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Autonomic and Behavioral Responses of Selectively Bred Hypercholinergic Rats to Oxotremorine and Diisopropyl Fluorophosphate

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REZVANI, A. H., D. H. OVERSTREET, A. EJANTKAR AND C. J. GORDON. *Autonomic and behavioral responses of selectively bred hypercholinergic rats to oxotremorine and diisopropyl fluorophosphate.* PHARMACOL BIOCHEM BEHAV **48**(3) 703–707, 1994.– The hypercholinergic Flinders Sensitive Line (FSL) rat was significantly more sensitive than the Flinders Resistant Line (FRL) rat to the biotelemetrically recorded hypothermic effects of oxotremorine, a direct-acting muscarinic agonist, and diisopropyl fluorophosphate (DFP), an anticholinesterase agent. The effects of these agents on heart rate and motor activity, also recorded biotelemetrically, indicate either small differences (DFP) or no significant effect (oxotremorine) between the lines. These findings confirm the dramatic differences in temperature responses to cholinergic compounds between FSL and FRL rats, for which they were selectively bred, but suggest that a general increase in the sensitivity of the FSL rats to all muscarinic-mediated responses may not occur.

Hypercholinergic FSL rats FRL rats Biotelemetry Core body temperature Heart rate
Motor activity Oxotremorine DFP

TWO lines of rats have been established by selective breeding for differential responses to the anticholinesterase agent, diisopropyl fluorophosphate (DFP) (10,11,12,14). The line more sensitive to DFP, the Flinders Sensitive Line (FSL), is also more sensitive to directly acting muscarinic agonists such as oxotremorine than its control counterpart, Flinders Resistant Line (FRL) (8,9). Hypothermic responses to DFP and muscarinic agonists have been key dependent variables throughout the selection of the two lines and the assessment of cholinergic sensitivity. However, there is little information on the cholinergic sensitivity of autonomic responses in undisturbed FSL and FRL rats. Handling and restraint can cause physiological and behavioral changes that lead to marked changes in autonomic responses in rodents (2,3).

Using biotelemetric techniques, it is possible to continuously monitor parameters sensitive to handling in unrestrained animals in their home cages (5). FSL rats have been used extensively as an animal model of depression. FSL and FRL rats have been compared under different experimental conditions (8–10). However, autonomic and behavioral responses of these two distinctive lines have not been studied under baseline conditions. The main purpose of the present study was to compare the autonomic and behavioral responses (heart rate, core body temperature, motor activity) of unrestrained, freely moving FSL and FRL rats under baseline conditions and following injections with an anticholinestrase agent (DFP) and a muscarinic agonist (oxotremorine).

METHOD

Animals

Five adult male FSL rats weighing 346 ± 25 g and five adult male FRL rats weighing 365 ± 8.0 g were selected from the 46th generation of FRL and FSL populations maintained

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at the Skipper Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill. Rats were housed individually in Plexiglas cages with wood shavings and had free access to food and water at an ambient temperature of 22 ± 1°C and 12 L : 12 D photoperiod (lights on at 2130 h). The Plexiglas cages were placed on metal racks. Animals were disturbed only for injections and twice weekly for the replacement of food, water, and bedding during the dark portion of the light/dark cycle. All experiments were performed at the same time during the months of July and August.

Surgery and Telemetry System

To measure core body temperature, heart rate, and motor activity, a transmitter weighing 7.0 g (Model TA-11ETA-F40-L20) was surgically implanted into the rats. The transmitter consists of a temperature-sensitive element and two protruding leads that detect the heart rate. Rats were anesthetized with a dose of 35 mg/kg b.wt. sodium pentobarbital injected IP. The fur over the ventral abdominal area was clipped and a 3-cm longitudinal incision was made along the midline of the abdomen about 1 cm below the sternum. The radiotransmitter was inserted into the abdominal cavity and sutured to the peritoneal wall with 4-0 silk thread. The two electrodes for determining heart rate were tunneled SC on opposite sides of the chest and sutured to the abdominal wall with 4-0 silk thread (5). After testing the radiotransmitter with an AM receiver, the skin was closed. At least 1 week for recovery was allowed before testing was commenced. The cages were positioned on a receiver that decoded the temperature and heart rate inputs. The receiver also detected the animal's movements in its home cage. The transmitters were actuated by passing a magnet along the rat's abdomen. Core body temperature, heart rate, and motor activity were collected automatically every 10 min and stored in a computer using the Data Quest IV Software (Data Sciences, Inc., St. Paul, MN).

Experimental Protocol

The study consisted of two phases: 1) recording of baseline circadian rhythms, and 2) recording of responses to oxotremorine and DFP. To compare the baseline circadian rhythms of FSL and FRL rats, their body temperature, heart rate, and motor activity were monitored continuously every 10 min for 5 consecutive days by radiotelemetry. Rats had free access to food and water in their home cages throughout the experiment.

To screen the rats for sensitivity to cholinergic agonists, the rats were first injected SC with 2 mg/kg methylatropine, a peripherally acting muscarinic antagonist. Then a dose of 0.2 mg/kg oxotremorine was administered (SC) 15 min later (13). Body temperature, heart rate, and motor activity were recorded every 10 min for the next 24 h by radiotelemetry. Three days were allowed for the rats to recover fully before conducting the next phase.

To determine the sensitivity of the FSL and FRL rats to DFP, the rats were injected with 1 ml of peanut oil as a vehicle and 3 days later with a dose of 1.0 mg/kg DFP (Sigma, St. Louis, MO). Core body temperature, heart rate, and motor activity were recorded every 10 min for the next 24 h.

Statistical Analyses

Core temperature, heart rate, and motor activity were analyzed by separate two-way analyses of variance (ANOVAs), with line and time as the two independent variables. For these analyses, it was convenient to collapse the data over hourly intervals rather than the 10-min intervals. Because the circadian patterns of temperature, heart rate, and motor activity were so similar in the two lines of rats, and ANOVAs revealed no significant line differences, no attempt was made to derive circadian parameters such as amplitude and period through cosinor analyses.

RESULTS

Circadian Rhythms

Rats of both FSL and FRL strains showed similar 24-h rhythms of core temperature, heart rate, and motor activity under the baseline condition. Both FSL and FRL groups exhibited higher body temperature, heart rate, and motor activity during the dark period than during the light portion of the light/dark cycle (Fig. 1). Analyses of these data over a 5-day period by two-way ANOVA revealed significant time effects for all three variables, but no significant genetic line differences.

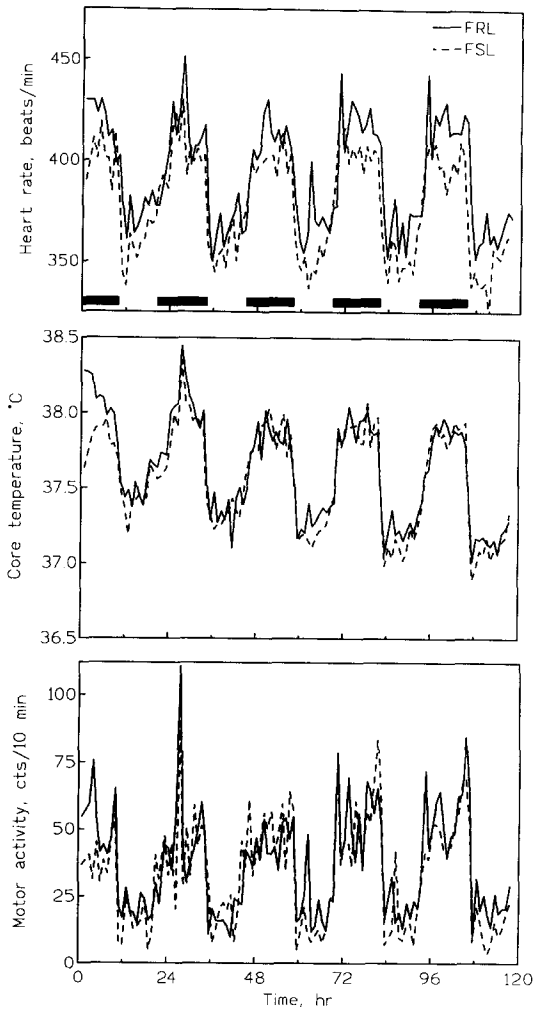

FIG. 1. Circadian rhythms of heart rate, core body temperature, and motor activity of FSL (---) and FRL (—) rats. (n = 5 for each group).

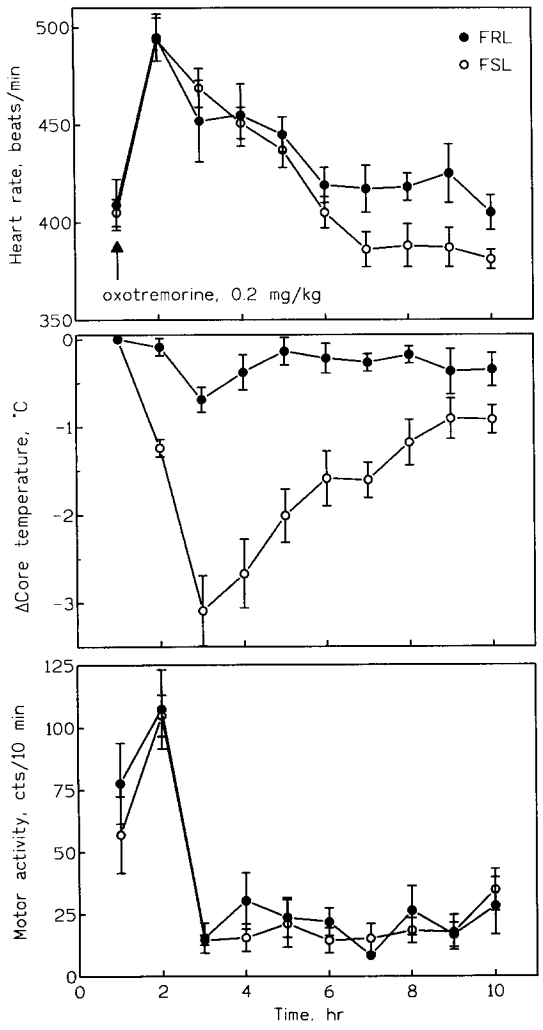

FIG. 2. Effects of an acute administration of oxotremorine on body temperature, heart rate, and motor activity in FSL (○) and FRL (●) rats. Data are mean ± SEM.

Oxotremorine Challenge

The acute injection of oxotremorine (0.2 mg/kg 15 min after 2 mg/kg methylatropine) significantly ($p < 0.001$) reduced the core body temperature in the FSL rats, but not in the FRL rats (Fig. 2). In the FSL rats oxotremorine produced a maximal drop in temperature of 3.1°C at 2 h after administration that did not return to normal for approximately 7 h. Analysis by two-way ANOVA showed significant line, time, and interaction effects ($p < 0.001$). These findings confirm that FSL rats exhibit a dramatic prolonged hypothermic response to oxotremorine compared with FRL rats.

In both lines, administration of oxotremorine initially produced an increase in both motor activity and heart rate (Fig. 2); however, motor activity decreased rapidly to subbaseline levels, and heart rate remained elevated for 4 h. There were no significant difference in the effects of oxotremorine on heart rate or motor activity between the FSL and FRL lines.

DFP and Peanut Oil

Injection of peanut oil, the vehicle for DFP, produced elevations in body temperature in both lines for about 1 h. The same pattern was evident for both motor activity and heart rate. There were no significant line differences in any of the these effects of this vehicle between FSL and FRL rats (data not shown).

An injection of DFP (1.0 mg/kg) significantly ($p = 0.04$) reduced core body temperature in FSL rats, but not in FRL rats (Fig. 3). The maximum drop in core temperature of 1.1°C in the FSL rats occurred about 4 h after DFP injection. The FSL rats remained hypothermic for at least 9 h after DFP administration. Thus, these data are comparable to the findings for oxotremorine (Fig. 1).

Motor activity and heart rate were significantly elevated after the administration of DFP (Fig. 3) in both FSL and FRL lines. A notable elevation in heart rate and motor activity of the FRL line over that of the FSL line was seen after DFP administration. Although there were no significant line effects of DFP on these parameters when the repeated-measure ANOVA was applied to these data over the same time period as that of core temperature, further post hoc analysis of the data measured between 4 and 10 h revealed significantly

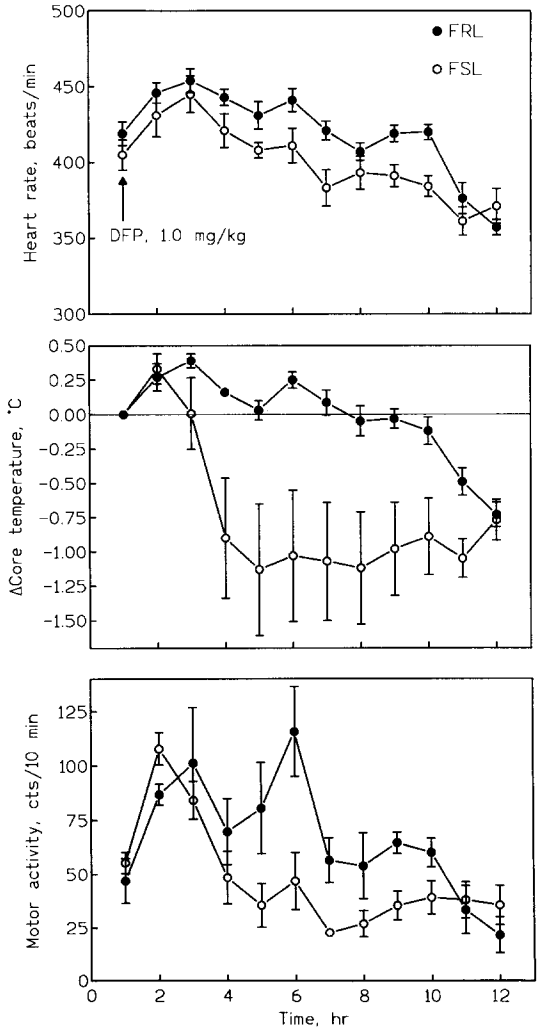

FIG. 3. Effects of an acute administration of DFP on heart rate, core body temperature, and motor activity in FSL (○) and FRL (●) rats. Data are mean ± SEM.

greater effects on motor activity ($p = 0.032$) and heart rate ($p = 0.012$) in FRL rats.

DISCUSSION

Using biotelemetry techniques to monitor temperature, heart rate, and motor activity, this study has demonstrated distinct genetic differences in the thermoregulatory responses to two types of cholinergic agents in genetically distinct FSL and FRL rats. The baseline circadian pattern of core body temperature, heart rate, and motor activity was nearly identical in the FSL and FRL rats (Fig. 1). The lack of line differences in circadian core body temperature in the present study is at odds with an earlier study, in which a phase advance of about 3 h in the temperature rhythm was reported in the FSL rats (15). This earlier study was conducted in rats from the West Coast in a colony of FSL and FRL rats derived from the Australian colony under a normal light/dark cycle (lights on at 0700 h). The present study was conducted on FRL and FSL colonies that were established by cross-fostering rats onto viral-free Wistar mothers under a reversed 12 L : 12 D cycle (lights on at 2200 h). More recently, studies conducted by Shiromani and Overstreet (submitted) in rats from the North Carolina viral-free colonies also failed to detect a circadian rhythm difference in baseline temperature between FSL and FRL rats. However, these studies did find that FSL rats were phase advanced relative to the FRL rats when animals were kept in constant darkness. It remains unclear why the earlier study found a line difference in the temperature rhythms and the latter two did not. It is possible that mycoplasma and other viral/bacterial infections present in the West Coast colonies may act as a nonspecific stressor that affects the FSL rats more than the FRL rats. Several studies have reported the FSL rats to be more sensitive to the effects of stressors than the FRL rats [see (8)].

The dramatic decrease in core temperature after oxotremorine was expected, and confirms the frequently found differences in muscarinic-induced hypothermic responses between FSL and FRL rats (2,8). Although the ANOVA indicates that there is no significant effect of oxotremorine on body temperature in the FRL rats, it should nonetheless be noted that a maximum drop of about 0.7°C was observed. This value is virtually identical to that typically found in the FRL rats by the standard thermistor probe methodology, as is the 3.1°C drop for the FSL rats (8). The very long duration of oxotremorine-induced hypothermia in FSL rats was unexpected, because oxotremorine is usually considered a short-acting agonist. It is possible that handling-induced stress in previous experiments might have masked the prolonged hypothermic effect of this agent. This prolonged hypothermic effect suggests that the biochemical differences between the FSL and FRL rats could include altered second messenger systems (e.g., G-proteins) as well as muscarinic receptors (8,10).

Given the dramatic line differences in the hypothermic effects of oxotremorine, it was surprising that there were no significant line differences for heart rate and motor activity. This indicates that oxotremorine-induced hypothermia is not related to the reduced motor activity in FSL rats. The patterns for heart rate and motor activity are virtually the same for the lines. Because oxotremorine did not significantly affect the temperature of FRL rats, it is reasonable to suggest that the apparent effects on heart rate and motor activity are due to handling and not to the drug. Therefore, the effects of handling on these two variables are sufficient to obscure any drug effects. Other studies using an open field apparatus have reported significant differences in the effects of oxotremorine on motor activity (9,13), but there have been no other studies on heart rate.

A single administration of DFP exerted a clear differential effect on core body temperature in the FSL and FRL rats, as expected from earlier studies (8). The core temperature of the FRL rats was virtually unchanged after DFP, and the FSL rats exhibited a moderate but long-lasting hypothermia (Fig. 3). These data confirm that the FRL rats are truly resistant to the effects of DFP. Other workers also have recently reported on DFP-induced hypothermia (1,4,6). The DFP-induced hypothermia has been attributed to the reduction of metabolic heat production and an increase in peripheral circulation that increases heat loss to the ambient environment (4). It is not clear at this stage whether the exaggerated hypothermia seen in the FSL rats after DFP administration (Fig. 3) is due to one or both of the above mechanisms. Post hoc analysis also indicated that heart rate and motor activity were reduced in the FSL rats for several hours after DFP administration. This reduction could be the result of DFP-induced hypothermia in this line; however, it should be noted that oxotremorine had a greater hypothermic effect than DFP, yet there were no differences in motor activity and heart rate. Hence, the inhibitory effects of DFP on heart rate and motor activity in the FSL strain are likely to involve mechanisms other than muscarinic activation.

In sum, using telemetry, it is shown that under normal conditions, the pattern of autonomic and behavioral responses are nearly identical in FSL and FRL rats. On the other hand, large differences in hypothermic responses to oxotremorine and DFP in the FSL and FRL rats were demonstrated. The effects of these agents on heart rate and motor activity are less clear-cut, but when they do exist, the FSL rats are more affected. This approach should be useful in assessing the effects of other drugs known to alter the autonomic and behavioral responses observed in this study. Furthermore, because FSL rats have been proposed to be a genetic animal model of depression (8), this approach can also be used to study the behavioral and autonomic responses of these rats under unrestricted conditions and when challenged with antidepressant drugs.

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