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Regional Analysis of 5-HT_{1A} Receptors in Two Species of *Peromyscus*

BRIDGET A. HULIHAN-GIBLIN,*[1] YOUNG D. PARK,* EDWARD B. PIVORUN† AND DAVID GOLDMAN*

**Laboratory of Neurogenetics, National Institute on Alcoholism and Alcohol Abuse, National Institutes of Health, Building 10, Room 3C-216, 9000 Rockville Pike, Bethesda, MD 20892*
†Clemson University, Department of Biological Sciences, 132 Long Hall, Clemson, SC 29634-1903

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HULIHAN-GIBLIN, B. A., Y. D. PARK, E. B. PIVORUN AND D. GOLDMAN. *Regional analysis of 5-HT_{1A} receptors in two species of Peromyscus.* PHARMACOL BIOCHEM BEHAV **45**(1) 143–145, 1993.—Two species of deermice, *Peromyscus maniculatus* (*P. man*) and *Peromyscus leucopus* (*P. leu*), were compared for differences in 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptor number and affinity. Both species enter into torpor; however, *P. man* enters spontaneous torpor with a higher frequency and for a longer duration than *P. leu*. Further, compared to *P. leu* a higher percentage of *P. man* exhibit daily torpor. Deer mice can be induced to enter torpor by a reduction in food supply, shortened photoperiods, and decreasing ambient temperature. Under these conditions, *P. man* enters into torpor more frequently, for longer durations, and with a higher percentage of individuals as compared to *P. leu*. [^{3}H]8-OH-DPAT was used to label 5-HT_{1A} brain receptors in three brain regions: the frontal cortex, brainstem, and striatum. In addition, the hypothalamus and hippocampus were examined for 5-HT_{1A} receptor differences; however, no measurable specific binding could be determined in these regions. In the frontal cortex, the B_{max} values were significantly lower in *P. man* compared to *P. leu*. There were no significant differences in the B_{max} values in the striatum and brainstem between *P. man* and *P. leu*. Further, there were no significant differences in the K_d values between the two species in any of the brain regions examined. The absence of any difference in receptor number or affinity in any of the brain regions examined, except the cortex, suggests that the 5-HT_{1A} receptor is most likely not involved in a more efficient pathway to torpor.

5-HT_{1A} receptor Serotonin Deermouse Torpor

TORPOR is a condition that occurs spontaneously in small mammals and is characterized by a decrease in body temperature, heart rate, and basal metabolism. This decrease allows animals to conserve energy during periods of low food supply. However, unlike in hibernation the decrease in body temperature and metabolism during torpor lasts for only a few hours and is immediately followed by a rapid return to normal body temperature (6,16).

Two southern species of deer mice, *Peromyscus maniculatus* (*P. man*) and *Peromyscus leucopus* (*P. leu*), were compared in this study. *P. man*, found mainly in the mountainous regions of the southeastern United States, displays significantly longer torpor bouts and with a much higher frequency than *P. leu*, which are found in the lower foothills (11). Deermice can be induced to enter torpor by reducing daily food rations, shortening photoperiods to 9 L : 15 D, and reducing ambient temperature to 10°C. Under these conditions, *P. man* enters torpor more readily than *P. leu* (14).

Recent studies suggest that entry into and maintenance of the hibernation state is regulated by increased activity of the serotonergic system (1). Torpor and hibernation are physiologically similar in that they both involve a lowering of body temperature along with a decrease in basal metabolism. During a torpor bout, levels of serotonin [5-hydroxytryptamine (5-HT)], its precursor, 5-hydroxytryptophan (5-HTP), and the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) are elevated in the suprachiasmatic nucleus (8). In addition, there is an increase in 5-HIAA in the median raphe nucleus, caudate putamen (8), and hypothalamus (9) in torpid animals compared to euthermic. Further, there is considerable evidence that the induction of hypothermia in mice is mediated through 5-HT_{1A} receptors. Following administration of 8-OH-DPAT, a known 5-HT_{1A} agonist, there is a dose-dependent decrease in body temperature (2–4,7). In the study presented here, we examined 5-HT_{1A} receptors in the frontal cortex, striatum, hippocampus, hypothalamus, and brain stem in normothermic individuals of *P. man* and *P. leu*.

METHOD

Reagents

5-Hydroxytryptamine creatinine sulfate and pargyline were purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]8-Hydroxy-DPAT (specific activity of 240 Ci/mmol)

[1] To whom requests for reprints should be addressed.

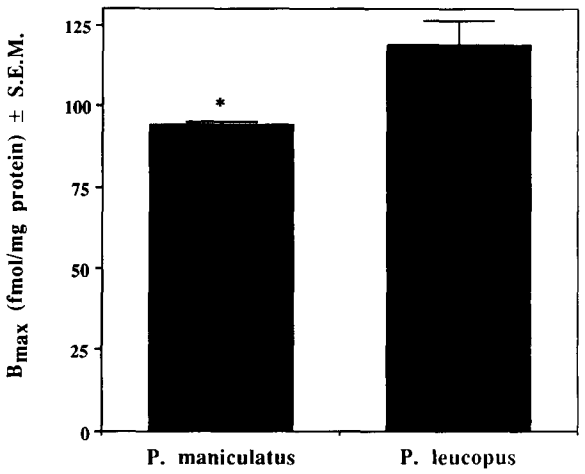

FIG. 1. [^{3}H]8-OH-DPAT was used to label 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor binding sites in the frontal cortex of *Peromyscus maniculatus* (*P. man*) and *Peromyscus leucopus* (*P. leu*). Through Scatchard analysis, it was determined that the B_{max} values were significantly less in *P. man* compared to *P. leu* (*$p < 0.01$). There were no significant differences in the K_d values. $n = 5$.

was purchased from Amersham Life Sciences (Arlington Heights, IL).

Animals

Male *P. man* and *P. leu* were obtained from colonies maintained by The Peromyscus Stock Center, University of South Carolina. The deermice are trapped in the wild in their natural habitat and then bred in a laboratory setting. Animals were killed by decapitation and then the heads, frozen and stored in dry ice, were shipped.

Binding Assay

The 5-HT$_{1A}$ receptor binding assay was performed according to previously described methods (5,12). The whole brain was rapidly removed and the frontal cerebral cortex, hippocampus, striatum, brainstem, and hypothalamus dissected out at 4°C. The brain regions were immediately frozen on dry ice and stored at −70°C. Brain tissue was homogenized on ice in 40 vol ice-cold 50 mM Tris-HCl buffer (pH 7.4), pelleted, and washed again. The homogenates were then incubated at 37°C for 10 min to remove any endogenous serotonin. Centrifugation and homogenization were repeated two additional times and the final resuspension was in 40 vol Tris incubation buffer, which consisted of 50 mM Tris-HCl, 4 mM calcium chloride, 0.1% ascorbic acid, and 10 nM pargyline. The total assay volume of 1.0 ml contained 500 μl Tris incubation buffer, 100 μl [^{3}H]8-Hydroxy-DPAT (between the range of 0.10–12.0 nM), 100 μl 10 μM 5-HT (displacing drug), and 400 μl tissue suspension. Tubes were vortexed after the final addition and assays were allowed to incubate for 30 min at 25°C in the dark. All assays were performed in triplicate. The reaction was terminated by the addition of ice-cold Tris-HCl buffer and was filtered through Whatman GF/C filters (Whatman, Clifton, NJ) using a Brandel 24-well cell harvester. The filters were then immersed in 10 ml Ready Gel (Beckman Instruments, Inc., Fullerton, CA) and counted overnight on a liquid scintillation counter (Beckman). Specific binding was approximately 60% of total binding. Protein concentration was determined by the Lowry et al. method (10).

Calculation and Statistical Analysis

Scatchard analysis was by computer-assisted linear regression written for the Apple Macintosh computer. Comparison of B_{max} and K_d values between species were made using Student's *t*-test (two tailed, unpaired). Probability levels of 0.05 or less were considered statistically significant.

RESULTS

The 5-HT$_{1A}$ receptor was characterized in two species of deer mouse: *P. leu* and *P. man*. Three brain regions (the frontal cortex, striatum, and brainstem) were compared for differences in receptor number and affinity. From Scatchard analysis, it was determined that the density of 5-HT$_{1A}$ binding sites in the frontal cortex, which were labeled by [^{3}H]8-OH-DPAT, was significantly lower in *P. man* as compared to *P. leu* (Fig. 1). The B_{max} value for *P. man* (94 fmol/mg protein) was 27% lower than the B_{max} value for *P. leu* (119 fmol/mg protein). There were no significant differences in B_{max} values between the two species in either the striatum or brain stem (Table 1). Linear regression analysis of the Scatchard plots for the three brain regions examined revealed no significant differences in the K_d (affinity) values between *P. man* and *P. leu*. Attempts to measure 5-HT$_{1A}$ receptor binding in the hypothalamus and hippocampus from both species were unsuccessful due to the low number of specific binding sites.

DISCUSSION

As discussed in the introductory section, several studies have shown that there are differences in daily torpor patterns among two southeastern species of *Peromyscus*. It has been suggested that an increase in brain serotonin may be involved in the entry and maintenance of the torpor state. Other studies have shown that activation of the 5-HT$_{1A}$ receptor may regulate hypothermic effects in mice. To determine if differences

TABLE 1

B_{max} AND K_d VALUES FOR [^{3}H]8-OH-DPAT BINDING IN BRAIN FROM *P. man* AND *P. leu*

Brain Region	*P. man* K_d	*P. leu* K_d	*P. man* B_{max}	*P. leu* B_{max}
Frontal cortex	1.37 ± 0.11	1.37 ± .089	94 ± 0.94	119 ± 7.1*
Striatum	0.26 ± 0.11	0.16 ± .001	85 ± 17.0	74 ± 3.0
Brain stem	0.29 ± .007	0.46 ± .071	38 ± 9.5	33 ± 3.0

The B_{max} (fmol/mg protein) and K_d (nM) values are the mean ± SEM ($n = 5$ for each brain region).

*$p < 0.01$.

in daily torpor patterns may be linked to the activity of specific serotonin receptors, we examined the 5-HT$_{1A}$ receptor in specific brain regions of normothermic *P. man* and *P. leu*.

Behavioral studies have shown that *P. man* experiences longer and more frequent spontaneous bouts of torpor than *P. leu*. This may be an evolutionary adaptive response to the variable temperatures and food availability in its natural mountainous habitat (14). A significant difference was found in the number of 5-HT$_{1A}$ receptors in the frontal cortex comparing the two species of deermice. The greater density of 5-HT$_{1A}$ cortical receptors in *P. leu* compared to *P. man* may be due to less serotonergic activity in this region. Lin and Pivorun (9) reported significant increases in 5-HIAA in the median raphe nucleus during torpor, which suggests an increase in activity of the serotonergic system. In our study, we determined that there are no significant differences in brainstem 5-HT$_{1A}$ receptors between the two species. There is considerable evidence that the 5-HT$_{1A}$ receptors on the cell bodies of neurons in the raphe are autoreceptors (13,15). Therefore, one could hypothesize that in the species that enters torpor more readily and with a higher frequency (*P. man*) there would be fewer 5-HT$_{1A}$ autoreceptors and thus more serotonin released compared to the species that enters torpor less frequently (*P. leu*). Because a difference in 5-HT$_{1A}$ receptors was not observed in the brainstem, the likelihood exists that some other site, such as one of the other 5-HT receptor subtypes and/or the 5-HT uptake site, is involved in determining the difference in torpor proneness between these two species.

Due to the importance of the hypothalamus in temperature regulation, it would be useful to determine if 5-HT$_{1A}$ receptors in deermice can be measured using quantitative autoradiographic techniques. In light of the evidence that the serotonergic system increases in activity in certain brain regions during torpor, future studies will determine the relative importance of other 5-HT receptors as well as the 5-HT uptake site in the regulation of torpor.

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