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Selective Breeding for Increased Cholinergic Function: Increased Serotonergic Sensitivity

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WALLIS, E., D. H. OVERSTREET AND A. D. CROCKER. *Selective breeding for increased cholinergic function: Increased serotonergic sensitivity.* PHARMACOL BIOCHEM BEHAV 31(2) 345–350, 1988.—The effects of the serotonergic antagonist cyproheptadine and the agonist l(m-chlorophenyl) piperazine (mCPP) on core body temperature, locomotor activity and operant responding for a water reward were determined in two lines of Sprague-Dawley rats selectively bred for differences in sensitivity to the anticholinesterase, diisopropyl fluorophosphate (DFP). Both cyproheptadine and mCPP induced a dose-dependent hypothermia that was significantly greater in the line of rat more sensitive to DFP (the Flinders Sensitive Line—FSL). On the other hand, the mild stimulant effects of cyproheptadine on operant responding and locomotor activity were similar in the two lines, whereas the marked inhibitory effects of mCPP on these two measures were significantly greater in the FSL rats. This study also confirmed that the FSL rats were significantly more sensitive to the hypothermic effects of oxotremorine, a muscarinic agonist, and showed that pretreatment with cyproheptadine reduced the hypothermic effects of oxotremorine to a similar extent in the two lines. These findings indicate that rats selectively bred for increased cholinergic function (FSL) also differ in their sensitivity to serotonergic agonists and antagonists, thereby extending the evidence for cholinergic-serotonergic interactions in the rat.

Selective breeding Cholinergic sensitivity Serotonergic sensitivity mCPP Cyproheptadine Oxotremorine

THE present study was designed to examine interactions between serotonergic and cholinergic systems. A selective breeding program at Flinders University has led to the establishment of two lines of rats with modified cholinergic systems (23–25, 31). These rats provide an ideal model for exploring interaction between the cholinergic system and other neurotransmitter systems (28). Pharmacological manipulation of serotonin (5-HT) would be expected to produce different effects on behavioural and physiological functions between the two lines of animals if acetylcholine (ACh) and 5-HT interact to modulate these functions.

Both ACh and 5-HT have been implicated in the control of temperature regulation, locomotor activity and operant behaviour, but the evidence has sometimes been contradictory. For example, intrahypothalamic 5-HT administration may cause decreases (2,17) or increases (3,21) in rat rectal temperature, whereas most investigators report hypothermia following administration of centrally acting muscarinic agonists (20, 22, 23, 27). There have also been some studies suggesting an interaction between hypothalamic serotonergic and cholinergic systems [e.g., (14)]. Although there are conflicting reports, there is a reasonable amount of literature consistent with the notion that both the cholinergic and serotonergic systems are behaviourally inhibitory: locomotor activity and operant responding may be reduced following administration of agonists and increased following administration of antagonists (8–10, 18, 28–30). In this context it is of interest that interactions between striatal serotonergic and cholinergic systems have also been reported (1, 5, 32). Overall, the data are consistent with the suggestion that the cholinergic and serotonergic systems act in parallel or are synergistic systems in the control of many functions.

The present study sought to examine further the interaction between ACh and 5-HT by determining the effects of 5-HT agonists and antagonists on body temperature, locomotor activity, and operant responding in two lines of rats

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TABLE 1

DOSE-RELATED EFFECTS OF CYPROHEPTADINE ON CORE BODY TEMPERATURE (°C) IN FSL AND FRL RATS

	Mean Deviation From Baseline Temperature Dose of Cyproheptadine		
Group	(0) Saline	1 mg/kg	5 mg/kg
Males			
FSL (n=8)	−0.1 ± 0.1	−0.8 ± 0.2	−1.3 ± 0.1
FRL (n=8)	−0.1 ± 0.1	−0.2 ± 0.1	−0.7 ± 0.1
Females			
FSL (n=8)	−0.0 ± 0.1	−0.7 ± 0.2	−1.4 ± 0.1
FRL (n=8)	−0.2 ± 0.1	−0.4 ± 0.1	−0.8 ± 0.1

Injections were given 10 min after the baseline recording of temperature. The second temperatures were recorded 90 min after the injections.

TABLE 2

EFFECTS OF mCPP ON CORE BODY TEMPERATURE (°C) IN FSL AND FRL RATS

	Mean Deviation From Baseline Temperature Dose of mCPP		
Group	(0) Saline	1 mg/kg	3 mg/kg
	10 Min After Injection		
Males			
FSL (n=8)	+0.2 ± 0.1	+0.0 ± 0.1	−0.4 ± 0.1
FRL (n=8)	+0.3 ± 0.1	+0.3 ± 0.1	−0.2 ± 0.1
Females			
FSL (n=8)	+0.0 ± 0.1	+0.1 ± 0.1	−0.9 ± 0.1
FRL (n=8)	+0.1 ± 0.1	+0.5 ± 0.1	−0.3 ± 0.1
	45 Min After Injection		
Males			
FSL	+0.2 ± 0.1	−0.1 ± 0.1	−1.2 ± 0.1
FRL	+0.2 ± 0.1	+0.1 ± 0.2	−0.7 ± 0.2
Females			
FSL	+0.1 ± 0.1	−0.3 ± 0.1	−1.9 ± 0.1
FRL	+0.0 ± 0.2	−0.1 ± 0.1	−1.1 ± 0.3

Saline or mCPP were injected 10 min after the baseline recording of temperature and subsequent temperatures were recorded at the times after injection indicated above.

which differ in cholinergic function. The results obtained are generally consistent with the model of ACh and 5-HT being parallel neurotransmitter systems.

METHOD

Animals

The animals used in the present experiments were from the 24th and 25th generations of the selectively bred lines of rats (FSL and Flinders Resistant Line—FRL). They were housed in a temperature-controlled room (22°C) under conditions of constant humidity (35%) and with free access to food and water except as described below. The lighting was continuous to minimise diurnal fluctuations in ACh (13).

Drugs

Oxotremorine sesquifumarate, atropine methyl nitrate and l(m-chlorophenyl) piperazine dihydrochloride (mCPP) were obtained from Sigma (St. Louis, MO). Cyproheptadine hydrochloride was obtained from F. H. Faulding and Co. Ltd. (Adelaide).

Cyproheptadine was selected as the 5-HT antagonist because it binds with high affinity to 5-HT_2 receptors (15,26) and there is previous information on its effects on the parameters selected for the present experiments (9,19). The agonist, mCPP, was selected because it is one of the most specific and potent 5-HT agonists (7).

Preliminary experiments were undertaken to establish suitable drugs, optimal doses and drug-time-response curves. Doses refer to the salts of the respective drugs. All injections of drugs and vehicle were given subcutaneously (SC) in a volume of 1 ml/kg.

Body Temperature

Core temperature was recorded to the nearest 0.1°C by inserting a thermistor probe 8 cm into the rectum for 30–60 sec and reading the output on a CRL digital recorder.

Operant Responding

Operant responding maintained by water reward was recorded in eight electronically monitored operant chambers, each equipped with a Gerbrands lever and surrounded by an insulated wooden cabinet. Programming of the chambers was achieved through the use of a TRS-80 microcomputer with Lehigh-Valley interfaces. The rats were maintained on a Fixed Ratio 5 schedule of reinforcement after training. The sessions were 15 min in duration and were followed by 15 min of water in the home cage.

Locomotor Activity

Spontaneous locomotor activity was measured for one-min periods under dim red light in a perspex open field chamber (30×60×30 cm with 10×10 cm squares on its base). Total line crossings and rears were recorded separately because there is an indication that the serotonergic system plays a different role in these responses (35).

Experiment 1

This experiment examined the dose-related effects of cyproheptadine (1 and 5 mg/kg) and mCPP (1 and 3 mg/kg) on body temperature, operant responding, and locomotor activity in eight rats from each line and sex, after the rats had attained a stable level of responding (less than 10% variation over 3 days) in the Fixed Ratio 5 operant task. The drug treatments were conducted over a period of 20 days, with three days between injections. The procedural details are given as footnotes to the tables of the relevant data.

Experiment 2

This experiment examined the effects of mCPP (1 mg/kg) and cyproheptadine (1 mg/kg) on core body temperature, in combination with oxotremorine in ten rats from each line and sex. The rats were familiarized with all the procedures for two weeks before the first injections. The procedural details

TABLE 3

EFFECTS OF CYPROHEPTADINE ON OPERANT RESPONDING AND LOCOMOTOR ACTIVITY IN FSL AND FRL RATS

	% of Saline Baseline Value			
	FSL Males	FRL Males	FSL Females	FRL Females
Bar Presses				
1 mg/kg	114.6 ± 6.1	109.2 ± 3.4	120.4 ± 8.9	116.7 ± 6.0
5 mg/kg	111.5 ± 2.3	113.5 ± 4.6	114.9 ± 8.5	120.9 ± 7.7
Lines Crossed				
1 mg/kg	89.2 ± 5.6	106.4 ± 8.7	109.9 ± 8.1	120.6 ± 14.2
5 mg/kg	88.0 ± 10.7	95.4 ± 3.9	90.4 ± 6.5	101.1 ± 10.1
Rears				
1 mg/kg	94.0 ± 12.9	99.8 ± 11.5	112.1 ± 12.5	114.9 ± 8.9
5 mg/kg	106.0 ± 16.3	84.7 ± 10.6	105.0 ± 12.3	123.6 ± 6.8

Operant responding was recorded between 150–165 min after the injection of cyproheptadine and line crosses and rears at 180 min after injection.

TABLE 4

EFFECTS OF mCPP ON OPERANT RESPONDING AND LOCOMOTOR ACTIVITY IN FSL AND FRL RATS

	% of Saline Baseline Value			
	FSL Males	FRL Males	FSL Females	FRL Females
Bar Presses				
1 mg/kg	53.0 ± 17.3	67.0 ± 6.7	68.7 ± 7.4	78.0 ± 4.1
3 mg/kg	2.3 ± 1.2	13.2 ± 5.9	13.7 ± 7.6	23.2 ± 5.9
Line Crosses				
1 mg/kg	59.0 ± 7.8	60.3 ± 5.5	52.6 ± 7.3	81.8 ± 7.2
3 mg/kg	25.8 ± 11.3	36.1 ± 2.5	25.7 ± 7.4	70.4 ± 11.4
Rears				
1 mg/kg	58.1 ± 7.5	71.0 ± 10.0	49.7 ± 8.1	70.0 ± 5.3
3 mg/kg	17.9 ± 6.0	23.3 ± 7.8	2.7 ± 1.5	28.0 ± 5.6

Activity was recorded at 11 min and operant responding was measured between 12.5 and 27.5 min after the injection of mCPP.

(e.g., injection doses and times) are given as footnotes to the tables of the relevant data.

Statistical Analyses

The temperature data were expressed as net changes from pretreatment temperatures recorded on the same day. The behavioural data were expressed as percentages of the responses after isotonic saline treatment, recorded 4 to 8 days previously.

The data were analysed by a series of three-way analyses of variance (ANOVA), with line, sex and treatment serving as the main effects. Student's *t*-tests for repeated or independent samples were used when appropriate.

RESULTS

Dose-Related Effects of Cyproheptadine and mCPP

Temperature. The effects of cyproheptadine on body temperature were dose-related, as illustrated in Table 1. These dose-related effects were confirmed to be significant in a 3-way ANOVA, $F(1,56)=32.91$, $p<0.001$. Cyproheptadine also produced significantly greater hypothermia in the FSL animals than in the FRL animals, $F(1,56)=31.28$, $p<0.001$. There were no significant sex differences or interactions.

The effects of mCPP on body temperature were more complex than those of cyproheptadine, as illustrated in Table 2. Temperatures were taken at both 10 and 45 min after the injection because of the preliminary results suggesting an early hyperthermia followed by a later hypothermia. Separate 3-way ANOVA's confirmed the significantly greater decrease in temperature produced by 3 mg/kg at both time points [$F(1,56)=99.959$, $p<0.001$, and 99.13, $p<0.001$, at 15 and 45 min, respectively]. There was also a significantly greater hypothermia induced by mCPP in the FSL rats at both time points [$F(1,56)=28.74$, $p<0.001$, and 45.14, $p<0.001$, at 15 and 45 min, respectively]. Other significant findings were sex × treatment and line × sex interactions at 10 min and sex differences at 45 min, with females being more affected than the males, $F(1,56)=10.89$, $p<0.05$.

Operant responding and activity. The effects of cyproheptadine on operant responding and locomotor activity are summarized in Table 3. Separate 3-way ANOVA's revealed very few significant effects on these variables. Cyproheptadine slightly increased operant responding in all groups but there were no significant treatment, line, sex or interac-

TABLE 5

EFFECTS OF CYPROHEPTADINE AND mCPP PRETREATMENT ON OXOTREMORINE-INDUCED HYPOTHERMIA IN FSL AND FRL RATS

Group	Mean Deviation From Baseline Temperature (°C)		
	Cyproheptadine	mCPP	Saline
Males			
FSL (n=10)	−1.8 ± 0.4	−3.0 ± 0.3	−3.0 ± 0.4
FRL (n=10)	−0.4 ± 0.1	−1.5 ± 0.3	−1.5 ± 0.2
Females			
FSL (n=10)	−2.0 ± 0.5	−3.9 ± 0.3	−3.4 ± 0.3
FRL (n=10)	−0.6 ± 0.1	−1.8 ± 0.1	−2.0 ± 0.1

Baseline temperatures were recorded 10 min prior to the beginning of the drug injections. Cyproheptadine or saline were given 140 min prior to the next recording; mCPP and/or methyl atropine, 70 min; and oxotremorine, 50 min.

tion effects. The lower dose of cyproheptadine tended to increase line crosses in most groups compared to the higher dose, $F(1,56)=4.03$, $p<0.05$, but there were no significant line, sex or interaction effects. Cyproheptadine also tended to stimulate rearing in females, $F(1,56)=4.57$, $p<0.05$, but there were no significant dose, line or interaction effects.

In contrast to cyproheptadine's mild effects on behaviour, mCPP had marked effects, as illustrated in Table 4. There were highly significant dose-dependent decreases in operant responding, $F(1,56)=114.95$, $p<0.001$, line crossings, $F(1,56)=17.69$, $p<0.001$, and rearings, $F(1,56)=82.41$, $p<0.001$. The FSL rats were also more significantly affected than their FRL counterparts for each measure [$F(1,56)=4.82$, $p<0.05$; 8.25, $p<0.01$; and 10.75, $p<0.001$, respectively, for operant responding, line crossings and rears]. Males were also more significantly affected than females for operant responding, $F(1,56)=5.82$, $p<0.05$, and line crossings, $F(1,56)=4.70$, $p<0.05$. The only significant interaction was a sex by line interaction for line crossings, $F(1,56)=7.49$, $p<0.01$, which was the consequence of the FRL females being much less affected by mCPP than the other three groups (see Table 4).

Drug Interactions on Body Temperature

The mean±s.e.m. baseline temperatures before saline treatment were 37.2±0.2, 37.7±0.1, 38.0±0.1, and 37.7±0.1°C for FSL males, FRL males, FSL females and FRL females, respectively. Independent sample t-tests revealed significantly lower temperatures in the FSL males compared to the FRL males ($t=3.33$, $p<0.01$) and FSL females ($t=4.01$, $p<0.01$).

The hypothermic effects of oxotremorine were more marked in the FSL males and females, as illustrated in Fig. 1. Statistical analyses (repeated measures t-tests) confirmed that oxotremorine significantly reduced rectal temperature in all groups 50 min after treatment. However, oxotremorine produced greater hypothermia in FSL males than in FRL males ($t=3.42$, $p<0.01$) and in FSL females than in FRL females ($t=6.60$, $p<0.001$). No significant sex differences were observed.

As illustrated in Fig. 1, maximum decrease in temperature was observed 50 min after the administration of oxotremorine. Consequently, analyses of the effects of cyproheptadine and mCPP pretreatment on oxotremorine-induced hypothermia were conducted on the net changes in temperature at 50 min after oxotremorine. These data are summarized in Table 5 and were analysed by separate 3-way ANOVA's (pretreatment × line × sex), which confirmed significant line differences [$F(1,63)=53.610$, $p<0.001$ for cyproheptadine and $F(1,59)=75.851$, $p<0.001$ for mCPP] in hypothermia and the absence of sex differences (both $p>0.05$). In addition, they revealed that cyproheptadine significantly reduced the hypothermic effects of oxotremorine, $F(1,63)=21.154$, $p<0.001$, whereas mCPP did not, $F(1,59)=2.345$, $p>0.05$. There were no significant interactions, suggesting that cyproheptadine's reduction of oxotremorine-induced hypothermia was similar in all groups (see Table 5).

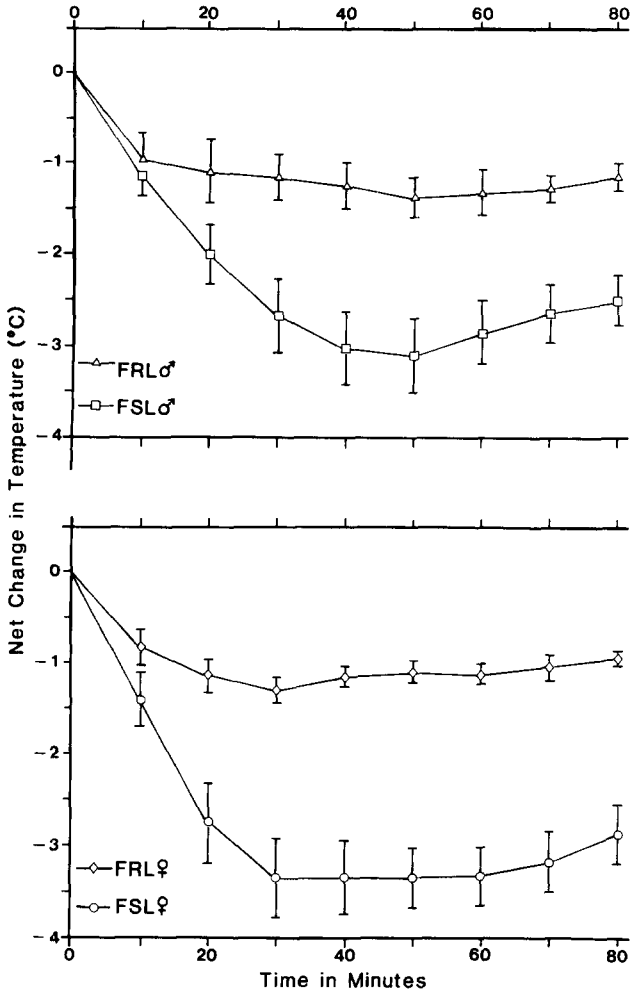

FIG. 1. Effects of oxotremorine on core body temperature in Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL) rats (N=10 in each group). Oxotremorine (0.2 mg/kg) was administered subcutaneously after recording of baseline temperature with a rectal thermistor probe. Methyl atropine (2 mg/kg) was given 15 min prior to the injection of oxotremorine to minimise its peripheral effects. Recordings were taken at 10 min intervals for 80 min after the injection of oxotremorine.

DISCUSSION

Temperature Regulation

Oxotremorine produced marked reduction in temperature in all rats (Fig. 1). This finding is consistent with a large body of evidence that muscarinic agonists induce hypothermia [e.g., (20, 22, 23, 27)]. Moreover, the present results confirmed the increased sensitivity of FSL rats to oxotremorine, a finding which may be related to the increased concentrations of muscarinic cholinergic receptors in these rats (24). Thus, other differences observed in these rats may be related to these differences in cholinergic sensitivity.

As indicated previously, there have been inconsistent reports on the effects of serotonergic agents on body temperature. The decreases observed after cyproheptadine in the present study (Table 1) are comparable to those found by Martin and co-workers (19). However, other workers have failed to see a change in rectal temperature after 1 (36) or 10 mg/kg (33) of cyproheptadine. Procedural differences may account for these findings. Similarly, the consistent decrease in temperature seen after 3 mg/kg mCPP (Table 2) is compatible with a number of reports of hypothermia in rats induced by serotonergic agonists (16,17) but is inconsistent with other literature that suggests that pharmacological stimulation of 5-HT receptors produces hyperthermia in rats (33, 34, 36).

These discrepant findings may be related to the proposal that 5-HT may have a dual role in both heat production and heat loss (17,21). A recent communication has further clarified this issue. By using agents which have some selectivity at the 5-HT_1 or 5-HT_2 receptor subtypes, Gudelsky and co-workers (12) suggested that 5-HT_2 receptors mediate hyperthermia while 5-HT_1 receptors mediate hypothermia. Since cyproheptadine has low affinity for the 5-HT_1 binding site but has high affinity for the 5-HT_2 site (15,26), it would be predicted to produce hypothermia, as observed in the present experiment. Conversely, mCPP appears to have low affinity at both sites and may simultaneously stimulate both heat loss and heat gain pathways weakly. Further studies with selective 5-HT_1 and 5-HT_2 agonists and antagonists are necessary to confirm these interesting new findings.

The above model may also be useful in accounting for the apparently paradoxical finding that both cyproheptadine, the antagonist, and mCPP, the agonist, produce significantly greater hypothermia in the FSL rats. If one postulates that cyproheptadine-induced hypothermia is a result of relative overactivity of the 5-HT_1 receptor subsystem because of the blockade of 5-HT_2 receptors and that mCPP-induced hypothermia is a consequence of its 5-HT_1 agonist action, then it would suggest that the FSL rats have more sensitive 5-HT_1 receptors. This hypothesis can be evaluated by further psychopharmacological and neurochemical studies, but provides a reasonable explanation of the present results on temperature regulation in the two lines of rats.

General Activity

The relatively weak effects of cyproheptadine on operant responding and line crossings are consistent with other recent reports of unchanged spontaneous locomotor activity (6). However, other workers have reported increased locomotor activity in rats after acute treatment with 5-HT receptor antagonists [e.g., (4)] and the present study showed a mild stimulant effect after the lower dose of cyproheptadine (Table 3). The increase in operant responding after cyproheptadine, although not dose-dependent, has also been observed by others, who also reported suppression of responding with higher (10 mg/kg) doses (9). Thus, 5-HT antagonists may increase behavioral output, but the exact results may be dependent upon the conditions of the experiment.

The effects of mCPP were more dramatic than those of cyproheptadine and were opposite in direction. Significant decreases in operant responding, line crossings, and rears were seen (Table 4). These findings support other reports (7,18) and are consistent with the hypothesis that 5-HT has an inhibitory role in behaviour (8).

There were highly significant differential effects of mCPP: the FSL rats were more sensitive to the behavioural inhibitory effects of this agent than were the FRL rats (Table 4). These findings could also be related to the increased 5-HT_1 receptor sensitivity postulated previously to underlie the effects of mCPP on body temperature. However, there is little information about which 5-HT receptor subtype mediates the behavioural effects of 5-HT. With the further development of selective agonists and antagonists [e.g., (11,12)], such information may soon come to light.

In conclusion, the present findings have confirmed the utility of using rat lines with genetically determined differences in cholinergic function to study interactions between the cholinergic and other neurotransmitter systems. The FSL rats are more sensitive to cholinergic agonists and also to serotonergic agonists, suggesting that 5-HT and ACh may be parallel systems in the regulation of many functions.

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