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BRIEF COMMUNICATION

Exposure to Constant Darkness Enhances the Thermic Response of the Rat to a Muscarinic Agonist

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FLEMMER, D. D., S. C. DILSAVER AND J. A. PECK. *Exposure to constant darkness enhances the thermic response of the rat to a muscarinic agonist.* PHARMACOL BIOCHEM BEHAV **38**(1) 227–230, 1991.—Bright artificial light is used to treat patients with major depression with a seasonal component ("Winter Depression"). Hyperactivity of muscarinic cholinergic systems is implicated in the pathophysiology of depression. Continual exposure to bright light for 7 days or during discrete portions of the photoperiod blunts the thermic response to a muscarinic agonist (oxotremorine) in the rat. Exposure to either 24 hours per day of bright light (in contrast to periods of circumscribed exposure) or darkness would tend to produce free-running. Observers have suggested that the reduced responsiveness to oxotremorine may result from the induction of free-running (the "free-running hypothesis"). The "free-running hypothesis" leads to the prediction that rats exposed to constant darkness will exhibit a reduction in thermic responsiveness to oxotremorine. The authors hypothesized that constant exposure to darkness would, contrary to the "free-running hypothesis," enhance the thermic response to oxotremorine. Rats (n = 12) exposed to constant darkness for 7 days exhibited supersensitivity to oxotremorine 5 days after return to standard light/dark cycle in the vivarium. This argues against the hypothesis that the induction of free-running enhances sensitivity to the thermic effects of oxotremorine.

Affective disorders Acetylcholine Bright light Cholinergic Muscarinic Receptors Seasonal affective disorder

"WINTER DEPRESSION" (officially referred to as "major depression with a seasonal pattern" in the *Diagnostic and Statistical Manual of the American Psychiatric Association*) is characterized by recurring episodes of depression which have their onset in the fall or winter and spontaneously remit in the spring or summer. This disorder is thought to be related to the timing of dawn, and/or the intensity, and duration of sun light (13, 15–17, 20, 21, 23). It is highly responsive to treatment with full-spectrum bright artificial light (20).

There is evidence that the pathophysiology of depression may involve the activation of central muscarinic mechanisms (4,14). Treatment with bright light during all or a fraction of the regular photoperiod (12), the first half of the dark phase (5:00 p.m. to 11:00 p.m.) (9) or for 24 hours daily for 7 days subsensitizes a central muscarinic mechanism involved in the regulation of core body temperature in the rat. This is a property unique to bright

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light; no other treatment for a psychiatric illness is known to have this effect [please see (3) for a review of this literature]. These findings raise a question about the consequences of continuous exposure to constant darkness.

In the context of this article the phenomenon of free-running is a state in which "circadian rhythms are governed by an endogenous circadian pacemaker . . . no longer entrained to the environmental light-dark cycle (15)." A "circadian rhythm" has a periodicity of close to or exactly 24 hours in length. Placement of the rat into constantly lighted or dark environment allows rhythms with periodicity set by to the light/dark cycle to assume the periodicity of the animal's endogenous pacemaker. Under this condition some circadian events will assume periodicities greater and others less than 24 hours (15). The former is by definition an "infradian" and the latter an "ultradian" rhythm.

The induction of free-running might be a sufficient cause for the induction of subsensitivity to the muscarinic agonist oxotremorine. This idea was raised after we demonstrated that constant exposure to bright light for an entire week was particularly potent in the induction of subsensitivity to oxotremorine (7). Constant exposure to darkness also induces free-running. Rats subjected to this treatment would demonstrate subsensitivity to oxotremorine if the induction of free-running is a sufficient means of affecting subsensitivity to a muscarinic agonist. We assessed this possibility in the study reported here.

METHOD

Variables

There are two dependent variables in this study. These are 1) mean change in core temperature over a 120-minute period and 2) thermic response 40 minutes after the injection of oxotremorine (base), 0.25 mg/kg IP in the adult male rat. The thermic response to oxotremorine is maximized about 40 minutes after its injection. The independent variable is the point of the sample in the course of the study. These points are preconstant darkness (or control state), 9 hours, and 2 and 5 days after return of the subjects to standard vivarium conditions.

Ideally, all of the variance in the dependent variables is related to variation of the independent variable and none to random fluctuation in the response of individual animals. Consequently random variation in the response of each rat across time was treated as a confounding variable and its contribution to the variance in the dependent variables was quantitated and corrected for in the analysis.

Method Used to Measure Core Temperature

Core temperature was measured telemetrically with the Model VM Mini-Mitter (Mini-Mitter Co., Sun River, OR). This device is hearing-aid battery powered radio transmitter sensitive to temperature. It is implanted into the peritoneal cavity during a surgical procedure of about 5 minutes duration. Methoxyflurane was used as an anesthetic agent. The Mini-Mitter yields reliable and valid measurements when used idependently by trained investigators (10).

The Mini-Mitters are calibrated by measuring the rate at which amplitude modulated (AM radio) waves are emitted at three different temperatures in a temerature controlled water bath (Precision Instruments, Model 50). The rate of emission of these waves is measured with a digital frequency counter (Global Specialties, EML Instruments). The rate of emission of pulses at each temperature is used to calculate a linear regression equation. Temperature is represented by "y" and rate of emission by "x." The correlation coefficients of these regression equations is usually .99. Trained investigators acting independently derive statistically idential regression equations when calibrating Mini-Mitters. The data demonstrating this and the procedure for the calibration the Mini-Mitter are available in the literature (10). The animals are allowed 5 days to recover from surgery prior to starting an experiment.

Definition of Mean Change in Core Temperature

Core temperature was measured in each animal prior to the injection of methylscopolamine nitrate. This is referred as "baseline" core temperature. Core temperature was then measured every 10 minutes for 120 minutes following the injection of oxotremorine. Mean change in core temperature is defined as the average of these 12 deviations from baseline.

Oxotremorine Challenges

Oxotremorine produces a dose-dependent thermic response in the rat. The animals were first challenged with 0.25 mg/kg of oxotremorine under standard conditions in the vivarium. These conditions include a 12 hour light/12 hour dark cycle (lights on at 6:00 a.m. and off at 6:00 p.m.). All oxotremorine challenges started at 3:00 p.m. Subjection of the animals to constant darkness started immediately following the initial challenge with oxotremorine. The duration of placement in constant darkness was 6 days and 14 hours. The animals were challenged with oxotremorine for the second time 9 hours after exposure to standard light conditions in the vivarium. The third and fourth challenges occurred 2 and 5 days after resuming the standard light/dark cycle.

Multiple challenges with oxotremorine (base), 0.25 mg/kg IP, every other day for 10 days (5 challenges) does not produce carryover effects (17). Thus, it is highly improbable that a carryover effect contaminated the results of this study.

Methylscopolamine nitrate, 1.0 mg/kg IP, was given to each animal 30 minutes before the injection of oxotremorine. This drug blocks muscarinic cholinergic receptors in the periphery but does not appreciably cross the blood-brain barrier due the presence of a quartenary (charged) nitrogen atom which renders it relatively insoluble in lipids.

Animals

Twelve (12) adult, male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing a mean 238.3 ± 7.0 g were used in this study.

Pharmacological Agents

Oxotremorine (base) and methylscopolamine nitrate were purchased from Sigma Chemical Company, St. Louis, MO. These drugs were prepared for use immediately prior to each challenge at concentrations of 0.25 and 1.0 mg/ml in distilled water, respectively. The dose of oxotremorine refers to the base form and that of methylscopolamine the nitrate salt.

Statistical Analysis

All statistical analyses were performed using SAS. Baseline core temperature, change in core temperature 40 minutes following the injection of oxotremorine, and the mean thermic response of each animal was measured four times. A separate two-way analysis of variance (ANOVA) for repeated measures was done for each of these measures. The factors were individual rat (subject) and challenge (i.e., prior to placement in the darkened environment, and at each of the points after return to the vivarium).

This analysis was used in place of a one-way ANOVA for repeated measures. It presents the advantage of allowing one to remove the contribution of between subject variance from the error variability.

A significant F value was followed by application of Fisher's Least Significant Difference Test. This result is produced by the LSMEANS statement in SAS (22). This is a post hoc test appropriate for comparing multiple means in one group of subjects to a control condition (in this case thermic response of the sample before exposure to constant darkness). The critical value of α was set at $p<0.05$ for both the ANOVA and post hoc tests.

The mean thermic response and the standard error of its mean (SEM) are presented for descriptive purposes.

RESULTS

The mean core temperature of the sample prior to the four oxotremorine challenges was 37.2 ± 0.1°C, 37.8 ± 0.2°C, 37.7 ± 0.2°C, and 37.4 ± 0.3°C, respectively. These means are significantly different, $F(3,33)=4.00$, $p=0.016$. The mean core temperature at the time of the first challenge differed from those at the time of the second ($p=0.004$) and third ($p=0.02$) challenges but not the fourth challenge ($p=0.26$).

Though mean core temperature differed significantly all are within the physiological range. The degree of variation observed here occurs under standard vivarium conditions. Examination of our laboratory notebooks for the period of 1987–present disclosed mean core temperatures for groups of the Sprague Dawley rat between 36.0 and 38.0°C (prior to 1989 we did not report mean core temperature for each individual pharmacological challenge).

Dependent Variable: Mean Change in Thermic Response

The two-way ANOVA for repeated measures indicated that random change in the thermic responses among subjects did not significantly contribute to the variance in mean thermic response, $F(11,33)=1.52$, $p=0.17$. However, the contribution to the variance in the outcome related to challenge number (i.e., point of the sample in the course of the study) was highly significant, $F(3,33)=12.09$, $p<0.0001$. Mean thermic response at baseline was -0.3 ± 0.11°C. The mean thermic response after 6 days and 14 hours of exposure to constant darkness (9 hours after return to standard vivarium conditions) was -1.0 ± 0.05°C ($p<0.0001$). The hypothermic responses 2 and 5 days after a return to standard vivarium conditions were -0.7 ± 0.09°C ($p<0.005$) and -0.4 ± 0.09°C (n.s.). Thus mean thermic response returned to baseline between 2 and 5 days after the animals were returned to standard vivarium conditions.

The results based on the consideration of mean thermic response are pictorially presented in Fig. 1.

Dependent Variable: Thermic Response 40 Minutes After Oxotremorine

The two-way ANOVA indicated that random flucuation in the response among subjects (rats) did not significantly contribute to the measurement of thermic response 40 minutes after the injection of the muscarinic agonist, $F(11,33)=1.31$, $p=0.26$. However, the component of variance in the thermic response of each animal at the 40-minute time point was highly significant with respect to the timing of each challenge, $F(3,33)=8.26$, $p=0.0003$. These two results suggest that the effect of treatment (placement in a constantly darkened environment) but not random variance in the response of each animal significantly contributed to the outcome of the study.

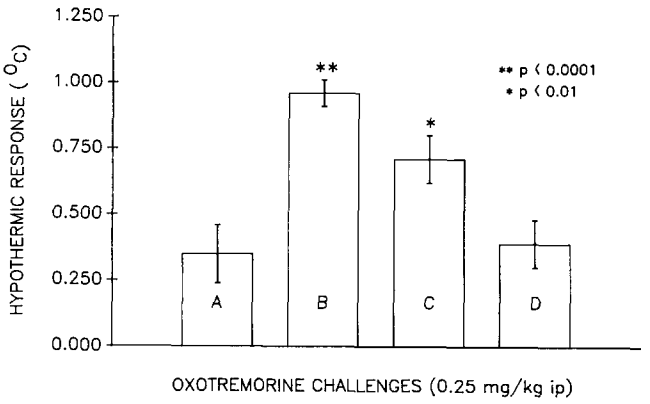

FIG. 1. This illustrates the mean thermic response ± SEM to oxotremorine (0.25 mg/kg IP): (A) at baseline; (B) 9 hours after return to standard vivarium conditions following 6 days and 14 hours of constant exposure to darkness; (C) 2 days (56 hours) after removal from a constantly dark environment; (D) 5 days after removal from a constantly dark environment.

The mean thermic response prior to placing the animals in a constantly dark environment was -0.8 ± 0.2°C. The mean thermic responses of the animals 9 hours after removal from the constant darkened conditions, and 2 and 5 days of exposure to the standard light/dark cycle were -1.8 ± 0.07°C ($p<0.0001$), -1.3 ± 0.12°C ($p<0.02$), and -0.99 ± 0.15°C (n.s.).

DISCUSSION

The contrast between the results of the experiment in which the rat is exposed to constant bright light for a week and the result obtained here are noteworthy. Either condition would result in free-running animals. However, constant exposure to bright light produces subsensitivity to the thermic effect of oxotremorine with or without an antecedent manipulation enhancing the sensitivity of the muscarinic cholinergic system [see (3) for a review]. This study demonstrates that constant exposure to darkness for seven days enhances the thermic response of supposedly free-

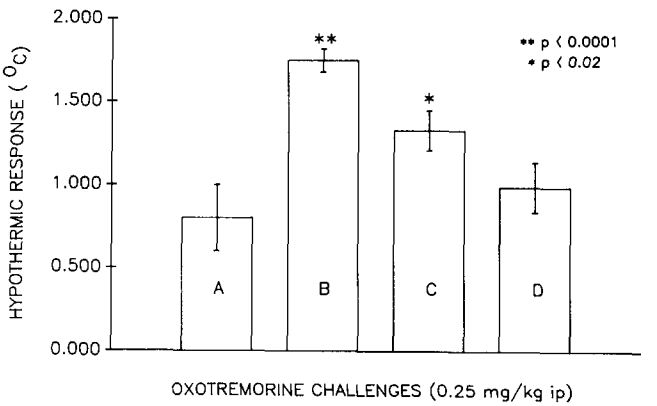

FIG. 2. This pictorially presents the mean thermic response ± SEM 40 minutes following the injection of oxotremorine (0.25 mg/kg IP): (A) at baseline; (B) 9 hours after return to standard vivarium conditions following 6 days and 14 hours of constant exposure to darkness; (C) 2 days (56 hours) after removal from a constantly dark environment; (D) 5 days after removal from a constantly dark environment.

running rats to a muscarinic agonist. Thus, free-running alone does not appear to be a sufficient cause for either a reduction or enhancement in thermic response of rats to oxotremorine.

Treatment of the rat with bright artificial light produces subsensitivity to the thermic response to nicotine. Constant exposure to darkness for a week produces the opposite effect (3). Thus, nicotinic and muscarinic systems exhibit parallel changes in sensitivity. Changes in the functional status of nicotinic mechanisms may compensate for changes in muscarinic systems and/or vice versa. These simultaneous decreases in the sensitivity of muscarinic and nicotinic cholinergic mechanisms may occur to maintain homeostasis (3–5).

Acetylcholine acting at nicotinic sites promotes and its action at muscarinic sites inhibits the release of norepinephrine in a portion of the hypothalamus (24). Similarly the action of acetylcholine at nicotinic sites triggers and its action at muscarinic sites inhibits the release of dopamine in the nigrostriatal tract (2). Nicotine and muscarinic agonists also have parallel effects in the periphery. Nicotine affects the release of norepinephrine in the myocardium and muscarinic agonists have the opposite effect.

An unusual line of rat known as the Flinders Sensitive Line (FSL) (19) exhibits simultaneous endogenous supersensitivity to muscarinic agonists and nicotine. This line was derived from the Sprague-Dawley rat by selectively breeding animals with maximum sensitivity to an anticholinesterase (diisopropylfluorophosphonate or DFP). The Flinders Resistent Line (FRL) was simultaneously developed to serve as a control line for experiments involving the FSL. These lines are now more than 40 generations removed from their progenitors. The FSL and FRL do not overlap in their thermic responsiveness to oxotremorine.

There is a left shift of the dose-effect curve describing the sensitivity of the FSL to the thermic effect of nicotine relative to the Sprague-Dawley rat (Dilsaver, Peck and Overstreet, unpublished data). Nicotine at a dose of 0.5 mg/kg IP produced a mean thermic response (as defined above) in the FSL ($n=8$) exceeding that produced by twice this dose in naive Sprague-Dawley rats in five of the six published reports coming from our laboratory. The FSL exhibited a response of -1.6 ± 0.2°C to nicotine (0.5 mg/kg) compared to a weighted mean for the Sprague Dawley rat of -1.3 ± 0.2°C ($n=60$, 1.0 mg/kg). It is not known whether high sensitivity to nicotine has adaptive significance in the FSL. It could allow compensation for deficits stemming from hyperactivity of muscarinic cholinergic mechanisms. However, it might simply be an artefact of the selection procedure used to derive the FSL. Regardless, the FSL is a line which demonstrates that simultaneous supersensitivity of muscarinic and nicotinic cholinergic systems is consistent with health of a mammalian species.

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