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A Comparison of Feeding and Locomotion Responses to Serotonin Agonists in Three Rat Strains

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AULAKH, C. S., J. L. HILL AND D. L. MURPHY. *A comparison of feeding and locomotion responses to serotonin agonists in three rat strains.* PHARMACOL BIOCHEM BEHAV 31(3) 567–571, 1988.—The hyperphagic effects of two selective 5-HT_{1A} agonists (8-OHDPAT and buspirone) in a free feeding paradigm and the locomotor suppressant effect of the serotonin agonist, m-chlorophenylpiperazine (m-CPP) were compared in three different rat strains: Wistar, Sprague-Dawley (SD), and Fawn-Hooded (FH) rats. Administration of various doses of 8-OHDPAT and buspirone produced significant increases in two-hour food intake only in Wistar and SD strains and not in the FH strain. Similarly, various doses of m-CPP produced significant decreases in locomotor activity only in Wistar and SD strains and not in the FH strain. Isolated FH animals gained significantly less body weight relative to both Wistar and SD animals. These findings demonstrate attenuated feeding and behavioral responses to serotonergic agonists in the FH strain relative to both Wistar and SD strains.

Fawn-Hooded m-CPP 8-OHDPAT Buspirone Food intake Locomotor activity Subsensitive

THE Fawn-Hooded (FH) rat strain is associated with a hemorrhagic disorder known as platelet storage pool deficiency, a genetic disorder analogous to that in the Chediak-Higashi syndrome of humans (22,27). Blood platelets from FH rats are deficient in storage, release, and uptake of the neurotransmitter serotonin (3,28).

Due to the striking similarities in the manner in which both the brain and platelets store and metabolize serotonin, platelets have been occasionally used as a model for the study of central serotonergic nerve function. Briley and co-workers (7) found that ^{3}H-imipramine (^{3}H-IMI) binding to platelets is similar to that for the brain, they have proposed that the ^{3}H-IMI binding site in both tissues is allosterically related to the serotonin uptake site. Some reports have suggested either absence (13) or diminished (3) ^{3}H-IMI binding sites on platelets and in brain tissue of FH rats, although these findings have been disputed (20,25). On the other hand, Joseph (21) did not observe any significant difference in brain serotonin concentrations between FH and Sprague-Dawley (SD) rats.

Recently, behavioral studies have demonstrated altered responses to serotonin agonists in FH rats relative to Wistar and SD rats (16,30). To extend these observations to other behavioral paradigms, we compared the effects of various doses of 8-OHDPAT, buspirone (5-HT_{1A} agonists) on food intake in freely fed animals and of m-chlorophenylpiperazine (m-CPP, a 5-HT_1 agonist) on locomotor activity in FH, SD, and Wistar rats. In addition, the effects of isolation on body weight gain were also compared in these three rat strains.

METHOD

Male rats of the Wistar, Sprague-Dawley (SD), and Fawn-Hooded (FH) strains weighing approximately 200 g at the beginning of the study were used. They were housed six per cage in temperature (24±1°C) controlled room with a 12-hr light/dark cycle (lights on 7:00 a.m.). The animals had free access to Purina rat chow and water at all times. Separate groups of animals were used for food intake, locomotor, and body weight gain studies.

Food Intake Study

The animals were habituated to eating food pellets placed on the cage floor (single cages) several times before saline or drug injection. On the experimental day, the rats were injected subcutaneously (SC) either with saline or various doses of 8-OHDPAT and immediately transferred to single cages containing a weighed amount of food on the floor. At the end of two hours, the remaining food was weighed; the difference from the original amount constituted the measure of food intake. After a two-week washout period, the same animals were injected SC with various doses of buspirone and two-hour food intake was measured as mentioned before. The food intake study was usually carried out between 10:30 a.m. and 1:00 p.m.

Locomotor Study

Eighteen animals (six animals from each strain) were used in this study. Saline or various doses of m-chlorophenylpipera-

zine (m-CPP) were injected intraperitoneally (IP) 10 minutes before the animals were placed in the activity boxes for locomotor assessment. All animals were injected first with saline followed by various doses of m-CPP with each dose separated by at least 72 hours.

Locomotor activity of individual rats was recorded daily for a period of 30 minutes at the same time of the day (10:00 a.m. to 1:00 p.m.) in the same test cages (Coulbourn Instruments; 30×25×29 cm) each equipped with five photocell detectors which are located 6 cm apart and 2 cm above the grid floor. The test cage was enclosed in a sound proof cubicle with a house light and a fan attached on the back side, and a small window for observation of the animal in the front. Interruptions of the photocell beams were recorded automatically by digital counters. Baseline activity was recorded for 5–7 days for all the animals before the start of drug treatment.

Body Weight Gain Study

Fifteen animals (five animals from each strain) were used in this study. The animals were transferred to single cages with food and water freely available. Their body weights were recorded at weekly intervals for a period of five weeks.

Drugs

m-CPP hydrochloride (Aldrich Chemical Co., WI), 8-OHDPAT HBr (Research Biochemicals Inc., MA), and buspirone HCl (Bristol Myers Co., IN) were all dissolved in saline. The doses of the drugs used in the text refer to salt.

Statistics

The data were analyzed using a variety of analysis of variance techniques. The food intake data were analyzed using two-way analysis of variance followed by post hoc *t*-tests comparing least squares means of the strain and dose treatment combinations. The locomotion data were analyzed using repeated measures design analysis of variance comparing strains across doses. Further analysis examined dose-response within each strain using repeated measures analysis of variance accompanied by contrasts of each dose with the saline control. The effect of isolation on body weight gain of the three strains was determined using an analysis of covariance which permitted the simultaneous testing of slopes and intercepts of the regression lines of the body weight data of the three strains across time. Statistical calculations were made using the General Linear Models Procedure of the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS

Food Intake

Prior to administration of any drug, the two-hour food consumption by rats of all three strains was not appreciably different. Administration of 8-OHDPAT (Fig. 1) and buspirone (Fig. 2) produced dose-related increases in two-hour food intake. The overall drug effect on two-hour food intake was highly significant for 8-OHDPAT, $F(2,45)=13.26$, $p<0.001$, and buspirone, $F(2,45)=8.65$, $p<0.001$. However, comparing within each strain, the overall 8-OHDPAT drug effect was significant in Wistar, $F(2,15)=4.25$, $p<0.05$, and SD, $F(2,15)=9.23$, $p<0.01$, rats only and not in FH, $F(2,15)=2.45$, $p>0.05$, rats. Similarly, the overall buspirone drug effect was significant in Wistar, $F(2,15)=8,24$, $p<0.01$, and SD, $F(2,15)=7.27$, $p<0.01$, rats but not in FH, $F(2,15)=0.79$, $p>0.05$, rats.

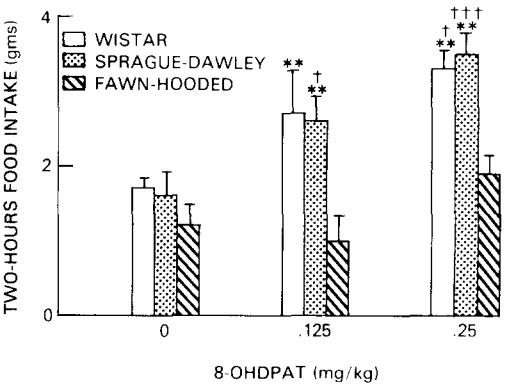

FIG. 1. The effects of various doses of 8-OHDPAT on 2-hour food intake in Wistar (n=6), Sprague-Dawley (n=6), and Fawn-Hooded (n=6) rats. Values are expressed as means±S.E.M. Values of drug-treated animals significantly different from saline-treated animals within each strain are represented by $\dagger p<0.05$; $\dagger\dagger\dagger p<0.001$. Wistar or SD animals significantly different from FH rats at various doses of 8-OHDPAT are represented by $**p<0.01$.

There were also overall significant strain differences for 8-OHDPAT, $F(2,45)=13.21$, $p<0.001$, and buspirone, $F(2,45)=36.6$, $p<0.001$. Further analysis revealed that FH animals consumed significantly less than either Wistar or SD animals at each dose of 8-OHDPAT (Fig. 1) and buspirone (Fig. 2), but there was no significant difference ($p>0.05$) between Wistar and SD strains. The overall interaction (strain × dose) was not significant for 8-OHDPAT, $F(4,45)=1.29$, $p>0.05$, but was significant, $F(4,45)=4.08$, $p<0.01$, for buspirone.

Locomotor Study

Administration of various doses of m-CPP produced dose-related decreases in locomotor activity (Fig. 3). The overall m-CPP drug effect in all three rat strains was highly significant, $F(3,45)=17.46$, $p<0.001$. However, comparing within each strain, the overall m-CPP drug effect was significant only in Wistar, $F(3,20)=3.48$, $p<0.05$, and SD, $F(3,20)=3.59$, $p<0.05$, rats and not in FH, $F(3,20)=1.57$, $p>0.05$, rats. There was neither a significant, $F(2,15)=0.28$, $p>0.05$, strain effect nor a significant, $F(6,45)=1.2$, $p>0.05$, strain × dose interaction.

Body Weight Gain Study

The effect of isolation on body weight gain in three rat strains is shown in Fig. 4. Analysis of covariance showed a significant, $F(2,84)=20.1$, $p<0.001$, strain × time interaction. Further analysis revealed that there was a significant difference in the slopes between the FH animals and Wistar ($t=4.39$, $p<0.001$) or SD ($t=6.16$, $p<0.001$ animals. On the other hand, the slopes for Wistar and SD animals did not differ significantly ($t=1.32$, $p>0.05$) from each other. There was no significant ($t=1.32$, $p>0.05$) difference in the intercepts between the FH and Wistar animals. However, the intercept for SD animals differed significantly from both FH ($t=7.98$, $p<0.001$) and Wistar ($t=9.31$, $p<0.001$) animals due to the fact that SD animals were about 67 g lighter than either Wistar or FH animals at the beginning of the study.

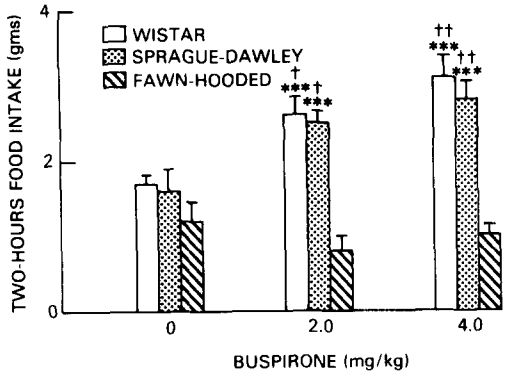

FIG. 2. The effects of various doses of buspirone on 2-hour food intake in Wistar (n=6), Sprague-Dawley (n=6), and Fawn-Hooded (n=6) rats. Values are expressed as means±S.E.M. Values of drug-treated animals significantly different from saline-treated animals within each strain are represented by †$p<0.05$; ††$p<0.01$. Wistar or SD animals significantly different from FH rats at various doses of buspirone are represented by ***$p<0.001$.

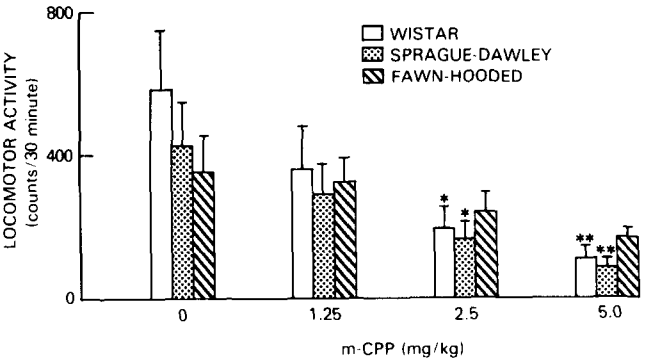

FIG. 3. The effects of various doses of m-CPP on locomotor activity in Wistar (n=6), Sprague-Dawley (n=6), and Fawn-Hooded (n=6) rats. Values are expressed as means±S.E.M. Values of drug-treated animals significantly different from saline-treated animals within each strain are represented by *$p<0.05$; **$p<0.01$.

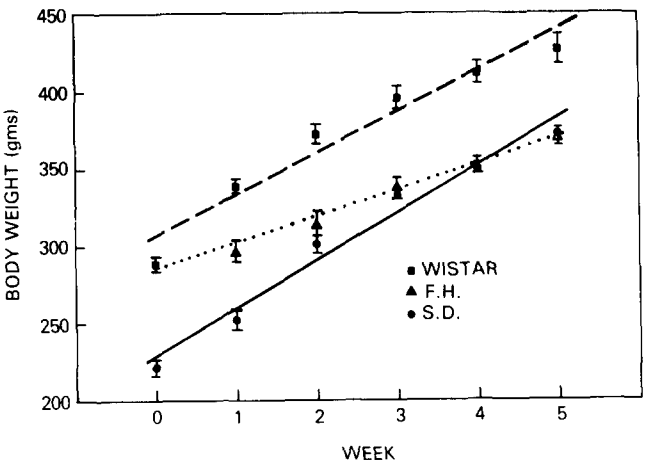

FIG. 4. The effect of isolation on body weight gain in Wistar (n=5), Sprague-Dawley (n=5), and Fawn-Hooded (n=5) rats. Values are expressed as means±S.E.M. The slope for FH animals differed significantly ($p<0.001$) from the slopes for Wistar and SD animals.

DISCUSSION

The demonstration of enhanced food intake in the freely fed animals following administration of serotonin (5-HT) agonists 8-OHDPAT and busiprone in the present study is consistent with previous reports using 8-OHDPAT (11,18) or buspirone (8). Radioligand studies have shown 8-OHDPAT (24) and buspirone (26) to have high affinity for 5-HT_{1A} binding sites. Administration of 8-OHDPAT also induced the "serotonin behavioral syndrome" in rats although the intensity of the syndrome was less at the lower dose. The "serotonin behavioral syndrome" in rats is mediated by activation of postsynaptic 5-HT_{1A} receptors (29). On the other hand, consistent with an earlier report (8), buspirone administration did not induce this syndrome in rats in the present study. Therefore, it is unlikely that enhanced food intake is related to the serotonin behavioral syndrome. Moreover, microinfusions of 8-OHDPAT into the dorsal and medial raphe nuclei increase food intake without producing the syndrome (18). 8-OHDPAT-induced hyperphagia has been suggested to be mediated via activation of 5-HT autoreceptors (18) and buspirone has also been suggested to enhance feeding by a similar mechanism (8).

There is substantial biochemical as well as behavioral evidence in favor of 8-OHDPAT being a presynaptic 5-HT agonist. The specific binding of [^{3}H]-8-OHDPAT in striatum is abolished by 5,7-dihydroxytryptamine (5,7-DHT) lesions (15). 8-OHDPAT has been shown to reduce K^+-evoked release of [^{3}H]-5-HT from cortical and striatal slices (17) although this finding has been disputed (23). In behavioral studies, inhibition of 5-HT synthesis by parachlorophenylalanine (PCPA) has been shown to attenuate 8-OHDPAT-induced hyperphagia but not the serotonin behavioral syndrome (12). 8-OHDPAT-induced hypothermia in rats is mediated by presynaptic 5-HT receptors, since lesioning of 5-HT terminals with 5,7-DHT or depletion of 5-HT stores by PCPA prevents it (14) although this finding has been disputed (19). Administration of 8-OHDPAT stimulates rat sexual behavior (2), whereas other 5-HT drugs which activate postsynaptic 5-HT receptors such as 5-HTP and lisuride decrease sexual behavior (1).

FH rats were found to be less sensitive to the hyperphagic effect of both 8-OHDPAT and buspirone than either Wistar or SD rats. As discussed earlier, 8-OHDPAT and buspirone enhance feeding in freely fed animals via activation of presynaptic 5-HT_{1A} receptors (8,18). Gudelsky *et al.* (16) found FH rats to be less sensitive to the hypothermic effect of 8-OHDPAT relative to SD rats. 8-OHDPAT-induced hypothermia in rats is mediated by activation of 5-HT_{1A} receptors located presynaptically (14) or postsynaptically (19). Thus, the present study suggests that presynaptic 5-HT_{1A} receptors mediating hyperphagia are subsensitive in the FH strain compared to Wistar and SD strains.

Consistent with an earlier report from this laboratory (4), administration of various doses of m-CPP produced dose-related decreases in locomotor activity in the present study. Recently, Curzon and Kennett (10) have suggested that m-CPP causes hypolocomotion by stimulating 5-HT_{1C} receptors. However, more studies with 5-HT antagonists of various

5-HT receptor subtypes are needed to validate this hypothesis. Administration of m-CPP produced significant decreases in locomotor activity only in Wistar and SD strains but not in the FH strain in the present study. In a previous report from this laboratory (30), we have demonstrated FH rats to be less sensitive to the food intake suppressant effects of 5-HT agonists including m-CPP. Serotonin agonists reduce food intake by stimulation of 5-HT_{1B} receptors (6,9). Failure of various doses of m-CPP to decrease locomotor activity significantly in the FH strain relative to both Wistar and SD strains in the present study suggests decreased sensitivity of 5-HT receptors mediating inhibitory effect on locomotor activity. It cannot be due to an m-CPP pharmacokinetic difference since we have demonstrated in a previous report that the brain concentrations of m-CPP were not significantly different in FH animals compared to Wistar or SD animals following IP administration of various doses of m-CPP (5).

It is noteworthy that Wistar and SD rats showed no significant differences to either the hyperphagic effect of various doses of 8-OHDPAT and buspirone or the locomotor suppressant effect of various doses of m-CPP. Both of these strains are widely employed in investigations of central serotonergic function. The comparable behavioral responses of the two strains to 8-OHDPAT, buspirone, and m-CPP in the present study suggests that conclusions about serotonergic function made from one of these strains can be extrapolated to the other.

The present study also demonstrates that when housed individually, FH rats gain significantly less body weight relative to both Wistar and SD rats in spite of free access to food and water at all times. In a previous report from this laboratory (30), we have demonstrated similar effects of isolation in the food-deprived paradigm where animals are given access to food for only four hours daily. Furthermore, separate groups of naive animals were used in the present study, whereas in the previous report, the same animals which were given various doses of 5-HT agonists were evaluated for total body weight gain during the study. Thus, the present study clearly demonstrates a strain difference effect of isolation alone. It is possible that this reduced ability to thrive under the stress of isolation may also be a manifestation of alterations in central serotonergic function in the FH rats.

In summary, this study is consistent with previous published reports demonstrating altered behavioral and feeding responses to serotonergic stimulation in the FH rat strain. Further analysis will seek to identify other altered behavioral responses to serotonergic agents in the FH strain and clarify the biochemical nature of these defects.

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