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Cholinergic/Serotonergic Interactions in Hypothermia: Implications for Rat Models of Depression

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OVERSTREET, D. H., L. C. DAWS, G. D. SCHILLER, J. ORBACH AND D. S. JANOWSKY. *Cholinergic/serotonergic interactions in hypothermia: Implications for rat models of depression.* PHARMACOL BIOCHEM BEHAV **59**(4) 777–785, 1998.—This article reviews published reports and presents new evidence that support a number of commonalties between lines of rats selectively bred for differences in cholinergic (muscarinic) and serotonergic (5-HT_{1A}) sensitivity. The Flinders Sensitive Line (FSL) rat, a genetic animal model of depression derived for cholinergic supersensitivity, is more sensitive to both cholinergic and serotonergic agonists, and exhibits exaggerated immobility in the forced swim test relative to the control, Flinders Resistant Line (FRL), rat. Similar exaggerated responses are seen in a line of rats recently selected for increased sensitivity to the 5-HT_{1A} agonist, 8-OH-DPAT (High DPAT Sensitive—HDS), relative to lines selectively bred for either low (Low DPAT Sensitive—LDS) or random (Random DPAT Sensitive—RDS) sensitivity to 8-OH-DPAT. For both the FSL and HDS rats, their exaggerated immobility in the forced swim test is reduced following chronic treatment with antidepressants. The present studies examined further the interaction between cholinergic and serotonergic systems in the above lines. Supersensitive hypothermic responses to 8-OH-DPAT were observed very early (postnatal day 18) in FSL rats, suggesting that both muscarinic and serotonergic supersensitivity are inherent characteristics of these rats. Scopolamine, a muscarinic antagonist, completely blocked the hypothermic effects of the muscarinic agonist oxotremorine in FSL and FRL rats, but had no effect on the hypothermic responses to 8-OH-DPAT, suggesting an independence of muscarinic and 5-HT_{1A} systems. On the other hand, genetic selection of genetically heterogeneous rats for differential hypothermic responses to the muscarinic agonist oxotremorine were accompanied by differential hypothermic responses to 8-OH-DPAT, suggesting an interaction between muscarinic and 5-HT_{1A} systems. Overall, these studies argue for an inherent interaction between muscarinic and 5-HT_{1A} systems, which probably occurs beyond the postsynaptic receptors, possibly at the level of G proteins.

Serotonin 8-OH-DPAT Muscarinic Oxotremorine Ontogeny FSL rat Depression Core body temperature

ALTHOUGH the underlying neurochemical components of depressive disorders are still largely unknown, it has been postulated that an interaction of, or dysbalance between, two or more neurotransmitter systems is involved [e.g., (6,26)]. Although other combinations have been put forward since Janowsky and colleagues originally proposed the cholinergic–adrenergic hypothesis of depression in 1972 (26), a hypothesis involving cholinergic–serotonergic interactions in depression has received relatively little attention to date (42,44). The present article reviews the evidence for an interaction be-

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tween serotonergic and cholinergic systems in two rat models of depression and describes new data supporting such an interaction in the regulation of temperature.

The involvement of the serotonergic system in depression and related affective disorders is now well recognized. Reports of alterations in serotonin (5-HT) receptors and/or receptor function in depressed individuals (2,6,8,31,67), down-regulation of the 5-HT receptor/second messenger systems by clinically effective antidepressants [e.g. (4,18,33)], and 5-HT_{1A} receptor agonists being potentially effective antidepressants (12,34) are just a small part of the evidence that has implicated 5-HT in the pathogenesis and treatment of depression. Furthermore, serotonergic "supersensitivity" has recently been reported in depressed individuals with, for example, increased 5-HT_2 receptor function, measured as increased phosphoinositide hydrolysis in the platelets of depressed humans, occurring after 5-HT_2 agonist administration (36).

In addition, central cholinergic neurotransmitter mechanisms have long been implicated in the pathogenesis of depressive disorders (25,26,28). It is well recognized that individuals with depressive disorders are more sensitive to the behavioral (i.e., depression-inducing) and physiological (e.g., elevation of adrenocortical hormones and growth hormone, induction of REM sleep) effects of muscarinic agonists than are normal controls [e.g. (7,27,37,52,60).

With respect to cholinegic and serotonergic interactions, it has been reported that brain regions that are integral in the regulation of mood and cognition, such as the cerebral cortex and hippocampus, are rich in muscarinic receptors (mAChR) (35) and receive a dense serotonergic innervation as well (64). Pharmacological studies suggest that both systems are involved in the regulation of passive avoidance behavior (51), which might relate to depression in humans (39). Biochemical (1,19) manipulations suggest that 5-HT release may be regulated by muscarinic receptors (19), whose plasticity is dependent upon the integrity of the serotonergic system (1). Thus, not only are the cholinergic and serotonergic systems anatomically related to each other, but they also interact in such a way that a dysbalance of one system may lead to a functional deficit in the other. The etiology of affective disorders may, therefore, be attributable to a dysbalance between these neurotransmitter systems, with, for example, cholinergic overactivity predisposing to depression but subsequent alterations in serotonergic function actually inducing the depressive episodes.

Potential genetic animal models of depression have been developed by selective breeding for differential responses to muscarinic and serotonergic agonists, respectively (45–47), and the implications of these models for a cholinergic/serotonergic interaction hypothesis of depression will be the focus of the present communication. The Flinders Sensitive Line (FSL) rats represent a cholinergic model of depression (39,46). These rats were originally selectively bred to be more sensitive to anticholinesterases than the control Flinders Resistant Line (FRL) rats (41,54). However, FSL rats are also more sensitive to the behavioral and physiological effects of directly acting muscarinic agonists (39,40,46). Furthermore, FSL rats also are more sensitive to a variety of serotonergic drugs, including those that target 5-HT_{1A} and 5-HT_2 receptors (42,56,66). Preliminary experiments have reported a positive correlation between increased behavioral sensitivity and increased 5-HT receptor number in the FSL rats (56).

More recently, randomly bred, genetically heterogeneous rats were used to selectively breed for differential hypothermic responses to the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT. The line that became more sensitive to 8-OH-DPAT (the High DPAT Sensitive—HDS line) exhibited a number of similarities to the FSL rats. In addition to their supersensitive responses to 5-HT_{1A} agonists, they exhibited exaggerated immobility in the forced swim test (46,49), and this immobility could be counteracted by chronic treatment with antidepressant drugs (24,49,58). Both HDS and FSL rats also exhibit higher consumption of sweet solutions (13,47,50), and both appear to have elevated numbers of cortical 5-HT_{1A} receptor binding sites (30,47,56). Thus, there are several intriguing parallels between the HDS rats, selectively bred for increased 5-HT_{1A} sensitivity, and the FSL rats, selectively bred for increased muscarinic sensitivity.

What is particularly intriguing about these genetic animal models of depression is that although the FSL and HDS rats were selectively bred for increased hypothermic responses to oxotremorine (66) and 8-OH-DPAT (44), respectively, they exhibit similar behavioral profiles and antidepressant-like responses to clinically effective antidepressant agents (24,46,47). Table 1 summarizes these similarities among depressed individuals and the FSL and HDS rats. Note that despite several similarities, neither the HDS nor the FSL rats resemble depressed individuals in serotonergic sensitivity. The predominant approach has been to challenge depressed and control individuals with serotonergic agonists and measure specific hormones, and the most common finding is for the depressed individuals to display a blunted response (31). Hormonal responses to serotonergic challenges have not been studied in the FSL or HDS rats, but these lines are supersensitive to the hypothermic effects of 8-OH-DPAT, as mentioned above (see Table 1). Therefore, these rat models do not mimic all aspects of depressed individuals. Nevertheless, they are innately more immobile in the forced swim test and are less immobile following chronic treatment with antidepressants (Table 1).

In contrast to what appeared to occur in the FSL rats (42,66), it initially appeared that selection for differential 5-HT_{1A} sensitivity was not accompanied by a parallel increase in muscarinic sensitivity as such (45). This observation, when coupled with the results of an interbreeding study that indicated little correlation between 5-HT_{1A} and muscarinic responses in genetically heterogeneous rats (44), suggested that the serotonergic and cholinergic systems were independently regulated. In the present set of experiments we sought to obtain data that would confirm or call into question the postulated independence of, or interaction between, the serotonergic and cholinergic systems, using drug-induced hypothermia as the index variable.

TABLE 1

SIMILARITIES AMONG DEPRESSED INDIVIDUALS AND FSL AND HDS RATS

Feature/Measure	Depressed Individuals	FSL Rats	HDS Rats
Increased cholinergic sensitivity	Yes	Yes	Yes
Increased 5-HT_{1A} sensitivity	*No*	*Yes*	*Yes*
Decreased locomotor activity	*Yes*	*Yes*	*No*
Increased REM sleep	Yes	Yes	N.D.
High sweet intake craving	Yes	Yes	Yes
Immobility after stress	Yes	Yes	Yes
Antidepressant response	Yes	Yes	Yes

Areas where there is a lack of agreement are highlighted in italics.

The three approaches used were: 1) a developmental profile of 8-OH-DPAT sensitivity in the FSL and FRL rats to determine if it is similar to the developmental profile observed in FSL rats for the muscarinic agonist, oxotremorine (11); 2) a classical pharmacological blockade study of the ability of scopolamine to counteract the hypothermic effects of oxotremorine and 8-OH-DPAT; 3) a short-term selective breeding study focusing on the development of oxotremorine sensitivity, with a parallel examination of changes in 5-HT_{1A} (i.e., 8-OH-DPAT) sensitivity.

METHOD AND RESULTS

Experiment 1. Developmental Profile of 8-OH-DPAT Sensitivity in FSL and FRL Rats

Developmental profiles have shown that the FSL rats are more sensitive to the hypothermic effects of oxotremorine than are their control counterparts, the FRL rats, as early as 2 weeks postnatal, the earliest age of practical testing (10,11). Using this developmental approach, the present study aimed to compile a profile for hypothermic sensitivity to the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in FSL and FRL rats of different ages.

Animals. Male and female FSL and FRL rats aged 15, 20, 25, 31, and 60 days of age were selected from the 47th generation of FSL and FRL colonies being maintained at the Flinders University of South Australia. Body weights ranged from a mean of 29.1 ± 0.8 g (n = 36 pooled genders) at 15 days of age to 267 ± 8 g for males (n = 42) and 188 ± 4 g for females (n = 37) at 60 days of age. Data for 150- and 250-day-old rats were derived from the 43rd and 46th generations, respectively. At 250 days of age males weighed 507 ± 12 g (n = 23) and females, 309 ± 8 g (n = 21). Until the time of weaning (30 days of age) all rats were housed with respective dams and removed only during test sessions. After this time they were housed in groups of six in large metal cages with free access to food and water. The colony room was maintained at 22 ± 1°C and 50% humidity, under a 12 L:12 D cycle. The number of rats used for each test ranged between 3 and 10 of each gender. All experiments were conducted between 0800 and 1300 h. This experiment was approved by the Institutional Animal Care Committee of Flinders University.

Core body temperature recording. Core body temperature was recorded by inserting a lubricated thermocouple probe (Eirelec 5000 hand-held thermometer), 1 to 5 cm into the rectum (i.e., the distance being proportional to the age and size of the rat). Temperature was recorded to the nearest 0.1°C and was stable within 1 min after insertion of the probe. Baseline temperatures were always obtained within the 2 h preceding drug challenge. The data are typically expressed as mean ± standard error of the mean (SEM) deviations from baseline where each animal served as its own control.

Procedure. Rats were weighed and baseline core body temperatures obtained on the morning of the drug challenge tests. Animals were quasi-randomly divided into two groups and subcutaneously injected with either isotonic saline (SAL) or 8-OH-DPAT (0.1 mg/kg). Core body temperature was recorded 30 min later. The two groups were selected in such a way that there was an equal representation from each litter in the two treatment groups. The dose of 8-OH-DPAT was selected on the basis of dose–response curves for 8-OH-DPAT-induced hypothermia in adult FSL and FRL rats (57); with 0.1 mg/kg producing near maximal hypothermia and clearly delineated sensitivity differences between the two lines. To minimize the possibility of drug tolerance occurring, the treatment groups were alternated so that there was a minimum of 10 days between exposure to either 8-OH-DPAT or SAL. As an additional check for tolerance development and for further clarification of the developmental profile, a subset of rats from each litter were left drug "naive." These rats were given 8-OH-DPAT once only at selected ages (17, 18, 24, and 30 days of age). 8-OH-DPAT hydrobromide was obtained from Research Biochemicals Incorporated (Natick, MA). The dose refers to the weight of the salt and was freshly prepared on each challenge day by dissolving in isotonic saline and kept on ice to avoid degradation.

Statistical analysis. The data were subjected to multiple analysis of variance using the statistical package SPSS-X on a UNIX system mainframe computer. Where there were no significant gender differences, data for male and females were pooled. Line, age, and treatment were the factors tested. The probability level for significance was set at $p < 0.05$. Prior to any inferential statistics analyses, data were tested for homogeneity of variance using Bartlett-Box F and Cochran's C-tests. Both confirmed homogeneity of variance.

Results. Within each line there were no significant gender differences in change in core body temperature after 8-OH-DPAT; therefore, the data for males and females were pooled. The results depicted in Fig. 1 highlight the pronounced differences in sensitivity between the FSL and FRL rats with respect to 8-OH-DPAT–induced hypothermia. The FSL rats displayed a significantly greater 8-OH-DPAT–induced hypothermic effect than the FRL rats at all ages tested with the exception of hypothermia at 15 days of age, where the lines were not different. This yielded a significant main effect of line, $F(1, 276) = 184.64$, $p < 0.001$. The magnitude of this difference varied with age, being maximal at 18 and 31 days of age, and yielded a significant main effect of age, $F(9, 276) = 43.88$, $p < 0.001$. Furthermore, an interaction effect between line and age was established (Fig. 1). FSL, relative to FRL rats, became more sensitive to the hypothermic effect of 8-OH-DPAT with age [line × age, $F(9, 276) = 5.87$, $p < 0.001$].

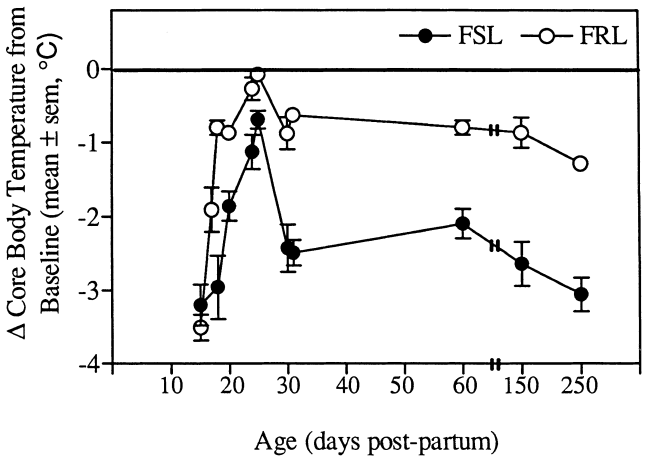

FIG. 1. Age-dependent changes in mean core body temperature after 0.1 mg/kg (SC) 8-OH DPAT. Data for male and female rats were pooled because no significant gender differences were established with respect to drug-induced change in core body temperature. There were 6 to 20 rats per group. Each animal served as its own control and change in temperature is with respect to normal baseline core body temperature.

8-OH-DPAT "naive" rats, depicted at 17, 18, 24, and 30 days of age in Fig. 1, did not deviate from the general developmental pattern observed in rats that received 8-OH-DPAT on three separate occasions at 10-day intervals.

Saline-injected controls exhibited minor fluctuations about a mean of 0°C change in core body temperature over all ages tested (data not shown), with the extreme ranges being from −0.4 to +0.2°C.

Experiment 2: Pharmacological Blockade of 8-OH- DPAT–Induced Hypothermia

This experiment explored the possibility that 5-HT_{1A} sensitivity, as shown by hypothermia, is caused by muscarinic sensitivity in the Flinders Line rats due to serotonergic neurons synapsing on cholinergic neurons, which transmit to the heat loss pathways. If this model of cholinergic neurons being the final common path to hypothermia is correct, then scopolamine, a centrally acting muscarinic antagonist, should block or partially counteract the hypothermic effect of 8-OH-DPAT, the 5-HT_{1A} receptor agonist, as well as that of oxotremorine, the muscarinic agonist. Experiments were conducted in adult FSL and FRL rats to investigate this hypothesis.

Animals. Male and female FSL and FRL rats were selected from the 48th generation of the breeding colonies maintained at Flinders University. The rats were between 75–80 days old at the beginning of the study and weighed approximately 350 g (for males) or 205 g (for females). The rats were housed and maintained as described above. This experiment was approved by the Institutional Animal Care Committee of Flinders University.

Procedure. The rats were randomly divided into eight treatment groups so that there was an even representation of litter mates in each group (7–16 rats per group). The groups received either isotonic saline (four groups) or scopolamine (0.2 mg/kg, four groups) pretreatments followed by saline, oxotremorine (0.19 mg/kg), or 8-OH-DPAT (0.1 or 0.5 mg/kg) 15 min later. Scopolamine hydrochloride and oxotremorine sesquifumarate were obtained from Sigma (St. Louis, MO), and 8-OH-DPAT was obtained from Research Biochemicals Incorporated (Natick, MA); doses refer to the salts for the drugs. Core body temperatures were recorded at baseline, when the animals were weighed, and at 30 min after the final injection. All injections were given SC in 1 ml/kg.

Statistical analyses. Results are expressed as mean ± SEM changes in °C from the corresponding baseline measures. The data were subjected to two-way ANOVAs, with line and treatment as the main factors. Post hoc analyses were performed using Scheffe's multiple contrast tests. Where appropriate, *t*-tests were performed to test the significance of selected pairs of data.

Results. The effects of scopolamine pretreatment on oxotremorine- and 8-OH-DPAT–induced hypothermia in the FSL and FRL rats are illustrated in Fig. 2. Two-way ANOVA indicated highly significant treatment ($F = 80.7$, $p < 0.001$) and line ($F = 142.8$, $p < 0.001$) effects. The saline vehicle produced a small hyperthermic response, while scopolamine slightly reduced temperature in the FSL rats and increased it in the FRL rats (Fig. 2A). Scopolamine significantly counteracted the decrease in body temperature induced by oxotremorine in both lines, as expected (Fig. 2A). In contrast, the hypothermia induced by either dose of 8-OH-DPAT was only slightly affected by scopolamine pretreatment (Fig. 2B). Thus, these findings indicate that scopolamine selectively blocks hypothermia induced by the muscarinic receptor agonist, oxotremorine.

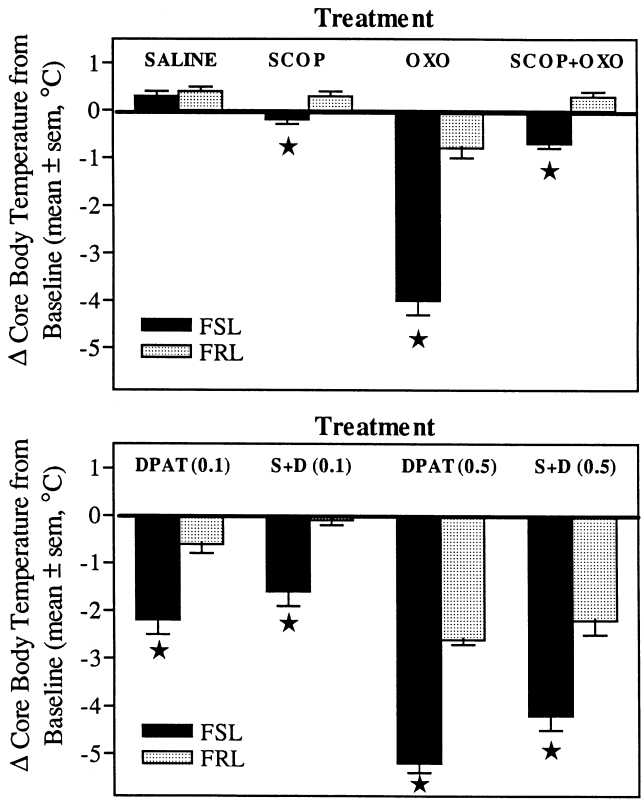

FIG. 2. Scopolamine blockade of hypothermia induced by oxotremorine but not 8-OH-DPAT. Scopolamine (0.2 mg/kg) or saline vehicle was given SC 15 min prior to the administration of saline vehicle, 8-OH-DPAT (0.1 or 0.5 mg/kg), or oxotremorine (0.19 mg/kg). Data represent the mean ± SEM changes in °C from baseline for 7–16 rats.

Experiment 3: Genetic Selection for Differential Hypothermic Responses to Oxotremorine

When the serotonergic sensitivity differences were first discovered in the FSL and FRL rats (66), 13 generations of selection for muscarinic sensitivity had occurred. Consequently, the apparent association between muscarinic and 5-HT_{1A} sensitivity may have occurred by chance and not by a genetic correlation. The fact that muscarinic and 5-HT_{1A} sensitivities are not significantly correlated in the previously mentioned intercross experiments between the FSL and FRL rats (43) would appear to support the lack of close genetic association between the cholinergic and serotonergic sensitivity. However, we have found dramatic differences in muscarinic sensitivity to oxotremorine in later generations of the randomly bred genetically heterogeneous rats that were selectively bred for differential hypothermic responses to 8-OH-DPAT, despite only small differences in the earlier generations (43,45). These studies thus provide mixed support for the hypothesis of a genetic association between muscarinic and 5-HT_{1A} sensitivities.

The present experiment sought to clarify this relationship by using randomly bred genetically heterogeneous rats to conduct a short-term selective breeding study in which serotonergic and cholinergic sensitivity were simultaneously evaluated in rats bred for differences in oxotremorine sensitivity.

Animals. The animals were selected from a genetically heterogeneous (N/Nih) breeding colony that was established in the Center for Alcohol Studies at the University of North Carolina (45). Since obtaining breeding stock from NIH, these rats have been maintained by breeding 10 pairs per generation, with no matings occurring between close relatives. Litter size averages 10–12 rats. The rats were housed in standard housing conditions under a reversed 12 L:12 D cycle, with lights off between 1000 and 2200 h. This experiment was approved by the Institutional Animal Care Committee of the University of North Carolina.

Procedure. The study began with an oxotremorine challenge at weaning (28–32 days of age). The rats were marked, weighed, and baseline temperatures were recorded with a rectal thermocouple probe attached to a Sensortek digital thermometer. They were then injected SC with a drug mixture containing oxotremorine (0.2 mg/kg) and atropine methyl nitrate (2 mg/kg). Core temperatures were recorded 30 min later and rats were selected for breeding according to their hypothermic responses. The male and female rat from each litter that exhibited the greatest decrease in temperature were used to establish the High Oxotremorine Sensitivity (HOS) group; those that exhibited the smallest decrease in temperature were used to establish the Low Oxotremorine Sensitivity (LOS) group.

Animals were paired for mating so that there were no close relatives. In the first generation progeny, the same procedures as described above were carried out at weaning: handling, weighing, recording of baseline temperature, injection of oxotremorine/methyl atropine mixture, recording of temperature at 30 min. Again, the most affected male and female were used to continue the HOS line and the least affected male and female were used to continue the LOS line.

The same procedure was followed once again for the second generation progeny, with one addition. Approximately 5 days after the oxotremorine challenge, the rats were given a single 0.5 mg/kg SC injection of 8-OH-DPAT, and core temperature was recorded 45 min later, as is typically done in the 8-OH-DPAT–selected rats (45,47).

To provide a reference group, data from the 7th generation of the HDS and LDS rats, selected for their differential hypothermic responses to 8-OH-DPAT, were included. The rats were first given 8-OH-DPAT (0.5 mg/kg, SC) at weaning and their temperatures recorded 45 min later. Then, approximately 5 days later, they were challenged with a mixture of oxotremorine and methyl atropine, as described above, and temperatures recorded 30 min later. Finally, in addition to the LOS and HOS groups, selectively bred for differences in oxotremorine-induced hypothermia, and the HDS and LDS groups, selectively bred for differences in 8-OH-DPAT–induced hypothermia, a group of randomly bred genetically heterogeneous (RDS) rats were included.

Oxotremorine sesquifumarate and atropine methyl nitrate were obtained from Sigma (St. Louis, MO) and 8-OH-DPAT was obtained from Research Biochemicals Incorporated (Natick, MA). Doses refer to the salts of the respective drugs.

Statistical analyses. The data were analyzed by one-way ANOVAs, with follow-up Newman–Keuls tests.

Results. Hypothermia induced by oxotremorine or 8-OH-DPAT was studied in similarly maintained animals from the 7th generation of selection of the 8-OH-DPAT–selected lines (HDS and LDS) and the 2nd generation of selection of the oxotremorine-selected lines (HOS and LOS). There were highly significant ($F = 57.18$, $p < 0.0001$) differences among the groups. In Fig. 3, it can be seen that there appeared to be

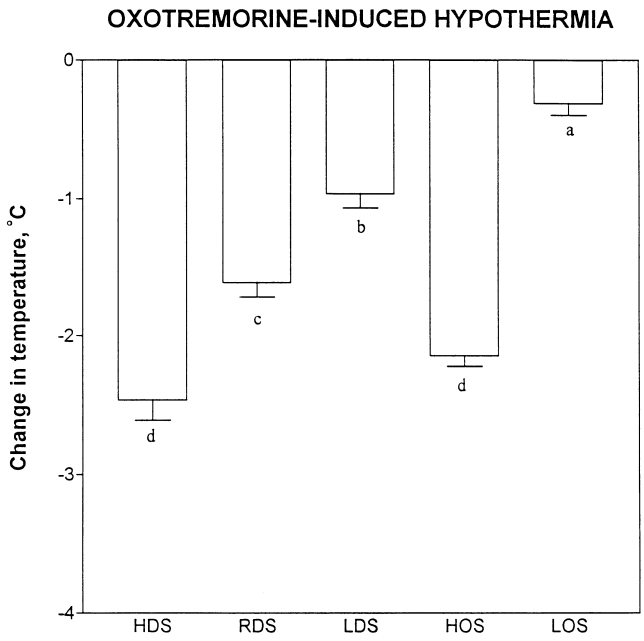

FIG. 3. Hypothermia induced by oxotremorine in rats selectively bred for differential hypothermic responses to oxotremorine or 8-OH-DPAT. A mixture of oxotremorine (0.2 mg/kg) and methyl atropine (2 mg/kg) was administered 30 min prior to the recording of core temperature by a rectal thermistor probe. The data represent the mean ± SEM changes in °C from baseline for 17–36 male and female rats. Groups with different letters are significantly different, $p < 0.01$, Newman–Keuls test.

rapid selection for the differential hypothermic response to oxotremorine, as the HOS rats exhibited a significantly greater hypothermic response than the LOS rats, with the randomly bred (RDS) rats intermediate. This figure also shows that there are significant differences in the hypothermic response to oxotremorine in the HDS and LDS rats, selectively bred for differential hypothermic responses to 8-OH-DPAT. The HDS rats were more sensitive to oxotremorine.

The converse data, illustrated in Fig. 4, present a rather similar picture. There are large and significant differences in hypothermic responses to 8-OH-DPAT in the lines selectively bred for differential responses to this agent (HDS and LDS), but there are also significant differences between the HOS and LOS lines, selectively bred for differences in oxotremorine-induced hypothermia ($F = 100.61$, $p < 0.001$). The HOS line is more sensitive to the hypothermic effects induced by 8-OH-DPAT. As for oxotremorine, the randomly bred RDS rats are intermediate between the HDS and LDS rats.

GENERAL DISCUSSION

The present findings, utilizing three diverse approaches, argue for an interaction between the cholinergic and serotonergic systems in these rat models of depression. However, the hypothesis that simple changes in 5-HT_{1A} or muscarinic receptors can account for the present findings cannot be supported, and alternative mechanisms must be considered.

Experiment 1 demonstrated that both FSL and FRL rats exhibited a hypothermic response to 8-OH-DPAT at the earliest age tested, suggesting that the 5-HT_{1A} system is already

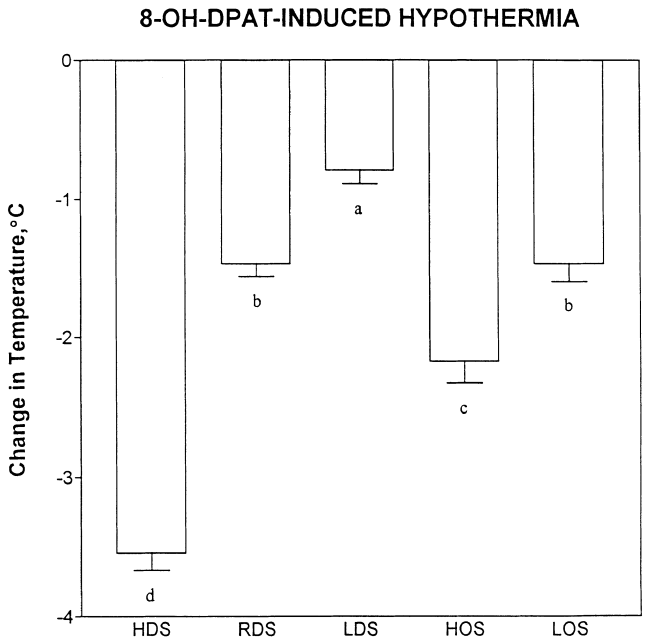

FIG. 4. Hypothermia induced by 8-OH-DPAT in rats selectively bred for differential hypothermic responses to oxotremorine or 8-OH-DPAT. 8-OH-DPAT (0.5 mg/kg) was administered 45 min prior to the recording of core temperature by a rectal thermistor probe. The data represent the mean ± SEM changes in °C from baseline for 20–31 male and female rats. Groups with different letters are significantly different, $p < 0.01$, Newman–Keuls test.

functional at 15 days of age. The ability of 8-OH-DPAT to exert an effect early in life is in good agreement with the literature (14,29,53,62,63). The present results are further supported by the observation that saline did not result in a significant change in core body temperature from baseline. The ability of saline-treated pups to maintain a constant body temperature when separated from their respective dams for extended periods of time indicates that the capacity to thermoregulate efficiently is present at 15 days of age and thus is not a confounding variable.

Because the FRL rats were considered control rats for the developmental (i.e., ontogenetic) study, it is appropriate to first compare the data derived from FRL rats with those in the literature for randomly bred Sprague–Dawley rats. There is a paucity of literature regarding 8-OH-DPAT–induced hypothermia in juvenile rats. However, the decrease in core body temperature (approx. −1.0°C) exhibited by adult FRL rats is comparable to reports where data have been obtained under similar conditions [e.g., (17,21,22)]. The greater hypothermia (−2.0 to 3.5°C) observed in rats aged 15–17 days is perhaps not surprising, because sensitivity to various serotonergic and cholinergic drugs, quantified using a variety of behavioral measures, have been reported to alter with age (53,61). For example, the 5-HT antagonist, metergoline, inhibited suckling in 3–4- and 7–8-day-old rat pups but had little influence on suckling behavior in older 21–24-day-old pups (53). Receptor number and/or affinity, coupling to second messengers, and integration of neurotransmitter systems often undergo dynamic changes during the first 2–3 postnatal weeks. These changes may not solely serve as precursors for the adult neurotransmitter systems but may also be related to the mediation of behaviors essential to the young pup (53). In summary, the FRL rats responded to a pharmacological manipulation of the serotonergic system that closely resembled that described in the literature. Any gross deviation from this ontogenetic profile exhibited by the FSL rats is, therefore, very likely to be a trait of these selectively bred rats.

Comparison of the developmental profiles for FSL and FRL rats revealed marked differences in their sensitivity to 8-OH-DPAT. FSL rats older than 15 days were supersensitive to the hypothermic effect of 8-OH-DPAT. Thus, 5-HT_{1A} supersensitivity, like muscarinic supersensitivity (10), appears to be an inherent characteristic of the FSL rats. Figure 1 illustrates the virtually parallel development of sensitivity to 8-OH-DPAT–induced hypothermia in FSL compared to FRL rats. The FSL rats, aside from exhibiting greater hypothermia, did not deviate from the ontogenetic pattern of the FRL rats. Both lines were least sensitive to the hypothermic effect of 8-OH-DPAT at 25 days of age. Sensitivity to 8-OH-DPAT then increased quite rapidly, and levels of adult responsivity were reached at 30 days of age. This pattern was not attributable to the emergence of tolerance to 8-OH-DPAT, because age-matched 8-OH-DPAT naive rats exhibited a hypothermia that fell within the bounds of the developmental profile derived from the main experimental group that received 8-OH-DPAT on three separate occasions. One functional implication of such change in sensitivity during development, as described earlier, may be the need for the suppression of neonate behaviors as adult behaviors emerge (55). The underlying neurochemical basis(es) for this period of relative insensitivity cannot be determined from these data alone, and so we can only speculate. However, it is noteworthy that the ontogenetic profile for sensitivity to the muscarinic agonist oxotremorine was similar in shape but the period of relative insensitivity occurred earlier (18 days of age) than for 8-OH-DPAT in FSL rats [(11); see Fig. 5].

Transient periods of altered sensitivity to drugs do not appear to be unique to a single neurotransmitter system and are probably attributable to the dynamic changes that take place during synaptogenesis in the early postnatal weeks. For example, the efficacy of receptor–second messenger coupling (5) and/or expression of genes involved in the manufacture of the various enzymes and receptors that combine to form the functional adult neural network undergo marked changes during the early period in development (19,22,23). The 5-HT_{1A} receptor gene is one such example. A high rate of expression is observed during a limited period in fetal life and again at 18 days of age, after which time the level of gene expression falls quite markedly (20). It is tempting to postulate that after 25 days of age, the FSL rats undergo a further period of 5-HT receptor gene overexpression, which may, at least in part, explain their "adult" supersensitivity to 8 OH-DPAT. Indeed, this is an appealing hypothesis, because preliminary receptor binding studies have indicated an approximately 20% increase in 5-HTlA receptor number in adult FSL compared to FRL rats (56) and in the HDS compared to LDS rats (30,47).

Although the neurochemical and/or metabolic changes that occur early in development are complex, it is clear that the FSL and FRL rats are different in their response to both 8-OH-DPAT and oxotremorine [(10,11); Fig. 5]. The most striking difference is the enhanced sensitivity of FSL rats to the hypothermic effect of these agonists. A more subtle difference, highlighted in Fig. 5, is the ontogenetic time frame for the periods of relative insensitivity to the hypothermic effect of 8-OH-DPAT and oxotremorine. As discussed earlier, both FSL and FRL rats are least sensitive to 8-OH-DPAT at 25

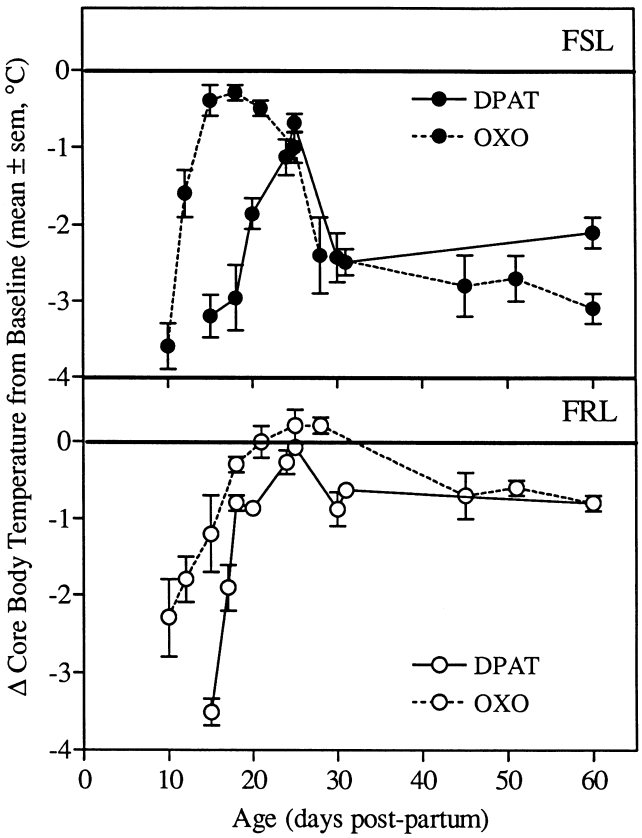

FIG. 5. A comparison of age-dependent changes in mean core body temperature after 0.1 mg/kg (SC) 8-OH-DPAT or 0.25 μmol/kg (SC) oxotremorine. Data for 8-OH-DPAT is duplicated from Fig. 1 and data for oxotremorine is modified from Daws and Overstreet (submitted). Data for male and female rats (within a line) were pooled because no significant gender differences were established with respect to drug-induced change in core body temperature. There were 5 to 20 rats per group. Each animal served as its own control and change in temperature is with respect to normal baseline core body temperature. Associated standard error of the means were not greater than 0.4°C and have been omitted for the sake of clarity. Solid symbols = FSL rats, open symbols = FRL rats; circles = 8-OH-DPAT, squares = oxotremorine.

days of age. The FRL rats are also least sensitive to oxotremorine-induced hypothermia at 25 days of age. This contrasts with the FSL rats, where the greatest insensitivity to oxotremorine-induced hypothermia occurs at 18 days of age. Thus, there are inherent differences not only in the sensitivity of these rat lines to muscarinic and serotonergic agonists, but also in the nature of their developmental profiles. Recent cross-breeding studies using the FSL and FRL rats suggest that muscarinic sensitivity is under the influence of additive and dominance genetic factors, whereas serotonergic sensitivity appears to be influenced by solely additive genetic factors (43,45). Together with the findings of the developmental studies, it appears that both cholinergic and serotonergic systems are instrumental in determining the altered phenotype of the FSL rats.

There is a growing body of literature regarding the genetics of affective disorder in humans (15), and recent studies have shown serotonergic dysfunction in prepubertal major depression patients (55) as well as cholinergic supersensitivity in children at risk for depression (59). The early emergence of serotonergic supersensitivity in the FSL rats provides yet another characteristic to add to the growing suite of parallels between the FSL rats and human depressives (39). Thus, the FSL animal model of conjoint cholinergic/serotonergic supersensitivity may well be heuristic in understanding the neurochemical causes of depressive illness, particularly with respect to a cholinergic–serotonergic balance hypothesis.

Unfortunately, there have been no studies to date that have examined cholinergic or serotonergic drugs on temperature regulation in humans despite its relative noninvasiveness. Instead, human studies have more often focused on changes in neuroendocrine or sleep measures after challenge with serotonergic or cholinergic agents (9,28,31). Also, none of these studies have used both serotonergic and cholinergic probes in the same group of subjects, so it is impossible to assess the value of the cholinergic serotonergic balance hypothesis at this time. The studies on the rat models presented here argue strongly for the necessity of such parallel studies in humans.

Experiment 2 demonstrated that scopolamine blocked the hypothermic responses induced by the muscarinic agonist oxotremorine but not the 5-HT_{1A} agonist 8-OH-DPAT (Fig. 2). Therefore, the serotonergic system probably is not linked in series with the cholinergic system in inducing hypothermia. There is other evidence from selectively bred rat lines that supersensitive hypothermic responses may not be dependent exclusively on changes in muscarinic or 5-HT_{1A} receptors. The supersensitive muscarinic response can be seen very early (Fig. 5), but the muscarinic receptor elevations in the hypothalamus do not appear until 60 days of age (10). Despite the large differences in hypothermic responses to 8-OH-DPAT in the HDS and LDS lines, there are no differences in hypothalamic 5-HT_{1A} receptors in these lines (30). Thus, the close developmental profiles of oxotremorine and 8-OH DPAT sensitivity and the parallel changes occurring during selective breeding must be accounted for by mechanisms other than simple changes in receptors.

However, the mechanism must be closely related to both cholinergic and serotonergic systems because, as Experiment 3 demonstrates, there were parallel changes in both oxotremorine- and 8-OH-DPAT–induced hypothermic responses when animals were selectively bred for differences in oxotremorine sensitivity (Fig. 3). This study provided, therefore, confirmatory evidence for the association of 5-HT_{1A} and muscarinic supersensitivity in the FSL rats (42,56,57,64). This experiment also demonstrated that the lines selectively bred for differential hypothermic responses to 8-OH-DPAT, the HDS and LDS rats, are now also differentially sensitive to the muscarinic agonist, oxotremorine (Fig. 4). These parallel changes in muscarinic and 5-HT_{1A} sensitivity during selective breeding for either muscarinic or 5-HT_{1A} sensitivity argue strongly for a common underlying mechanism.

One potential mechanism that could account for the above observation is an alteration in G proteins or in some other aspect of the second-messenger systems. According to various biochemical and molecular studies, both muscarinic M2 receptors and 5-HT_{1A} receptors interact with a Gi protein which contributes to the inhibition of cyclic AMP (38). In contrast, the muscarinic M1 receptor and the 5-HT_2A receptor are positively linked to the phosphatidyl inositol second-messenger system (38). It is not clear at present whether the hypothermic effects of oxotremorine or OH-DPAT are mediated through a Gi protein. However, if they were and these proteins changed as a consequence of selective breeding, then the parallel changes in 5-HT_{1A} and muscarinic sensitivity could be explained.

Furthermore, there is also considerable interest in the possibility that G proteins may be involved in the etiology and phenomenology of depression in humans (3,48), and that alterations in G protein function may accompany chronic treatment with antidepressant drugs (3,32,33). Thus, it is possible that both FSL and HDS rats exhibit exaggerated immobility in the forced swim test and other behavioral analogs of depression because selective breeding for the increased hypothermic responses to their respective drugs has resulted in a similar change in G protein function. An investigation of this hypothesis in the FSL and HDS models of depression may, in turn, help clarify the mechanisms underlying human depression, in particular, the growing body of evidence implicating altered G protein function in affective disorders (3,32,33,48).

This article would not do justice to the impressive literature on depressive disorders if it did not conclude with this cautionary note. Although almost all of the available evidence accumulated to date is consistent with cholinergic supersensitivity in depressive disorders (28), there is a wealth of information suggesting an association of serotonergic subsensitivity with affective disorders, and not serotonergic supersensitivity as indicated above. This serotonergic subsensitivity is most commonly observed as a blunted hormonal response to serotonergic drugs, such as serotonin reuptake inhibitors, in depressed individuals [e.g. (16,65)]. However, a recent review article has indicated that the findings with directly acting 5-HT receptor agonists are much less consistent with respect to hormonal subsensitivity, and has suggested that the apparent subsensitivity can be explained by a reduction in the release of 5-HT, rather than any change in 5-HT receptors (9). At present, there are no data on hormone levels in the FSL and HDS rats after challenges with serotonergic agents, so it is not possible to indicate how closely these animal models resemble depressed individuals with respect to hormonal subsensitivity. Similarly, as indicated above, there is no information on the effects of cholinergic and serotonergic drugs on temperature regulation in humans. Clearly, further studies must be performed before the serotonergic/cholinergic balance hypothesis can be accepted.

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