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A brain microdialysis study on 5-HT release in freely moving rat lines selectively bred for differential 5-HT$_{1A}$ receptor function

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Abstract

Breeding for high and low hypothermic responses to systemic administration of a serotonin$_{1A}$ (5-HT$_{1A}$) receptor agonist (8-hydroxy-2-(di-n-propylamino)tetralin, 8-OH-DPAT) has resulted in high DPAT-sensitive (HDS) and low DPAT-sensitive (LDS) lines of rats, respectively. These lines also differ in several behavioral measures associated with stress. In the present microdialysis study we observed that basal 5-HT concentrations in the prefrontal cortex and dorsal hippocampus did not differ significantly between HDS and LDS rats. Thus, behavioral differences between the HDS and LDS lines might not be attributed to differences in basal 5-HT release. However, both lines had lower basal levels of 5-HT release than their randomly bred control group (random DPAT-sensitive, RDS) in the prefrontal cortex (mean ± SEM, pg/20 µl, was 3.0 ± 0.4 for LDS, 3.8 ± 0.3 for HDS and 6.4 ± 0.6 for RDS; $F(2,59) = 5.8$, $P<0.005$). The administration of (±)-fenfluramine (10 mg/kg) induced a greater increase in hippocampal 5-HT levels in HDS rats (500%) as compared with LDS (248%) or RDS (243%) rats ($P<0.0001$). There were no significant differences in the prefrontal cortex among lines, with a fenfluramine-induced 5-HT increase of about 900% in the three groups. This differential response to fenfluramine may be due to functional alterations of hippocampal 5-HT reuptake sites in the HDS line.

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- Fenfluramine
- 8-OH-DPAT

Introduction

The prototypical serotonin$_{1A}$ (5-HT$_{1A}$) receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) induces hypothermic responses in the rat. Selective breeding for high and low 8-OH-DPAT sensitivity to this hypothermic response has led to the establishment of the high DPAT-sensitive (HDS) and low DPAT-sensitive (LDS) lines of rats. HDS rats exhibit greater immobility in the forced swim test than either the LDS rats or the randomly bred control (random DPAT-sensitive, RDS) (1,2). HDS rats consistently exhibit lower social investigation than LDS rats, whereas these lines do not differ in locomotor activity (3). Furthermore, after 14 days of daily handling and injections, the HDS line showed lower exploration of open arms in the elevated plus-maze

than the LDS line but, again, there was no difference in locomotor activity (4). Overall, these studies suggest that these lines respond differently to stress. It is remarkable that the HDS line is more stressed or anxious and also exhibits greater susceptibility to behavioral despair compared to the LDS line (1-4). Autoradiographic studies of the medial prefrontal and cingulated cortices have revealed that HDS rats had more 5-HT_{1A} binding sites than LDS rats (1). Administration of 8-OH-DPAT into the dorsal hippocampus decreased social interaction in the LDS line but not the HDS line, suggesting functional differences in the dorsal hippocampal 5-HT system. However, intrahippocampal administration of the 5-HT_{1A} receptor antagonist, WAY 100,635, had no effect in either line (3). Although these data suggest no line difference in 5-HT release in the hippocampus, the actual measurement of 5-HT levels in the hippocampus is essential to confirm such a notion. Based on these behavioral and neurochemical findings, it was considered of interest to measure basal 5-HT levels in each of these lines and to explore whether the serotonergic systems of HDS and LDS lines react differently to a serotonergic drug challenge. Fenfluramine was chosen because it releases 5-HT from nerve endings by blocking its uptake and by an exocytotic-like mechanism (5,6). Using a brain microdialysis technique in freely moving animals, basal concentrations of 5-HT and changes in its levels induced by systemic injections of fenfluramine (10 mg/kg) were studied in two 5-HT terminal areas, the medial prefrontal cortex and dorsal hippocampus, of these rat lines.

Material and Methods

The HDS and LDS rat lines (12th and 13th generations) and their randomly bred control (RDS) came from the breeding colony at the University of North Carolina Center for Alcohol Studies (2). The animals, weighing 270-350 g, were housed in groups of four and allowed 2 weeks to recover from shipping before surgery. Food and water were freely available and room temperature was maintained at 22-25ºC. Lights were on from 7 pm to 7 am. Rats were anesthetized by *ip* co-administration of ketamine hydrochloride (110 mg/kg) and pentothal (15 mg/kg). A 10-mm long, 21-gauge stainless steel tube was implanted 2.6 mm anterior, 0.5 mm lateral and 1.5 mm ventral (for the prefrontal cortex) and 4.1 mm anterior, 2.4 mm lateral and 1.2 mm ventral (for the dorsal hippocampus) to the bregma, the midsagittal suture and the skull surface, respectively. The guide shaft was attached to the skull with stainless steel screws and acrylic cement. Microdialysis was performed after 7 days of postoperative recovery. On the experimental day, the rats were transferred from their home cage to a novel arena for the microdialysis procedure. The arena consisted of a Perspex cage (37 x 37 x 35 cm) with sawdust on the floor. Food and water were freely available during the experiment. Because 5-HT basal levels are near detection limits (0.2-0.5 pg/20 µl for our HPLC-EC instrument) and it has been previously shown that 5-HT levels are higher during the dark period on a regular 12-h/12-h light-dark cycle (7), dialysis was performed during the dark period.

Laboratory-made microdialysis probes (8) protruded 5 mm out of the tip of the guide shaft. The effective lengths of the cellulose fiber were 2 and 4 mm for the dorsal hippocampus and frontal cortex, respectively. Artificial cerebrospinal fluid (135 mM NaCl, 3.7 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 10 mM $NaHCO_3$, pH 7.4) was injected into the probe with a syringe pump at a flow rate of 1 µl/min.

The HPLC system was a double-piston Water model 510 HPLC pump (Millipore, Waters, CA, USA) with a standard head and a model 7125 valve (Rheodyne Co., Cotati, CA, USA) equipped with a 20-µl loop. Separations were made using a 10-cm long, 3.2-mm bore, ODS Brownlee column containing

3-µm particles (Perkin Elmer, Applied Biosystems, Woburn, MA, USA). The mobile phase was 0.116 M sodium acetate buffer with 100 µM EDTA, 1 mM octanesulfonic acid and 3% acetonitrile (v/v), pH 2.9.

5-HT was detected with a Water model 464 electrochemical detector (Millipore) equipped with a glass carbon electrode, a stainless steel auxiliary electrode and an Ag-AgCl reference electrode. The chemicals were oxidized at 600 mV applied between the working and the reference electrode. The analytes were measured by comparing the peak heights of the samples with standard solutions.

Dialysis perfusion was started during the dark period of the cycle between 8 and 9 am. Samples were taken between 11 am and 4 pm every 20 min and immediately analyzed for 5-HT. After collecting four consecutive samples that showed a stable 5-HT baseline, an *ip* injection of 10 mg/kg (±)-fenfluramine hydrochloride (Sigma, St. Louis, MO, USA) was given to the rat and four further consecutive samples were collected thereafter.

Basal data were calculated as relative to the 5-HT (20 pg/20 µl) standard peak height. Four basal measurements for each rat were subjected to one-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test. Because basal values differed among groups, all data following injections of fenfluramine were normalized to percentage of the mean basal concentrations. To compare changes in 5-HT levels between lines after fenfluramine injection, all normalized data from the three lines were subjected to mixed two-way ANOVA, with time and rat line as the repeated measures and independent factors, respectively. Concentrations at specific time points were compared by the Tukey *post hoc* test.

At the end of the experiment, the tracks of the probes were localized by birefringence on unstained wet brain sections according to the Paxinos and Watson (9) rat brain stereotaxic atlas. Only data from animals with the probe track in the correct position were included in the study (animals/group for the frontal cortex, HDS = 6, LDS = 5, RDS = 5, and for the dorsal hippocampus, HDS = 5, LDS = 5, RDS = 5).

Results

The means ± SEM of 5-HT basal concentrations (pg/20 µl) for the rat lines are shown in Figure 1. One-way ANOVA showed that overall differences between lines for basal concentrations were highly significant for both prefrontal cortex [$F(2,63) = 18.0$, $P<0.0001$] and dorsal hippocampus [$F(2,59) = 5.8$, $P<0.005$]. Multiple comparison tests showed that basal levels for the prefrontal

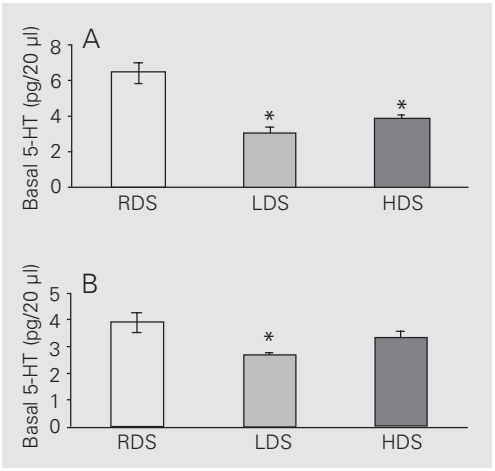

Figure 1. Basal serotonin (5-HT) concentrations. Basal 5-HT concentrations (mean ± SEM, pg/20 µl) of the high DPAT-sensitive (HDS), low DPAT-sensitive (LDS) and random DPAT-sensitive (RDS) lines in dialysates from the prefrontal cortex (A) and dorsal hippocampus (B) obtained during the dark period for 5-6 animals/group. *P<0.05, compared with RDS (Newman-Keuls test).

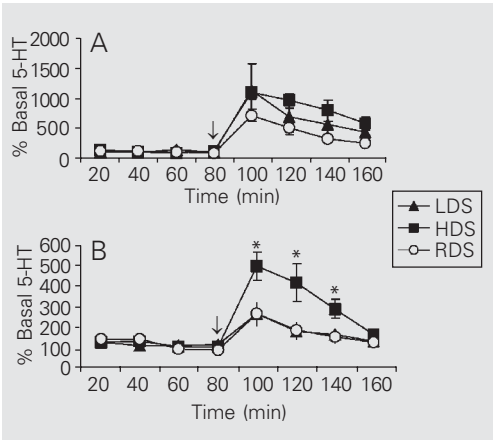

Figure 2. The fenfluramine test. Effect of fenfluramine (10 mg/kg, *ip*, arrow) on serotonin (5-HT) concentration in dialysates obtained during the dark period from the prefrontal cortex (A) and dorsal hippocampus (B) of rats selectively bred for high (HDS), low (LDS) and random (RDS) 8-OH-DPAT sensitivity. Data are reported as percentage (mean ± SEM) of basal concentration for 5-6 animals/group. *P<0.05, HDS compared with either LDS or RDS groups (Tukey test).

cortex did not differ between HDS and LDS, whereas both lines had lower levels than RDS rats (Figure 1A). In contrast, basal concentrations in the dorsal hippocampus only differed between the LDS and the RDS lines (Figure 1B).

Fenfluramine significantly increased extracellular concentrations of 5-HT in both the prefrontal cortex [HDS, $F(7,42) = 25.6$, $P<0.0001$; LDS, $F(7,35) = 4.7$, $P<0.01$; RDS, $F(7,35) = 33.9$, $P<0.0001$] and dorsal hippocampus [HDS, $F(7,35) = 16.3$, $P<0.0001$; LDS, $F(7,35) = 4.8$, $P<0.01$; RDS, $F(7,35) = 9.8$, $P<0.0001$]. The largest increase was observed in sample 5 (first sample after fenfluramine injection) when compared with the four basal samples (*post hoc* test, $P<0.05$).

In the dorsal hippocampus, the lines differed in their response to fenfluramine [time x line factor, $F(14,84) = 7.0$, $P<0.0001$; line factor, $F(2,12) = 20.9$, $P<0.0001$] and the *post hoc* test revealed that the HDS group had higher hippocampal 5-HT concentrations than either the LDS or RDS rats in three consecutive post-injection samples (see Figure 2). In contrast, in the frontal cortex, the repeated measures test did not show significant line differences in response to fenfluramine [time x line factor, $F(14,91) = 1.0$, NS; line factor, $F(2,13) = 0.9$, NS].

Discussion

The fact that basal 5-HT levels were lower in both selectively bred lines (HDS and LDS) compared with the RDS rats indicates that selection for either high or low 8-OH-DPAT sensitivity leads to decreased basal 5-HT levels in the hippocampus and frontal cortex. The basis for this lowered basal 5-HT release in both lines is not known, but might be related to the fact that both lines are more immobile than the RDS rats in the forced swim test (2). The lowered 5-HT levels cannot be related to 5-HT_{1A} receptors or responses to 8-OH-DPAT because HDS rats have greater cortical 5-HT_{1A} binding and greater hypothermic responses to 8-OH-DPAT than LDS rats (1,2).

The present finding of regional differences in fenfluramine-induced release of 5-HT supports previous reports. It has been proposed that there are two anatomically and functionally distinct sets of serotonergic neurons projecting to the forebrain. These systems originate from separate nuclei in the brainstem, the dorsal (DRN) and median (MRN) raphe nuclei and project preferentially to the prefrontal cortex and dorsal hippocampus, respectively (10). There is evidence that these two systems differ in their pharmacological properties such as the vulnerability to the toxic actions of amphetamine derivatives (11). Furthermore, it has been suggested that fenfluramine has a greater acute effect on DRN than MRN terminals. Systemic administration of fenfluramine increased 5-HT concentrations in dialysates collected from the amygdala (a DRN terminal area) but not from the dorsal hippocampus (an MRN terminal area) (12). The increase in 5-HT observed in the three lines (HDS, LDS and RDS) following the fenfluramine challenge was less in the dorsal hippocampus (about 350%) than in the prefrontal cortex (about 900%). Thus, the present findings are consistent with the hypothesis that fenfluramine has a greater action on DRN than on MRN terminal areas.

Pharmacological studies have shown that fenfluramine releases 5-HT from nerve endings by blocking its uptake and by an exocytotic-like mechanism (5,6). Because 8-OH-DPAT, the agent used to select the lines, has been found to have *in vivo* 5-HT uptake blocking properties in the hippocampus (13), numerical and functional differences in 5-HT reuptake sites may underlie the line differences in response to the fenfluramine challenge. Further binding studies are needed to clarify this point. Interestingly, the HDS rats had a comparatively greater increase in 5-HT levels following fenfluramine injection into the dorsal hippocampus (500%)

than either LDS (248%) or RDS rats (243%). Therefore, it is possible that HDS rats have functional alterations of 5-HT reuptake sites in the hippocampus. The HDS rats have been reported to exhibit an abnormal response to 8-OH-DPAT following intrahippocampal administration. Unlike the LDS rats, which exhibit a typical anxiogenic response in the social interaction test, the HDS rats do not (3). Thus, taken together, these results indicate that HDS rats exhibit abnormal responses following intrahippocampal administration of either 8-OH-DPAT or fenfluramine due to functional alterations of 5-HT reuptake sites.

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