Pharmacology Biochemistry and Behavior, Vol. 45, pp. 785–789, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Diurnal Rhythms of 5-HT_{1A} and 5-HT_2 Receptor Binding in Euthermic and Torpor Prone Deermice, *Peromyscus maniculatus*

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

**Laboratory of Neurogenetics, NIAAA, NIH Clinical Center, Room 3C-216, 9000 Rockville Pike, Bethesda, MD 20892*
†Department of Biological Sciences, Clemson University, Clemson, SC 29634-1903

Received 13 October 1992

HULIHAN-GIBLIN, B. A., E. B. PIVORUN AND D. GOLDMAN. *Diurnal rhythms of 5-HT_{1A} and 5-HT_2 receptor binding in euthermic and torpor prone deermice, Peromyscus maniculatus.* PHARMACOL BIOCHEM BEHAV **45**(4) 785–789, 1993.—Deermice display both spontaneous and induced daily torpor bouts, attaining minimum body temperatures of 15–20°C. There is evidence that brain serotonin may be involved in the initiation and/or maintenance of torpor. Inhibition of serotonin [5-hydroxytryptamine (5-HT)] synthesis markedly reduces the duration and depth of torpor. Because a certain percentage of deermice will not enter torpor under any circumstances, we were able to compare 5-HT receptor subtypes in deermice that readily enter into torpor (TP) and in non-torpor prone (NTP) animals. Deermice were trapped in the wild and subjected to food rationing and low ambient temperature and then sacrificed either in a normothermic or torpid state at 11:00 p.m. or 11:00 a.m. Whole brain was assayed for 5-HT_{1A} and 5-HT_2 receptor differences using [^{3}H]8-OH-DPAT and [^{3}H]ketanserin, respectively. The B_{max} values for 5-HT_{1A} receptors were significantly greater in both TP and NTP animals sacrificed at 11:00 p.m. compared to animals sacrificed at 11:00 a.m. In contrast, the density of 5-HT_2 receptors was significantly greater in animals sacrificed at 11:00 a.m. compared to animals sacrificed at 11:00 p.m. This is consistent with the opposing functions of these receptors in the regulation of temperature and sleep. The affinity (K_d) of each receptor was unchanged. A comparison of TP and NTP animals sacrificed at the same time of day revealed no significant differences in either B_{max} or in K_d values, indicating that differences in 5-HT_{1A} and 5-HT_2 receptors may not explain the heterogeneity of deermice in their ability to enter torpor. However, the diurnal fluctuation in 5-HT receptors described here may be involved in the serotonergic regulation of hormone rhythmicity and the onset of torpor in deermice.

Deermouse Torpor Serotonin Diurnal rhythms 5-HT_{1A} receptor 5-HT_2 receptor

THE deermouse, *Peromyscus maniculatus,* displays both spontaneous and induced daily torpor bouts. Daily torpor is a regulated decrease in body temperature and activity occurring in several species of small mammals in response to changes in ambient temperature and restriction of food resources. There is considerable evidence that entry into hibernation involves central serotonergic pathways and the manipulation of serotonergic brain levels implicates serotonin [5-hydroxytryptamine (5-HT)] as an essential neuromodulator for hibernation (3). Due to the physiological similarities between hibernation and torpor, it is believed that brain serotonin is also involved in the regulation of daily torpor. Changes in levels of biogenic amines associated with daily torpor bouts have been reported by Lin and Pivorun (12), who found that in the suprachiasmatic nucleus (neuronal circadian pacemaker) 5-HT, its precursor 5-hydroxytryptophan (5-HTP), and the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) are all increased during torpor. Further, a significant increase in 5-HIAA levels in the caudate putamen, median raphe nucleus (12), and hypothalamus (13) was measured in torpid animals compared to euthermic animals.

The serotonergic system has been extensively studied with regard to its involvement in the regulation of circadian rhythms (1,9,10,15). A circadian rhythm for the 5-HT_1 binding site in Wistar rats was found, with maximal binding during the dark phase and minimal binding in the light (20,21). In contrast, the 5-HT_2 receptor exhibits peak binding in Sprague-Dawley rats after lights are on and lowest levels of binding during the dark phase (2). In a related field, it has been reported that 5-HT_2 receptors play an important role in the regulation of sleep. In sleep and wakefulness studies, activation of rat 5-HT_2 receptors has been found to produce an increase in wakefulness and a decrease in deep slow-wave sleep (6).

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

The 5-HT_{1A} and 5-HT_2 receptors appear to be functionally opposed to one another in the regulation of temperature changes. In rats, stimulation of 5-HT_{1A} receptors produced a dose-dependent hypothermia while 5-HT_2 agonists led to a dose-dependent hyperthermia (8). Another case in which these two serotonin receptor subtypes have been reported to be functionally opposed is in food intake studies. Agonists of the 5-HT_{1A} receptor have been shown to stimulate food intake (4,5) while administration of 5-HT agonists presumably stimulating 5-HT_2 receptors causes anorexia (17).

From the aforementioned reports, it is clear that 5-HT receptors are involved in a number of functions critical for the onset of torpor, including the regulation of circadian rhythms, sleep, temperature control, and food intake. The onset of torpor is accompanied by a decrease in body temperature and is induced by food rationing or low food supplies in nature. In addition, torpor occurs at a set time during the day (early morning) and is similar to sleep in that there is a decrease in basal metabolism. In the study presented here, deermice caught in their natural habitat were induced to enter into torpor in the laboratory setting. To gain more information on whether 5-HT receptors are involved in the regulation of torpor, both the 5-HT_{1A} and 5-HT_2 receptors were analyzed for any differences among the non-torpor prone (NTP), torpor-prone (TP), and torpid (T) animals at two time points, 11:00 a.m. and 11:00 p.m.

METHOD

Animals

Deermice (*P. maniculatus*) were trapped in their natural habitats and maintained at room temperatures of 22–25°C until used in this study. Animals of both sexes were transferred to environmental chambers kept at 10°C under a 9 L : 15 D photoperiod (light on at 8:00 a.m. EST). After acclimating to these conditions for 4–6 weeks, individually caged animals were placed on a 3/4 food rationing regimen. Cotton was provided as nesting material and animals had free access to water. Following 1 week of food rationing, animals were sacrificed in either a normothermic or torpid state by cervical dislocation. Body temperatures were measured using a computerized thermometer (Model HH-71T, Omega, Stamford, CT) with a needle probe inserted into the abdominal cavity immediately after cervical dislocation. The criterion for torpor was a core body temperature of less than 30°C. All torpid mice displayed body temperatures between 15–25°C. NTP animals were awake when sacrificed at 11:00 p.m. and 11:00 a.m. Animals were considered NTP when they did not enter into torpor after 1 week of 3/4 food rationing. TP animals were sacrificed at 11:00 p.m. and were awake when sacrificed. T animals were sacrificed in deep torpor at 11:00 a.m. Brains were immediately dissected out, frozen, and stored at −80°C until analyzed.

Radioligand Binding Assay

Tissue preparation. Whole brain was homogenized on ice in 40 vol of 50 mM Tris-Cl buffer, pH 7.4, using a Polytron homogenizer. After the homogenates were centrifuged at 40,000 × *g* for 15 min, the pellets were resuspended in the same volume of buffer, pelleted, and washed again. The homogenates for the 5-HT_{1A} receptor assay were incubated between washes for 10 min at 37°C to remove endogenous serotonin. The final resuspension for the [^{3}H]8-OH-DPAT binding assay was in 40 vol of Tris-Cl buffer containing 10 μM pargyline, 4 mM $CaCl_2$, and 0.1% ascorbic acid. The final tissue concentration for the [^{3}H]ketanserin binding assay was in 80 vol of Tris-Cl buffer.

Reagents

[^{3}H]8-OH-DPAT, specific activity 190 Ci/mmol, was purchased from Amersham (Arlington Heights, IL). [^{3}H]Ketanserin (specific activity 60 Ci/mmol) was obtained from New England Nuclear (Boston, MA). The biochemical reagents, 5-HT creatinine sulfate and pargyline HCl, were from Sigma Chemical Co. (St. Louis, MO). The methysergide maleate was kindly donated by Sandoz Research Institute (East Hanover, NJ).

[^{3}H]8-OH-DPAT binding. The procedure for measuring [^{3}H]8-OH-DPAT binding to 5-HT_{1A} receptors in the brain was adapted from previously described methods (7,16). For Scatchard analysis, the total assay volume of 1.0 ml consisted of 500 μl buffer, 100 μl [^{3}H]8-OH-DPAT (0.19–15.3 nM), 100 μl displacing drug, and 400 μl tissue suspension. The tubes were incubated for 30 min at 25°C in the dark; all assays were run in triplicate. The reaction was terminated by the addition of ice-cold Tris-Cl buffer and the suspension then rapidly filtered through Whatman GF/C filters (Whatman, Clifton, NJ) using a Brandel 24-well cell harvester. The filters were dried and immersed in 10 ml Ready Gel (Beckman Instruments, Inc., Fullerton, CA) and counted overnight on a liquid scintillation counter (Beckman). Specific binding was defined using 10 μM 5-HT and represented 70–80% of total binding.

[^{3}H]Ketanserin binding. The assay for [^{3}H]ketanserin binding to 5-HT_2 receptors was performed according to the method of Leysen et al. (11). The assay tubes were incubated for 15 min at 37°C in the dark and the reaction was terminated as above. At each concentration of [^{3}H]ketanserin (0.17–14.9 nM), 2 μM methysergide was added to half the tubes to determine nonspecific binding; specific binding represented 40–60% of total binding. Protein determinations were by the method of Lowry et al. (14).

Data Analysis

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

RESULTS

NTP deermice were sacrificed at 11:00 p.m. or 11:00 a.m., as were TP animals. However, TP animals sacrificed at 11:00 a.m. are in torpor when sacrificed and are thus referred to as T. 5-HT_{1A} and 5-HT_2 receptor differences were determined in the whole brain using the ligands [^{3}H]8-OH-DPAT and [^{3}H]ketanserin, respectively.

As shown in Fig. 1, the B_{max} values for [^{3}H]8-OH-DPAT binding to 5-HT_{1A} receptors were significantly greater in NTP animals sacrificed at 11:00 p.m. (91 fmol/mg protein) vs. NTP animals sacrificed at 11:00 a.m. (60 fmol/mg protein). A representative Scatchard transformation of [^{3}H]8-OH-DPAT binding in NTP animals sacrificed at 11:00 A. M. vs. those sacrificed at 11:00 p.m. is shown in Fig. 2. TP animals sacrificed at 11:00 p.m. (108 fmol/mg protein) displayed significantly greater B_{max} values compared to both T (69 fmol/mg protein) and NTP (60 fmol/mg protein) animals sacrificed at 11:00 a.m. (Fig. 1).

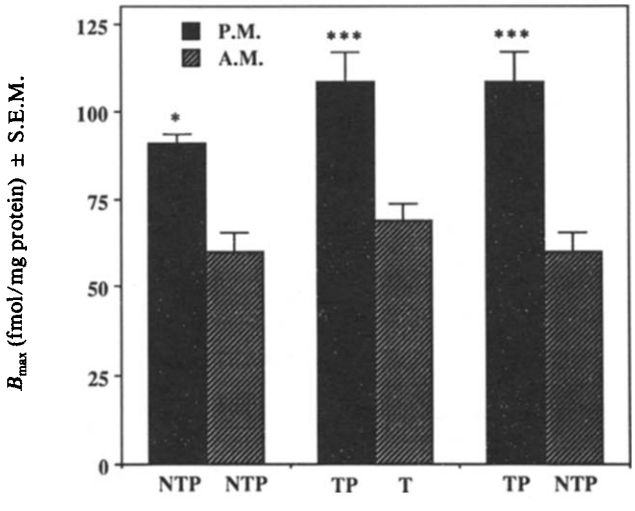

FIG. 1. B_{max} values from Scatchard analysis of [^{3}H]8-OH-DPAT binding to 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptors in deermouse brain. Each bar represents the mean ± SEM from 6–14 animals that were either non-torpor prone (NTP), torpor prone (TP), or torpid (T). Animals sacrificed at 11:00 p.m. (solid bars) had significantly more 5-HT$_{1A}$ receptors than those sacrificed at 11:00 a.m. (hatched bars). *$p < 0.02$; ***$p < 0.001$.

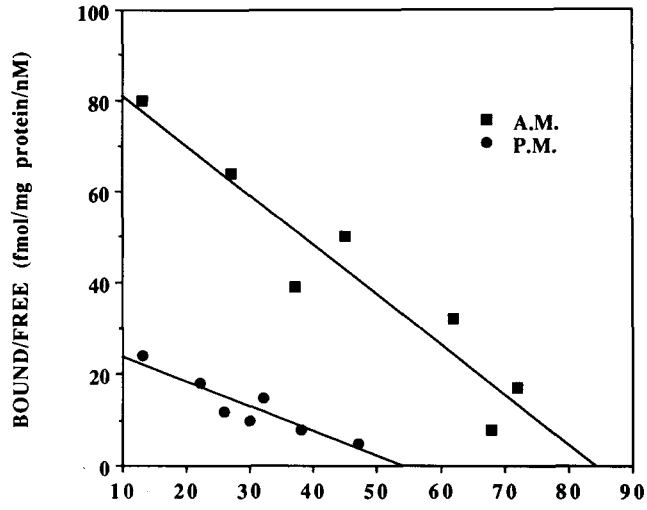

FIG. 3. Representative Scatchard transformation of [^{3}H]ketanserin binding to membranes from non-torpor prone animals in three to five independent experiments. The B_{max} for animals sacrificed at 11:00 p.m. (●) was significantly less compared to the B_{max} for animals sacrificed at 11:00 a.m. (■).

The 5-HT$_2$ receptors also displayed diurnal variation. However, 5-HT$_2$ receptor density varied inversely as compared to 5-HT$_{1A}$ receptor number. Figure 3 is a representative Scatchard plot of [^{3}H]ketanserin binding in NTP animals sacrificed at 11:00 A.M vs. those sacrificed at 11:00 p.m. The B_{max} values for the 5-HT$_2$ receptor were significantly greater in NTP animals sacrificed at 11:00 a.m. (82 fmol/mg protein) compared to NTP animals sacrificed at 11:00 p.m. (48 fmol/mg protein) (Fig. 4). Further, the B_{max} values for NTP animals sacrificed at 11:00 a.m. (82 fmol/mg protein) were significantly greater than the B_{max} values for TP animals sacrificed at 11:00 p. m. (56 fmol/mg protein; Fig. 4).

There were no significant differences in the dissociation constants (K_d values) comparing T, TP, and NTP animals for either the 5-HT$_{1A}$ or 5-HT$_2$ receptors (Table 1). Comparing

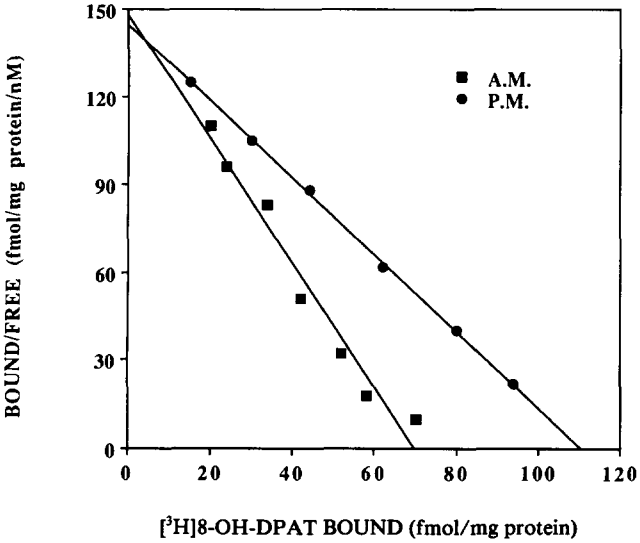

FIG. 2. Scatchard analysis of [^{3}H]8-OH-DPAT binding to membranes from non-torpor prone animals shows that the B_{max} for animals sacrificed at 11:00 a.m. (■) is significantly less compared to the B_{max} for animals sacrificed at 11:00 p.m. (●). The data are representative of three to five independent experiments.

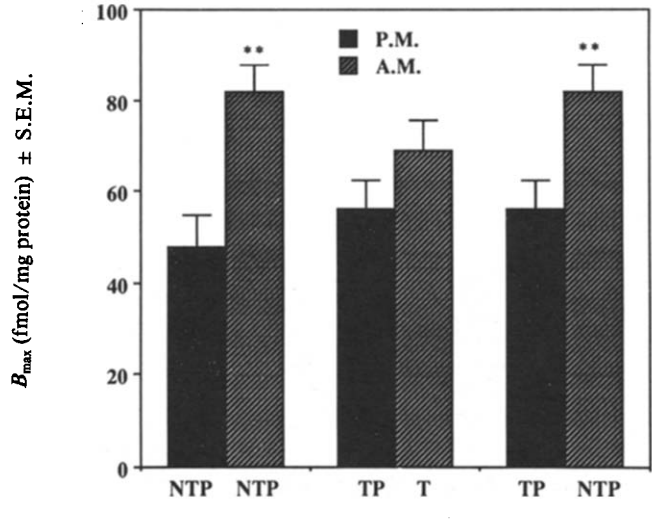

FIG. 4. [^{3}H]Ketanserin was used to label 5-hydroxytryptamine$_2$ (5-HT$_2$) receptors in the deermouse brain. Scatchard analysis revealed a significantly greater number of 5-HT$_2$ receptors in non-torpor prone (NTP) animals sacrificed at 11:00 a.m. (hatched bars) compared to both NTP and torpor-prone (TP) animals sacrificed at 11:00 p.m. (solid bars). Each bar represents the mean ± SEM from six to seven animals that were either NTP, TP, or torpid (T). **$p < 0.01$

TABLE 1

K_d VALUES (nM) FOR [^{3}H]8-OH-DPAT AND [^{3}H]KETANSERIN BINDING IN THE DEERMOUSE BRAIN IN NTP, TP, AND T ANIMALS

	NTP p.m.	TP p.m.	T a.m.	NTP a.m.
5-HT_{1A}	1.18 ± 0.06 (6)	1.64 ± 0.25 (6)	1.47 ± 0.32 (14)	1.93 ± 0.27 (7)
5-HT_2	1.07 ± 0.44 (6)	1.18 ± 0.29 (6)	1.42 ± 0.13 (7)	0.99 ± 0.13 (6)

All values are mean ± SEM. Numbers in parentheses = n. There were no significant differences in K_d values among the four groups in either the 5-HT_{1A} or 5-HT_2 Scatchard analysis.

animals sacrificed at the same time of day (11:00 p.m. or 11:00 a.m.), there were no significant differences in the B_{max} values for either 5-HT_{1A} (Fig. 1) receptors or 5-HT_2 receptors (Fig. 2) between NTP and T deermice. There was a trend for the 5-HT_{1A} receptors in TP animals sacrificed at 11:00 p.m. to be greater than in NTP animals; however, this was not a significant difference.

DISCUSSION

The serotonergic system is believed to be involved in the onset and maintenance of torpor in small mammals. The results obtained in this study demonstrate that there is significant diurnal variation in both 5-HT_{1A} and the 5-HT_2 receptors in the deermouse brain and that the density of these two receptors varies in inverse fashion. Deermice are heterogeneous in their ability to enter torpor. As there were no significant differences in 5-HT_{1A} or 5-HT_2 receptor number or affinity between NTP and TP animals sacrificed at the same time of day, it may be that these receptors do not determine which deermice are torpor prone and which are not. However, given the considerable involvement of the serotonergic system in hibernation and torpor it would be more prudent to compare 5-HT receptors in specific brain regions between TP and NTP animals before a definitive role for serotonin receptors in the regulation of torpor can be determined. Differences in receptor number and/or affinity may be masked in the whole brain but readily measured in discrete brain regions.

Although significant differences in 5-HT receptors were not detected between NTP and TP animals, the marked diurnal variations are consistent with the involvement of the serotonergic system in circadian rhythm. Previous studies in rodents have produced different findings in rats and hamsters vs. laboratory mice, while the deermouse had not been studied. In the rat, both 5-HT_{1A} (20,21) and 5-HT_2 receptor (2) binding exhibit diurnal variation. In addition, 5-HT_{1A} receptors have been found to mediate circadian activity rhythms in both the rat (18) and hamster (19). However, in mice it was found that the functional response to 5-HT_{1A} receptor stimulation does not vary throughout the day (15). In other words, no diurnal variation in the mouse 5-HT_{1A} receptor was observed. These conflicting reports may be due to differences among species and/or specific brain regions. Our results demonstrate that in the deermouse there clearly are significantly more 5-HT_{1A} receptors in the dark phase compared to the light phase. The observation that the density of 5-HT_2 receptors changes in the opposite direction is an indication that this reflects a physiological process in which these two receptors are functionally opposed to one another.

In summary, we detected large, functionally opposed diurnal variation in both 5-HT_{1A} and 5-HT_2 receptors in the deermouse. These findings strongly support a role for the serotonergic system in the regulation of circadian rhythmicity in the deermouse and potential involvement of 5-HT receptors in the entry of deermice into torpor. However, the lack of a difference in whole-brain density of 5-HT_{1A} and 5-HT_2 receptors between TP and NTP animals might indicate that a difference in 5-HT receptors does not account for the heterogeneity of deermice in their ability to enter torpor.

ACKNOWLEDGEMENTS

This work was done while the author held a National Research Council-NIAAA Research Associateship. The authors thank the Sandoz Research Institute for the generous donation of methysergide maleate.

REFERENCES

1. Block, M.; Zucker, I. Circadian rhythms of rat locomotor activity after lesions of the midbrain raphe nuclei. J. Comp. Physiol. 109: 325–347; 1976.
2. Bruinink, A.; Lichtensteigen, W.; Schlumpf, M. Ontogeny of diurnal rhythms of central dopamine, serotonin, and spirodecanone binding sites and motor activity in the rat. Life Sci. 33:31–38; 1983.
3. Canguilhem, B.; Miro, J. L.; Kempf, E.; Schmitt, P. Does serotonin play a role in the entrance into hibernation? Am. J. Physiol. 251:R755–761; 1986.
4. Dourish, C. T.; Cooper, S. J.; Gilbert, F.; Coughlan, J.; Iverson, S. D. The 5-HT_{1A} agonist 8-OH-DPAT increases consumption of palatable wet mash and liquid diets in the rat. Psychopharmacology (Berl.) 94:58–63; 1988.
5. Dourish, C. T.; Hutson, P. H.; Curzon, G. Para-chlorophenylalanine prevents feeding induced by the serotonin agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT). Psychopharmacology (Berl.) 89:467–471; 1986.
6. Dugovic, C.; Wauquier, A.; Leysen, J. E.; Marrannes, R.; Janssen, P. A. J. Functional role of the 5-HT_2 receptors in the regulation of sleep and wakefulness in the rat. Psychopharmacology (Berl.) 97:436–442; 1989.
7. Gozlan, H.; El Mestikawy, S.; Pichat, L.; Glowinski, J.; Hamon, M. Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. Nature 305:140–142, 1983.
8. Gudelsky, G. A.; Koenig, J. I.; Meltzer, H. Y. Thermoregulatory responses to serotonin (5-HT) receptor stimulation in the rat:

Evidence for opposing roles of 5-HT_2 and 5-HT_{1A} receptor. Neuropharmacology 25:1307–1313; 1986.
9. Honma, K.; Wantanabe, K.; Hiroshige, T. Effects of parachlorophenylalanine and 5,6-dihydroxytryptamine on the free-running rhythms of locomotor activity and plasma corticosterone in the rat exposed to continuous light. Brain Res. 169:531–544; 1979.
10. Levine, J. D.; Rosenwasser, A. M.; Yanovski, J. A.; Adler, H. T. Circadian activity rhythms in rats with mid brain raphe lesions. Brain Res. 384:240–249; 1986.
11. Leysen, J. E.; Niemegeers, C. J. E.; Van Nueten, J. M.; Laduron, P. M. [^{3}H]Ketanserin (R41 468) a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. Binding properties, brain distribution, and functional role. Mol. Pharmacol. 21:301–314; 1982.
12. Lin, L. H.; Pivorun, E. B. Analysis of serotonin, dopamine and their metabolites in the caudate putamen, the suprachiasmatic nucleus and the median raphe nucleus of euthermic and torpid deermice, *Peromyscus maniculatus*. Pharmacol. Biochem. Behav. 33:309–314; 1989.
13. Lin, L. H.; Pivorun, E. B. Hypothalamic monoamines and their metabolites in the deermouse, *Peromyscus maniculatus*, during daily torpor. J. Neural Trans. 194:1–9; 1989.
14. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265; 1951.
15. Marsden, C. A.; Martin, K. F.; Webb, A. R. Absence in mice of a diurnal variation in 5-HT_{1A} receptor function. J. Pharm. Pharmacol. 37(suppl.):155P; 1985.
16. Peroutka, S. J. Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT_{1B} and 5H-HT_{1C} binding sites in rat frontal cortex. J. Neurochem. 47:529–540; 1986.
17. Schechter, L. E.; Simansky, K. J. 1-(2,5-Dimethoxy-4-iodophenyl)-2-amino-propane (DOI) exerts an anorexic action that is blocked by 5-HT_2 antagonists in rats. Psychopharmacology (Berl.) 94:342–346; 1988.
18. Shibata, S.; Tsuneyoshi, A.; Hamada, T.; Tominaga, K.; Watanabe, S. Phase-resetting effect of 8-OH-DPAT, a serotonin$_{1A}$ receptor agonist, on the circadian rhythm of firing rate in the rat suprachiasmatic nuclei in vitro. Brain Res. 582:353–356; 1992.
19. Tominaga, K.; Shibata, S.; Ueki, S.; Watanabe, S. Effects of 5-HT_{1A} receptor agonists on the circadian rhythm of wheel-running activity in hamsters. Eur. J. Pharmacol. 214:79–84; 1992.
20. Weseman, W.; Rotsch, M.; Schulz, E.; Sturm, G.; Zofel, P. Circadian rhythm of serotonin binding in rat brain. I. Effect of light–dark cycle. Chronobiol. Int. 3:135–139; 1986.
21. Weseman, W.; Weiner, N.; Hoffman-Bleihauer, P. Modulation of serotonin binding in rat brain by membrane fluidity. Neurochem. Int. 9:447–454; 1986.