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Pharmacology, Biochemistry and Behavior 67 (2000) 701–708

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

Selective breeding of 5-HT_{1A} receptor-mediated responses: application to emotion and receptor action

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Accepted 21 July 2000

Abstract

Rat lines that were selectively bred for high (high DPAT-sensitive, HDS) or low (low DPAT-sensitive, LDS) hypothermic responses to the specific 5-HT_{1A} receptor agonist, 8-hydroxy-di-*n*-propylaminotetralin (8-OH-DPAT), differ in receptor binding and certain behaviors related to anxiety and depression. After reviewing this literature, the present communication summarizes new experiments designed to clarify and extend the nature of the pharmacological and biochemical differences between the lines. A challenge with the 5-HT_2 receptor agonist, DOI, produced similar degrees of head shakes and skin crawls in the HDS and LDS rats, suggesting similar sensitivity of 5-HT_{2A} and 5-HT_{2C} receptors. In contrast, DOI-induced flat body posture (FBP), which has been linked to 5-HT_{1A} receptor stimulation, was observed more readily in the HDS rats. The HDS and LDS rats exhibited similar degrees of increase in 8-OH-DPAT-stimulated [^{35}S]GTPγS binding in several brain regions. This result suggests that the dramatic differences in hypothermia in HDS and LDS rats cannot be related to 5-HT_{1A} receptor-mediated action on G proteins. Overall, these findings indicate that the selective breeding for 5-HT_{1A}-mediated hypothermia has been fairly selective, and that differences in emotionally relevant behaviors between these two rat lines can strongly be associated with an unidentified component of the 5-HT_{1A} receptor signaling pathway.

Keywords: 8-OH-DPAT; DOI; 5-HT_{1A}; 5-HT_2; HDS; LDS; Hypothermia; 5-HT receptors; Selective breeding; Agonist-stimulated [^{35}S]GTPγS autoradiography; Review; Behavior

1. Introduction

There is significant evidence from human patients and animal models for a role of the serotonin-1A (5-HT)$_{1A}$ receptor in mental disorders including alcoholism [13,31, 33,47,48,54,55,59], anxiety [12,15,17,18,29], and clinical depression [4,10–12,28,29,36,37,45,52,57]. Genetic modifications of the 5-HT_{1A} receptor offer the potential to provide confirmatory information about the involvement of these receptors in psychiatric disorders. The present communication summarizes information accumulated using a classical selective breeding approach to modify 5-HT_{1A} receptor function. Rats were selectively bred for either high (high DPAT-sensitive, HDS) or low (low DPAT-sensitive, LDS) hypothermic responses to the selective 5-HT_{1A} receptor agonist 8-hydroxy-di-*n*-propylaminotetralin (8-OH-DPAT) [35,40]. The next sections review the establishment of the lines and their behavioral and pharmacological profiles. Next follows description of the results of new experiments designed to assess 5-HT_2 receptor function in the HDS and LDS lines and the role of [^{35}S]GTPγS binding in the 5-HT_{1A} responses. Finally, the behavioral profile of the HDS rat is compared to that of the 5-HT_{1A} knockout mouse. These findings generally support the hypothesis that 5-HT_{1A} receptor abnormalities are associated with anxiety and depression.

1.1. Establishment of HDS and LDS rat lines

The HDS and LDS rat lines were selected from National Institutes of Health (NIH) heterogeneous stock rats [22] using a within-family selection procedure. A moderate dose of the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.5 mg/kg) was chosen to select for rats with either weak or strong hypothermic responses to the drug. From the

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0091-3057/00/$ – see front matter
PII: S0091-3057(00)00415-9

initial population of 30 male and female NIH rats, 10 of each sex were randomly selected to form the randomly bred reference group (random DPAT-sensitive, RDS). Of the remaining 20 rats, the 10 males and females with the largest and smallest hypothermic responses to 8-OH-DPAT, respectively, were randomly mated to establish the HDS and LDS group. For subsequent generations, a strict within-family selection was followed to reduce the risk of fixing genes irrelevant to the hypothermic response to 8-OH-DPAT. The most and least, respectively, hypothermic male and female from each of the 10 litters in the HDS and LDS lines were saved. Mating pairs were not closely related. The RDS line was continued by random selection of progeny within litters and mating pairs that were not closely related [40].

By the fourth generation, the HDS rats substantially diverged from the their HDS and RDS parental means (1.8°C and 1.6°C, respectively) with a decrease of 2.7°C. The LDS rats exhibited a smaller but still significant divergence as well, with a decrease of 1.2°C. It was concluded that selection for this 5-HT_{1A} receptor-mediated response was rapid and likely mediated by one or a limited number of genes [40]. Furthermore, based on the ability of the 5-HT_{1A} receptor antagonists pindolol and WAY100635 but not the 5-HT_7 receptor antagonist ritanserin to antagonize 8-OH-DPAT-induced hypothermia, it is clear that the physiological responses to the agonist is mediated by the 5-HT_{1A} receptor [10,39]

1.2. Correlated behavioral responses

A key purpose of the development of the HDS and LDS lines was to determine whether behavioral differences might emerge in parallel with the changes in 5-HT_{1A} receptor sensitivity. It is well known that conclusions based on a single pair of selected lines can only be tentative. However, several procedures were followed in this selection study to reduce the risk of spurious associations. These included use of family selection, avoidance of close relatives in the mating scheme, recording of behaviors early in the selection process before any potential fixation of irrelevant genes [39,40], and recording of the behavioral measures over several consecutive generations.

The initial behavioral findings suggested that the HDS rats exhibited abnormal behavior related to depression and reward but not to anxiety. Compared with the LDS and RDS rats, the HDS rats were more immobile in the forced swim test, a procedure used to detect antidepressant drugs, and drank more saccharin solution in a two-bottle choice paradigm [39]. However, the three lines did not consistently differ in the open field test of locomotor activity, the elevated plus maze test of anxiety, or alcohol preference tests [39]. Because these tests were carried out in the first eight generations and were consistent from generation to generation, they may represent true behavioral correlations of the 5-HT_{1A} receptor. In general, these results support the literature implicating the 5-HT_{1A} receptor in depression [11,12,45,58]. However, the failure to find differences in the elevated plus maze of anxiety were not consistent with the literature [12,29].

A possible reason for the lack of differences in the plus maze between the HDS and LDS rats is the fact that behavior in this task is not strongly influenced by serotonergic agents [16,20]. Indeed, when the HDS and LDS rats were tested in the social interaction test of anxiety, a task sensitive to serotonergic agents [16,20], the HDS rats interacted much less and consequently were deemed more anxious [21]. This same study confirmed the lack of differences in the elevated plus maze.

1.3. Pharmacological profile of HDS and LDS rats

Since their original generation, a number of experiments have been conducted in HDS and LDS rats to determine responses to pharmacological agents in different models. For example, to extend observations from the forced swim test, experiments with the differential reinforcement of low rate (DRL) 72-s operant schedule (a purported behavioral screen for antidepressant drugs) [10,34] were designed for HDS and LDS animals from the 14th generation. Previous work had shown greater hypothermic responses and high DRL reinforcement rates in Harlan vs. Holtzman Sprague–Dawley rats and greater DRL responses to serotonergic drugs in Holtzman compared to Harlan Sprague–Dawley rats. It was hypothesized, based on these previous observations, that HDS rats would show higher rates of responding than LDS rats and that 8-OH-DPAT would increase responding in LDS rats only. The hypothesis was confirmed, with LDS animals demonstrating lower rates of reinforcement and showing greater improvements in reinforcement rates after treatment with 8-OH-DPAT, ketanserin, and fluoxetine [10] In contrast, the noradrenergic reuptake inhibitor desipramine caused antidepressant-like responding in the DRL test for both HDS and LDS rats. These results suggest that the HDS animals are already responding as if they had received antidepressant drugs at pre-drug baselines, an effect mimicked in the LDS animals only after treatment [10]. Thus, the selection of 8-OH-DPAT sensitivity allows for isolation of antidepressant-like drug effects from HDS animals in the forced swim test and from LDS animals in the DRL test.

Although both HDS and LDS animals exhibit anxiogenic responses in the social interaction test after an acute dose of the serotonin-selective reuptake inhibitor (SSRI) fluoxetine [17], administration of this antidepressant for 14 days prior to testing did not produce an anxiolytic response in the social interaction test in either line. Prior chronic fluoxetine produced an anxiogenic effect in the plus maze in the HDS line but not the LDS line. These observations suggest that an elevation of 5-HT activity can be anxiogenic and differentially expressed based on selective breeding of 5-HT_{1A} responses.

Chronic antidepressant drug treatment normalized responding in two lines of rats exhibiting enhanced immobility in the forced swim test [24,39,46]. In these experiments, both fluoxetine and desipramine administered chronically before testing were found to reduce immobility in the HDS rats while desmethylimipramine reduced the immobility in the Flinders Sensitive Line (FSL), bred originally for supersensitivity to the anticholinesterase DFP. The FSL line also exhibited an enhanced hypothermic response to the 5-HT_{1A} receptor agonist 8-OH-DPAT. Further examination of this potential cholinergic/serotonergic interaction revealed that the muscarinic cholinergic antagonist scopolamine blocked the hypothermic effects of a muscarinic agonist oxotremorine but not 8-OH-DPAT. Furthermore, HDS rats exhibited stronger hypothermic responses to oxotremorine compared to LDS and RDS rats. Finally, separate lines of rats selectively bred for higher hypothermic responses to oxotremorine (high oxotremorine sensitivity — HOS) demonstrated greater hypothermic responses to 8-OH-DPAT. These findings suggest the involvement of a cholinergic/serotonergic interaction in selective breeding for 5-HT_{1A} receptor-mediated hypothermic responses [38].

Administration of serotonergic agents directly to the central nervous system has been accomplished in the HDS and LDS lines in an attempt to confirm the brain's role in the behavioral and hypothermic responses to these drugs. First, 8-OH-DPAT administered intracerebroventricularly induced a strong hypothermic response in HDS rats [25]. The response to 20 μg icv was comparable to the response to the 0.25 mg/kg dose administered subcutaneously. Additionally, when the 5-HT_{1A} receptor antagonist WAY100635 was applied directly to the dorsal hippocampi of these animals, no change in social interaction score was found for HDS or LDS rats. This finding suggests that differences in basal 5-HT tone in the hippocampus do not mediate the social interaction differences between these two lines [21]. However, direct injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT into the dorsal hippocampus revealed an anxiogenic response in the LDS line and no response in the HDS line [17,21]. Chronic fluoxetine did not modify the acute anxiogenic response to intra-dorsal hippocampal 8-OH-DPAT in LDS rats or the lack of response to hippocampal 8-OH-DPAT in HDS rats. These results suggest that the effect of elevating 5-HT tone with fluoxetine is not modifying the hippocampal 5-HT_{1A} receptor in either line.

The initial differences in hypothermia and behavioral responses in the forced swim test and saccharin preference test encouraged investigations into differences in the numbers of 5-HT_{1A} receptors in specific regions of brain in these animals. Autoradiographic analyses of ^{3}H-8-OH-DPAT binding [42] revealed few changes in receptor numbers other than moderately elevated binding in the medial prefrontal, superficial frontal, and deep frontal cortical regions [25]. Correlations between individual hypothermic responses and ^{3}H-8-OH-DPAT binding were significantly positive in these regions, a finding that suggests a role for 5-HT_{1A} receptor number in the hypothermic phenotype. Modest increases in binding of ^{3}H-ketanserin to 5-HT_2 receptors were found only in the superficial frontal cortex. No binding differences were found for these two ligands in the hypothalamus, a region long known to influence body temperature [6,8], hippocampus, dorsal or medial raphe, and many other regions. Therefore, it is unlikely that the hypothermic differences in these lines are explained wholly by differences in receptor numbers in specific regions of the brain.

A simple explanation of the physiological differences among the lines based on monoamine levels is also unlikely. Control data for levels of 8-OH-DPAT in brain tissues did not reveal any differences among the lines in the frontal cortex, hippocampus, mesencephalon, and hypothalamus. The differences appear not to be related to differential pharmacokinetics among the three lines [10]. Furthermore, measures of monoamines in these brain regions were slightly higher only for HVA in the hypothalamus and hippocampus of HDS rats. No other differences in 5-HT, 5-HIAA, dopamine, norepinephrine, and DOPAC were found.

In summary, this review describes the generation and use of 8-OH-DPAT sensitive and insensitive rats in behavioral, physiological, and receptor-based experiments. The evidence provided to date indicates that the HDS animals exhibit strong hypothermic responses to 8-OH-DPAT, modestly higher numbers of 5-HT_{1A} receptors in limited regions of the brain, depression-like responding in the forced swim and DRL-72 tests, and elevated anxiety in tests known to be influenced by serotonin system (i.e., the social interaction test). Although major differences in receptor numbers among brain regions of HDS and LDS animals were not found, it seems clear from the results described above that some aspect of signaling within the 5-HT_{1A} receptor system has been altered by the selective breeding. Two receptor-based mechanisms were targeted for further investigation of their involvement in the differential 8-OH-DPAT-mediated hypothermia. First, since binding of the 5-HT_{1A}-selective ligand ^{3}H-8-OH-DPAT only partially supported the role of receptor number in the hypothermic effects, the effect of selective breeding on 5-HT_{1A} receptor-mediated G-protein activation was investigated. Second, although no extensive differences were found in ketanserin binding in HDS vs. LDS rats, there are reports of functional 5-HT_2/5-HT_{1A} receptor interactions that may play a role independent of receptor numbers [27,30,32]. Thus, a second experiment was designed to test for line-related differences in behavioral responses to the selective 5-$HT_{2A/2C}$ receptor agonist DOI. Classic 5-HT_2 behavioral responses, head shakes and skin crawls, were recorded as was flat body posture (FBP), a classic 5-HT_{1A} behavioral response. It was predicted that if 5-HT_2 receptors were differentially influ-

encing the response to 8-OH-DPAT in the HDS animals as a compensatory response to the selective breeding, then behavioral responses to DOI should be differentially expressed in HDS vs. LDS rats.

2. Materials and methods

2.1. Animals and tissue preparation

Six adult (200–300 g) male HDS, five RDS, and seven LDS rats from generations 13 and 15 were the subjects of the 8-OH-DPAT-stimulated [^{35}S]GTPγS autoradiographic study, while 17 HDS and 16 LDS animals from generations 20 and 23 were used in the DOI behavioral studies. As previously described [39,40], each of these lines was selectively bred from 10 sets of parents obtained from NIH HS (heterogeneous stock) with the greatest/random/least hypothermic responses, respectively, to 0.5 mg/kg sc 8-OH-DPAT as measured 45 min after dosing. Hypothermic responses to 8-OH-DPAT in littermates of the animals used for [^{35}S]GTPγS experiments were 5.54 ± 0.08°C for the HDS, 0.83 ± 0.05°C for the LDS, and 1.75 ± 0.07°C for the RDS rats. Because group hypothermic responses have been stable for more than 15 generations, rats from the generations used in the DOI study were not tested for hypothermic responses. All progeny used in these experiments were maintained in temperature- (22°C) and humidity-controlled environments on a reverse light cycle (lights off from 1000–2200 h).

2.2. Agonist-stimulated [^{35}S]GTPγS autoradiography

Brains from the HDS and LDS lines were rapidly removed after decapitation and frozen in isopentane at −35°C. Twenty-micron coronal sections at several brain levels were cut on a cryostat and thaw mounted onto gelatin coated slides. Alternate sections were collected in triplicate on paired slides to allow processing of basal and 8-OH-DPAT-stimulated [^{35}S]GTPγS binding in adjacent sections. Slides were dried under vacuum and stored dessicated at −80°C until use. Autoradiographic assays of 8-OH-DPAT-stimulated [^{35}S]GTPγS binding were performed as previously described [49,51]. Briefly, slides were incubated in assay buffer (50 mM Tris–HCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, pH 7.4) for 10 min at 25°C, followed by incubation in 2 mM GDP in assay buffer at 25°C for 15 min. Sections were then incubated in 5 μM 8-OH-DPAT with 2 mM GDP and 0.04 nM [^{35}S]GTPγS in assay buffer for 2 h at 25°C. Basal binding was determined in the absence of agonist. Slides were rinsed twice for 2 min each in cold 50 mM Tris buffer and once for 30 s in deionized water and dried overnight. Slides were then exposed to Reflections film in the presence of ^{14}C microscales for 48 h. Films were digitized with a Sony XC-77 video camera and analyzed densitometrically using the NIH IMAGE program for Macintosh Computers. Data are expressed as nCi [^{35}S]/g tissue by using brain paste assay for conversion of ^{14}C to ^{35}S and reported as mean values ± S.E.M. from triplicate sections of five to seven animals [50].

2.3. Drugs and chemicals

[^{35}S]GTPγS (1250 Ci/mmol) and Reflections film were purchased from New England Nuclear (Boston, MA). *R*(+)-8-hydroxy-2(di-*n*-propylamino)tetralin HBr (8-OH-DPAT) was purchased from Research Biochemicals International (Natick, MA). All other chemicals were obtained from Sigma (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

2.4. Behavioral test of DOI

All HDS and LDS used in this experiment received a 1.25 mg/kg ip dose of DOI (in saline) and were immediately placed into a 3 × 3 ft enclosure for observations [3]. Rats were monitored for 30 min, during which a blind observer recorded each head shake (“wet dog shake”) and skin crawls (“paraspinal muscle contractions” or spinal myclonus) [3,43]. FBP was scored in a binary fashion as the presence or absence of the behavior during the monitored period.

2.5. Statistics

[^{35}S]GTPγS binding was expressed as net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding, calculated by subtracting basal [^{35}S]GTPγS binding from 8-OH-DPAT-stimulated [^{35}S]GTPγS binding. Differences in net-8-OH-DPAT-stimulated [^{35}S]GTPγS binding between the HDS and LDS

Table 1
8-OH-DPAT-stimulated [^{35}S]GTPγS binding in select brain regions of HDS and LDS rats[a]

Region	Rat line	DPAT	Basal	Net
Septum	LDS	465 ± 39	241 ± 25	225 ± 22
	HDS	514 ± 18	275 ± 20	239 ± 14
Cingulate Cx	LDS	362 ± 19	237 ± 15	125 ± 10
	HDS	423 ± 15	258 ± 14	165 ± 12
Hypothalamus	LDS	541 ± 20	494 ± 20	47 ± 10
	HDS	588 ± 22	544 ± 25	45 ± 14
Hippocampus	LDS	425 ± 26	263 ± 12	162 ± 30
	HDS	400 ± 26	250 ± 20	150 ± 21
Entorhinal Cx	LDS	377 ± 20	291 ± 16	86 ± 9
	HDS	397 ± 18	299 ± 14	97 ± 9
Dorsal raphe	LDS	469 ± 22	314 ± 17	154 ± 17
	HDS	460 ± 19	342 ± 34	118 ± 16

[a] Data are means ± S.E.M. of five to seven animals expressed as nCi/g tissue for ^{35}S. 8-OH-DPAT stimulated GTPγS binding (Net binding) was calculated by subtracting basal (unstimulated) from DPAT-stimulated values. No statistically significant differences ($P > .05$) were found in Net binding for the two lines of rats within a given brain region (protected *t* tests). LDS, low DPAT-sensitive; HDS, high DPAT-sensitive; DPAT, 8-OH-DPAT; Cx, Cortex.

rats were compared with protected *t* tests. Head shakes and skin crawls were totaled for each line and means compared with *t* tests. A Pearson correlation coefficient with Fishers *r* to *z* was calculated for the relationship between these two measures across all 33 subjects in the HDS and LDS groups. The presence or absence of FBP was compared across groups with chi-square.

3. Results

3.1. Agonist-stimulated [^{35}S]GTPγS autoradiography

Basal and 8-OH-DPAT-stimulated [^{35}S]GTPγS binding were analyzed densitometrically in regions known to contain 5-HT_{1A} receptors (see Table 1). Net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding was particularly high in the septum, hippocampus, dorsal raphe nucleus, and cingulate cortex. In contrast, much lower levels were found in the hypothalamus and entorhinal cortex. Although trends toward lower levels of 8-OH-DPAT-stimulated [^{35}S]GTPγS in the dorsal raphe nucleus and higher levels in the cingulate cortex were found for HDS rats, none of the average net values obtained differed significantly between the groups.

3.2. Behavioral responses to DOI

Three behavioral responses were monitored for 30 min after DOI injection. Head shakes and skin crawls were counted and averaged across the HDS and LDS lines. Each line exhibited 16 and 70 head shakes and skin crawls, respectively; fairly typical numbers for this test. There were no significant differences between the lines on these two measures. Furthermore, these two responses induced by DOI did not correlate with each other (Pearson correlation coefficient of .234 with Fishers *r* to *z* ($P = .19$). Finally, certain of the classic signs of the serotonin syndrome such as Straub tail and reciprocal forepaw treading were not evident in either line. However, a measure of the presence and absence of FBP revealed a strong difference between the lines with most of the HDS animals and few of the LDS exhibiting the behavior. These data are depicted in greater detail in Table 2.

Table 2
Behavioral effects of the 5-HT_2 agonist DOI in HDS and LDS rats

	Head shakes[a]	Skin crawls[a]	FBP[b]
HDS ($n = 17$)	15.2 ± 2.8	70.3 ± 7.2	14
LDS ($n = 16$)	16.1 ± 2.4	71.1 ± 10.4	3

[a] Data are means ± S.E.M. Rats were treated with 1.25 mg/kg DOI and observed for 30 min. *t* tests revealed no differences in either head shakes or skin crawls between the HDS and LDS rats ($P > .05$).

[b] Data are number of animals exhibiting FBP. FBP scores were significantly different from each other based on a chi-square test predicting that equal numbers of animals in each line (half) would exhibit the flat body response ($\chi^2 = 13.5$, $P < .0003$).

4. Discussion

The results of the study show that rats selectively bred for strong or weak hypothermic responses to the 5-HT_{1A} receptor agonist 8-OH-DPAT do not differ in 5-HT_{1A} receptor-stimulated G-protein activity or in classic 5-HT_2 receptor-mediated behaviors. However, FBP, a classic sign of the 5-HT_{1A} receptor-mediated serotonin syndrome, was induced by the 5-HT_2 receptor agonist DOI much more consistently in HDS than LDS rats. The anatomical distribution of 5-HT_{1A} receptor-mediated [^{35}S]GTPγS binding is consistent with previous reports showing that 8-OH-DPAT-stimulated [^{35}S]GTPγS binding is high in regions known to have high levels of 5-HT_{1A} binding [25,51]. Although no changes were found in the overall level of 5-HT_{1A} receptor-activated G-proteins between groups, it is possible that changes occur in coupling to specific type(s) or subtype(s) of G-proteins. This possibility is supported by previous reports of agonist-selective G-protein activation for G-protein-coupled receptors, including 5-HT_{1A} receptors [19]. It is also possible that differences in signal transduction between the rats may be found at the effector level. The behavioral data underscore the specificity of the selective breeding for 5-HT_{1A} receptor-mediated effects. These results also extend previous findings in these animals in which the hypothermic effect of 8-OH-DPAT was most likely centrally mediated [25] and was blocked by the 5-HT_{1A} receptor antagonist pindolol but not by 5-$HT_{2/7}$ receptor antagonist ritanserin [39].

Three logical targets have been investigated in order to elucidate the signaling mechanism(s) responsible for the profound hypothermic differences between the HDS and LDS rat lines: differences in 5-HT_{1A} receptor number and/or affinity, changes in 5-HT_{1A}/5-HT_2 receptor interactions, and 5-HT_{1A} receptor-mediated G-protein coupling. In earlier work, partial support was found for differences in 5-HT_{1A} receptor number, but not in regions thought to regulate body temperature. The possibility exists that projections from regions containing high levels of 5-HT_{1A} receptors in HDS rats may influence the differential hypothermic responses [9,53]. Furthermore, the higher [^{3}H]8-OH-DPAT binding in the medial prefrontal cortex is interesting given the possible role of this region in humans with major affective disorders [2]. Combined with the notion of a limited receptor reserve for 5-HT_{1A} receptors [35,60], the changes in forebrain ^{3}H-8-OH-DPAT binding may in part explain differential anxiety and depression-like behaviors seen in these rats [21,38]. In the present work, interactions of 5-HT_{1A} receptors with 5-HT_2 receptors did not seem likely since no significant differences were found in 5-HT_2-mediated head shakes or skin crawls. However, the FBP component of the postsynaptic 5-HT_{1A} receptor mediated serotonin syndrome was elevated by DOI in the HDS rats. This finding is reminiscent of the somewhat stronger hypothermic response to DOI shown in the HDS animals (unpublished

observations). Thus, although selective breeding for differential 5-HT_{1A} sensitivity does not appear to influence basal 5-HT_{1A} receptor effects on some 5-HT_2 receptor-mediated responses, this selective breeding appears to have influenced the effect of 5-HT_2 receptors on 5-HT_{1A} responses.

Interactions of 5-HT_{1A} and 5-HT_2 receptors may have clinical relevance in depression [4,7] and appear to regulate specific behavioral and physiological responses of rats [26,32,56]. The ratio of 5-HT_{1A}/5-HT_2 receptor number and/or function may have more relevance to this interaction than absolute receptor numbers, and the interaction may differ depending on the measure used. For example, studies of receptor agonists have shown that 5-HT_{1A} and 5-HT_2 receptors interact negatively on locomotor activity, lordosis, and hypothermia [5,27,56], and positively on myoclonus, specific neuroendocrine responses, head shakes, and forepaw treading [5,14,30,43]. Conversely, when endogenous 5-HT tone at 5-$HT_{2A/2C}$ receptors was reduced with ritanserin, 8-OH-DPAT mediated serotonin syndrome was increased [1]. From previous studies of 5-HT_{1A} and 5-HT_2 receptor number in HDS and LDS rats, no consistent differences in the ratio of these receptors could be found in a number of brain regions. Thus, changes in the ratio of receptors are not likely to explain the elevated FBP response found in the present study. Furthermore, when FBP was induced by 8-OH-DPAT in normal rats [43], DOI did not modify the response. Thus, there appears to be a unique interaction of 5-HT_{1A} and 5-HT_2 receptors limited to FBP in HDS and LDS rats. This interaction does not depend on receptor number, but it does depend on basal or endogenous differences in 5-HT_{1A} receptor sensitivity in the absence of 8-OH-DPAT treatment.

The rat model of supersensitivity to 8-OH-DPAT is a unique tool to probe the function of the 5-HT_{1A} receptor and provides for potentially heuristic comparisons with models of 5-HT_{1A} receptor over- or under-expression. Although 5-HT_{1A} receptor-overexpressing mice are not yet available for comparison, the behavioral and physiological responses in the HDS rats compared with knockout mice lacking the 5-HT_{1A} receptor [23,41,44] may be illustrative here. Based upon the lack of 5-HT_{1A} receptors in the knockout mice and elevated receptor sensitivity of HDS rats, one might predict opposite 5-HT_{1A}-mediated behavioral responses in these animals. Table 3, which compares the two models on a variety of behavioral and physiological responses, shows that there are several opposite responses. It would be valuable to obtain the missing information in the two models to assist in interpretations of the functional roles of the 5-HT_{1A} receptor in these models. So far, we can offer the tentative conclusion that the HDS 5-HT_{1A} receptor supersensitivity model and the 5-HT_{1A} receptor knockout model provide complementary information on the role of the 5-HT_{1A} receptor in behavior.

In summary, rats selectively bred for differential hypothermic responses to the 5-HT_{1A} receptor agonist 8-OH-DPAT exhibit no differences in 8-OH-DPAT-stimulated [^{35}S]GTPγS binding and no differential head shake or skin crawl responses to the 5-HT_2 agonist DOI. Furthermore, these data indicate that head shakes and skin crawls are not correlated. This finding suggests that while the head shakes are mediated by 5-HT_{2A}, the skin crawls may be mediated by 5-HT_{2C} and/or other serotonergic receptors. However, DOI induced greater FBP in HDS rats, a finding that suggests an interaction of the 5-HT_2 receptor on 5-HT_{1A} receptor-

Table 3
Behavioral and physiological measures in high 5-HT_{1A} agonist sensitive HDS rats (relative to LDS rats) and 5-HT_{1A} receptor knockout (KO) mice (relative to wild type)

Measure	HDS rats	KO mice
Forced swim mobility	Decreased [39]	Increased [41,44]
Tail suspension mobility	Not tested	Increased [23]
DRL-72	Increased reinforcement [10]	Not tested
Plus/zero maze anxiety	No differences [39]	Anxiety-like [23]
Plus/zero maze activity	Unchanged [39]	Unchanged [23,44]
Active avoidance	Unchanged [39]	Not tested
Hypothermia to DPAT	Strong [10,25,39]	Not tested/reduced [23]
FBP to DOI	Increased (data herein)	Not tested
Head shake/skin crawl to DOI	Unchanged (data herein)	Not tested
Social interaction	Anxiety-like [20]	Not tested
Social interaction, acute fluoxetine	Anxiety-like [17]	Not tested
Open field anxiety	Not tested	Anxiety-like [41,44]
Open field activity	Reduced [17]/unchanged [39]	Reduced [44]/unchanged [23,41]
Novelty suppressed feeding	Not tested	Anxiety-like [61]
Novel object exploration	Not tested	Anxiety-like [23]
Ultrasonic vocalizations	No differences (unpublished)	Not tested
Aggression	Not tested	Less aggressive? [61]
Motor coordination	Not tested	Unchanged [41]
Saccharin intake	Increased [39]	Not tested
Ethanol preference	Unchanged [39]	Not tested

mediated behaviors. These new data combined with previous observations suggest that the selective breeding has led to changes specifically in the 5-HT_{1A} receptor in the brain that may be independent of differences in monoamine levels and downstream of receptor binding and G-protein coupling in regions of the brain thought to mediate hypothermia. Differential activities of adenylate cyclase and/or phosphorylation states of elements within the 5-HT_{1A} receptor signaling cascade between the HDS and LDS lines should be examined for their contribution to these phenotypes. Continued efforts to isolate the mechanism involved should contribute to our understanding of the role of the 5-HT_{1A} receptor in anxiety, depression, and chronic drug exposure.

Acknowledgments

The authors gratefully acknowledge the expert technical assistance of Dana Rotella and Leslie Vogt. These studies were supported by NIDA00287, NIAAA00253, and NIAAA00214.

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