophysiology of chronic stress involves activation of muscarinic mechanisms (5). The capacity of chronic stressors to activate central muscarinic mechanisms may partially explain the epidemiological finding of a relationship between stressful life events and the onset of depressive (27) and manic (24) episodes. Mania may be indirectly related to the activation of muscarinic mechanisms. Stressors may trigger rebound subsensitization of muscarinic mechanisms and activation of aminergic systems (11). Implication of muscarinic mechanisms in the pathophysiology of the stress response in no way precludes the involvement of aminergic, peptidergic, or other systems.

Bright artificial light is the only treatment for a depressive disorder producing subsensitivity of a central muscarinic mechanism (8, 12, 13). Amitriptyline supersensitizes a central muscarinic mechanism to the hypothermic effects of a muscarinic agonist (8, 12, 13, 16, 17). Six hours of treatment with bright light during the regular photoperiod completely blocks this effect of amitriptyline (8,15). Chronic forced swim stress depletes brain biogenic amines and produces learned helplessness (33) and supersensitivity of a central muscarinic mechanism to a muscarinic agonist (5, 9, 18). These findings prompted us to test the hypothesis that treatment with full-spectrum bright artificial light blocks the capacity of forced swim stress to produce supersensitivity to a muscarinic agonist.

The results of this study without the details of methodology and discussion recently appeared in a review article on the entitled "Neurobiology of Bright Light (8)."

METHOD

Dependent Variable

The dependent variable in this study is mean change in core

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temperature following the intraperitoneal injection of oxotremorine. The core temperature of each rat was measured prior to the injection of the peripheral muscarinic receptor antagonist methylscopolamine nitrate, 1 mg/kg IP. Oxotremorine (base), 0.25 mg/kg IP, was administered (i.e., at t=0) 30 minutes following the injection of methylscopolamine. Core temperature was measured every 10 minutes for 120 minutes following the injection of oxotremorine. The mean change relative to t=0 is calculated by adding the deviations from t=0 and dividing by 12 [see (10) for further details].

Measurement of Core Temperatures

Core temperature was measured telemetrically with an intraperitoneally implanted thermosensor known as a Mini-Mitter (Mini-Mitter Co., Sun River, OR). This sensor is sensitive to a change in temperature of 0.1°C (10). The reliability and validity of this method are established (14).

Calibration of Mini-Mitters

The thermosensors are calibrated by measuring the rate at which they emit pulses of amplitude modulated (AM) radiowaves over 10-second intervals in a temperature controlled waterbath (Precision Instruments, Model 50) at multiple temperatures (10,14). The pulses are detectable with an AM receiver attached to a digital frequency counter (Model 5001 Universal Counter Timer, Global Specialties, New Haven, CT). The rate at which pulses are emitted is directly proportional to temperature.

Oxotremorine Challenges

Oxotremorine produces dose-dependent hypothermia in the rat (8, 17, 28, 29). The animals used in this study were challenged with 0.25 mg/kg of oxotremorine at baseline (prior to being subjected to forced swim stress) at 3:00 p.m. Subsequent challenges started at the same time of day. We previously demonstrated that a chronic stressor dramatically enhances the response to this low dose, thus magnifying the difference between baseline and the posttreatment (stress) state (5, 10, 18).

The animals were challenged with oxotremorine at baseline (prior to the first swim stress session) and 7 hours after the 5th, 10th, and 15th sessions of forced swim stress. Multiple challenges with oxotremorine, 0.25 mg/kg IP, every other day for 10 days (5 challenges) do not produce carryover effects (10,30).

Animals

The Sprague-Dawley rats used in this study were obtained from Harlan Laboratories (Indianapolis, IN).

Pharmaceuticals

Oxotremorine (base) and methylscopolamine nitrate were purchased from Sigma Chemical Company (St. Louis, MO). These drugs were prepared in distilled water at concentration requiring the administration of 1 ml/kg.

Statistical Analysis

The mean thermic response ± the standard error of the mean (SEM) at each point in the study are presented for descriptive purposes. The mean thermic responses from each of the four oxotremorine challenges entered into a one-way analysis of variance (ANOVA) for repeated measures. A significant ANOVA prompted us to perform multiple paired Student's *t*-tests.

Forced Swim Stress

The rats were subjected to forced swim stress in a sink which was 137 cm long, 55 cm wide, and 38 cm deep at 7:00 a.m. for 10 minutes at 12°C. The first 5 sessions were followed with return to their cages in which the light-dark cycle was 12 hr-12 hr. The next 5 sessions were followed by exposure to full-spectrum bright artificial light (7,400 lux) between 8:30 a.m. and 3:30 p.m. The following sessions were followed by a return to standard vivarium conditions. Full-spectrum bright artificial light was emitted from a bank of eight 122-cm long Vitalite tubes suspended 50 cm above the animals. This light unit (Duro Test Co., North Bergen, NJ, Model 5599) is used to treat Seasonal Affective Disorder. Temperature under the light unit is about 23°C.

RESULTS

The mean core temperature of the sample of the rats prior to the first oxotremorine challenge was 37.2 ± 0.12°C. This is within the physiological range. The mean weight of the sample was 256.7 ± 13.0 g.

The ANOVA for repeated measures indicated that the mean hypothermic response of the sample differed across time (i.e., the first second, third, and fourth oxotremorine challenges), $F(3,76) = 14.29$, $p<0.0001$. The mean hypothermic response of the sample prior to swim stress was 0.21 ± 0.05°C. The mean hypothermic response following 5 days of swim stress was 0.85 ± 0.05°C (4.1-fold greater than baseline). This differed from the hypothermic response at baseline, $t(19) = 9.40$, $p<0.0001$. Thus, chronic inescapable swim stress was associated with the development of supersensitivity to the hypothermic effect of the muscarinic agonist. The mean hypothermic response following 5 additional days of inescapable swim stress and concurrent treatment with bright light was 0.26 ± 0.14°C (1.24 times above baseline). This mean did not differ from baseline, $t(19) = 1.03$, $p>0.30$. The mean hypothermic response following 5 additional days of forced swim stress (without bright light) was 0.58 ± 0.09°C (2.8-fold greater than the baseline response to oxotremorine). This differed from baseline, $t(19) = 2.95$, $p<0.05$. Thus, the sample once again exhibited supersensitivity to the hypothermic effect of oxotremorine. Figure 1 illustrates the results of the study.

DISCUSSION

Bright artificial light administered for 8 hours daily during the regular photoperiod counteracted the capacity of concurrently applied inescapable swim stress to supersensitize rats to the hypothermic effects of the muscarinic agonist oxotremorine. Oxotremorine is thought to produce hypothermia through its effects on hypothalamic nuclei (29). Light reaching the retina can directly affect the hypothalamus via the retinohypothalamic pathway. Thus, our finding can be explained neuranatomically.

The data presented here are consistent with our previous findings (8, 12–15). They 1) support the hypothesis that a forced stressor can activate the muscarinic cholinergic system and 2) are consistent with results indicating that bright light is a treatment which generates a potent force tending to subsensitize the muscarinic cholinergic system. We previously found that chronic twice daily (9:00 a.m. and 9:00 p.m.) inescapable swim stress supersensitizes rats to the hypothermic effect of oxotremorine (5,18). The data in this report indicates that once-daily inescapable swim stress is sufficient to produce supersensitivity to this effect.

It is highly improbable that the results presented here are due to habituation to the forced stress. Habituation occurring contemporaneously with treatment with bright light could explain blunting

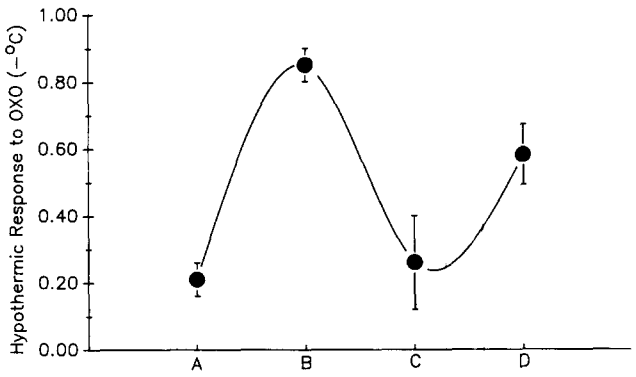

FIG. 1. Telemetric thermosensors which emit "amplitude modulated" (AM) radio waves were implanted into the peritoneal cavities of 20 adult male Sprague-Dawley rats. The animals were allowed five days to recover from this minor surgical procedure. On Day 0 the baseline (prestress) mean thermic response to oxotremorine, 0.25 mg/kg IP, was determined. Mean thermic response as plotted on the y-axis was calculated by averaging the 12 differences between core temperature immediately prior to the injection of oxotremorine (at t=0) and core temperature 10, 20, 30 . . . 120 minutes after the injection of this agonist. Methylscopolamine (a muscarinic receptor antagonist which does not appreciably cross the blood-brain barrier) was injected 30 minutes before the administration of oxotremorine to block its effects on peripheral muscarinic receptors. All challenges with oxotremorine started at 4:00 p.m. The day following measurement of the baseline thermic response to oxotremorine the rats began a 15-day course of daily forced swim stress. Sessions of swim stress started at 7:00 a.m. and were conducted for 10 minutes at 12°C. Five days of forced swim stress produced supersensitivity to the thermic effect of oxotremorine. The rats were subjected to full-spectrum bright light between 8:30 a.m. and 3:30 p.m. on Days 6–10 of the study. This treatment was associated with return to the level of sensitivity to oxotremorine measured at baseline. Treatment with bright light then ceased and daily inescapable swim stress was continued for 5 more days (Days 11–15). The thermic response to oxotremorine was once again enhanced on Day 15 relative to baseline. (A) Baseline; (B) 5 days forced swim stress; (C) 5 additional days forced swim stress concurrent with BAL treatment; (D) 5 additional days forced swim stress; no BAL treatment.

of the thermic response to oxotremorine occurring on the tenth day of forced swim stress. However, the reemergence of supersensitivity to oxotremorine following the fifteenth day of inescapable swim stress is not consistent with the hypothesis that the findings are due to habituation. Further, the results of a recent study designed to assess the effect of twice daily forced swim stress of 10 minutes duration at 10–12°C (the conditions of forced swim used in the experiment described in this article) for 14 days indicated that the Sprague-Dawley rat remains profoundly sensitive to the motoric inhibiting effects of low dose arecoline (0.125 mg/kg) relative to saline, $t(17) = -2.22$, $p<0.04$ (Dilsaver *et al.*, submitted). A study assessing the effect of once-daily forced swim stress of 10 minutes duration at 12°C for 5 days on the inhibition of crossings in response to multiple doses of arecoline in the Sprague-Dawley rat disclosed a similar effect of arecoline (base) at a dose of 0.125 mg/kg IP (presented at the 26th Annual Meeting of the American College of Psychopharmacology; Miller *et al.*, submitted).

The significance of these findings may partially lie in the capacity of muscarinic mechanisms to mediate effects of stress on muscarinic mechanisms in individuals with affective disorders (3, 4, 6, 7, 19, 20–23). Hypotheses, models or theories linking the neurobiology of stress with processes potentially involved in the pathogenesis of affective illness could be of great heuristic value to both preclinical and clinical investigators in various disciplines. These models could suggest behavioral, pharmacological, electrophysiological, neurochemical, and molecular studies. The stressor applied in this study has long been used to produce what is widely regarded to be an animal model of depression and is known to deplete brain stores of biogenic amine. The recent observation that inescapable swim stress (7, 8, 14) and footshock (7–9) render a central (supposedly hypothalamic) muscarinic mechanism involved in the regulation of core temperature supersensitive to a muscarinic agonist provide a new focus for interest in the learned helplessness model. Pharmacological treatments reverse some of the effects of stressors producing learned helplessness (33), and it is now clear that an effect of inescapable stress on a muscarinic mechanism is reversed by exposure to bright artificial light, a nonpharmacological treatment for wintertime depression.

The mechanisms through which a chronic stressor enhances and bright light diminishes responsiveness to the thermic effects of oxotremorine is not yet known. We were unsuccessful in our attempts to determine whether chronic forced stress increases the maximum density or affinity of quiniclidinyl-benzilate (QNB) binding sites in the hypothalamii of rats treated with bright light during the regular photoperiod (M. Girouxm, E. Malatynska and S. Dilsaver, submitted). The effect of bright light could be highly selective. A form of quantitative autoradiography could disclose a particular hypothalamic locus exhibiting upregulation of muscarinic receptor radioligand binding sites. It is also possible that bright light does not affect a change in binding parameters but alters another important parameter such as receptor sensitivity by uncoupling the muscarinic receptor to second messenger generating mechanisms. It would be interesting to learn whether forced stress increases the transcription of messenger coding for subunits of the muscarinic receptor and that bright light blocks such this effect.

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