Pharmacology Biochemistry & Behavior, Vol. 32, pp. 491–493. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Circadian Rhythm of Serotonin Receptor in Rat Brain

J. AKIYOSHI, H. KURANAGA, K. TSUCHIYAMA AND H. NAGAYAMA

Department of Neuropsychiatry, Medical College of Oita, Hazama-Machi, Oita 879-56, Japan

Received 18 September 1986

AKIYOSHI, J., H. KURANAGA, K. TSUCHIYAMA AND H. NAGAYAMA. *Circadian rhythm of serotonin receptor in rat brain.* PHARMACOL BIOCHEM BEHAV 32(2) 491–493, 1989.—B_{max} and K_d for the serotonin receptors (5-HT-1) as well as the ratios of 5-HT-1A and 5-HT-1B receptors were assessed at 3-hour intervals over a 24-hour period in the cortex of rats that were housed under a 12-hour lighting cycle, with the light turned on at 18:00. The circadian rhythm of the B_{max} for the high- and low-affinity sites in 5-HT-1 receptor became evident. The peak of the B_{max} for the high- and low-affinity sites occurred between 21:00 and 00:00. No circadian rhythm was observed for K_d at each site for the 5-HT-1 receptors. The ratios of B_{max} for the high- and low-affinity sites of the 5-HT-1 receptors were constant at 8.6±1.4% and 91.4±1.4% respectively over the test period. The ratios of 5-HT-1A and 5-HT-1B receptors were constant at 36.8±1.3% and 63.2±1.2% respectively over the test period. No circadian rhythm was observed for K_d. These results suggest that the B_{max} for the 5-HT-1 receptors may have the same circadian rhythm as high- and low-affinity sites and the B_{max} for the 5-HT-1A and 5-HT-1B receptors may also have circadian rhythm.

Circadian rhythm Serotonin 5-HT-1A receptor 5-HT-1B receptor

CHRONOBIOLOGICAL studies revealed the presence of rhythm in various intracerebral receptors. Reports have been made on alpha- and beta-adrenergic, dopamine, opiate, cholinergic and benzodiazepine receptors (6, 7, 10, 15). The only previous study on the rhythm of serotonin (5-HT) receptors is Weseman *et al.* (18), in which rats were submitted to a special procedure to keep unspecific stress at a minimum. They were placed for 30 min/day into the slowly rotating cylinder. We attempted to determine the presence or absence of original rhythm, using a procedure which did not disturb it.

High-affinity cortical binding sites for [^{3}H]-5-HT have been known to exist for many years. Only recently, however, have subtypes of [^{3}H]-5-HT binding sites been recognized. High-affinity [^{3}H]-5-HT binding sites were divided between 5-HT-1 and 5-HT-2 binding sites in rat brain (13). The 5-HT-1 binding sites were further subdivided between 5-HT-1A and 5-HT-1B binding sites in rat brain (12). In this paper the B_{max} and K_d of the 5-HT-1 receptors and the ratios of 5-HT-1A and 5-HT-1B receptors were measured.

METHOD

Male Wistar rats weighing 250–350 g were kept in a semisound-proof room illuminated only by artificial light from 18:00 to 6:00 for 12 hr and were maintained in total darkness for the next 12 hr. The room temperature was kept at 24°C and the humidity at 50%. Free access to food and water was provided. Room cleaning was done at random times. These manipulations were carried out with the aid of dim red lights. The rats were kept for at least 4 weeks under these conditions before any treatment began. They were killed at 3-hr intervals by decapitation. The brain was immediately dissected on ice using the method of Glowinski *et al.* (4), then frozen with liquid nitrogen and preserved at −80°C. Only the cerebral cortex was used for the experiment. The 5-HT-1 binding assay was performed, using [^{3}H]-5-HT. Frozen cortical samples were homogenized by polytron, an ice-cooled 50 mM Tris-HCl buffer solution amounting to 50 times that of the specimen (pH 7.4, at 37°C) and the preparation centrifuged for 15 min at 48,000 × g. The pellet was resuspended in the original volume of Tris-HCl buffer and again centrifuged at 48,000 × g for 15 min. The washed pellet was resuspended in 50 vol. of 50 mM Tris-HCl buffer.

The standard assay buffer consisted of 50 mM Tris-HCl, pH 7.4 containing 4 mM $CaCl_2$, 5.7 mM ascorbic acid and 10 μM pargyline. Membrane pellets were resuspended. 1) In saturation studies of [^{3}H]-5-HT binding, to each assay tube was added 0.15 ml of this preparation (0.2 mg as protein), 0.1 ml of [^{3}H]-5-HT (the final concentration varied from 0.25 to 18 nM) and 0.1 ml of a buffer solution or 5-HT (the final concentration 40 μM) (3). 2) In competition experiments performed in the presence of spiperone (12), to each assay tube was added 0.5 ml of this receptor preparation (0.67 mg as protein), 0.2 ml of [^{3}H]-5-HT (1.6 nM), 0.2 ml of spiperone and 1.1 ml of buffer solution or 5-HT (the final concentration 10 μM) after incubation for 10 min, the sample was passed through Whatman GF/C and washed 3 times with 4 ml of the buffer solution. The filter was moved to a vial containing Aquasol-2, and after being left to stand for 12 hr, measurements were made using a liquid scintillation counter. Protein assay was performed by the Bio-Rad method (1). Three to six animals per group were used. Analysis of the binding curves was performed by Scatchard representation

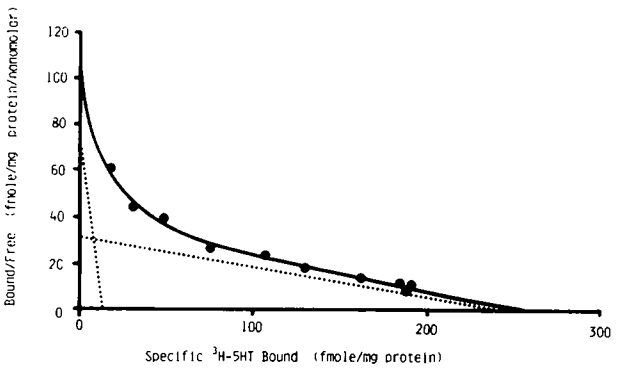

FIG. 1. Scatchard plots of [³H]-5-HT binding to rat cortical membranes. The concentration of [³H]-5-HT ranged from 0.25 to 18 nM. Specific binding is defined as the difference between total binding and that amount bound in the presence of 40 μM unlabelled 5-HT.

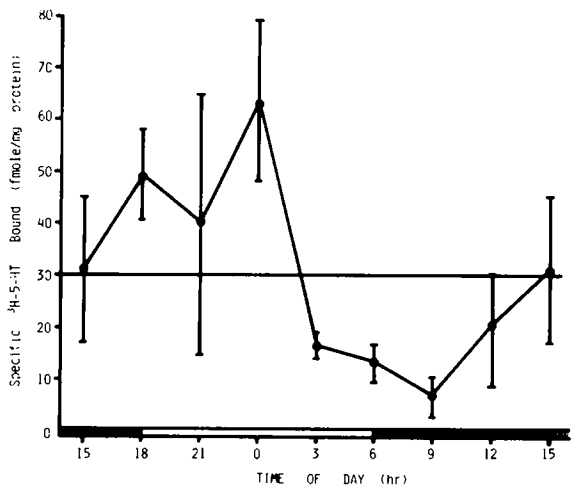

FIG. 2. Circadian rhythm of B_{max} for the high-affinity sites of 5-HT-1 receptor. The light period is 18:00–06:00 and the dark period 06:00–18:00. $F(7,18)=2.772$ by one-way ANOVA ($p<0.05$). The vertical lines extending from each point represent ± 1 SEM.

(16) or by a nonlinear regression analysis (2). 5-HT-1A or 5-HT-1B binding sites were defined as [³H]-5-HT sites which were sensitive to low or high concentration of spiperone, respectively.

RESULTS

The Scatchard plots revealed two distinct types of binding sites (Fig. 1). Each B_{max} for the high- and low-affinity sites of the 5-HT-1 receptor showed a significant circadian rhythm (least-squares method; $p<0.05$) (Figs. 2,3). The peaks of the B_{max} for the high- and low-affinity sites were between 21:00 and 00:00. The ratios of B_{max} for the high- and low-affinity sites of the 5-HT-1 receptors were constant at $8.6\pm1.4\%$ and $91.4\pm1.4\%$ respectively over the test period. No substantial circadian rhythm was found in K_d for the high- and low-affinity sites. The spiperone inhibition of [³H]-5-HT binding showed a biphasic curve (Fig. 4). Nonlinear regression using one- or two-site models supported these conclusions. The ratios of 5-HT-1A and 5-HT-1B receptors were constant at $36.8\pm1.3\%$ and $63.2\pm1.2\%$ respectively over the test period. No substantial circadian rhythm was found in K_d at any site.

DISCUSSION

The major finding of the present study is that the B_{max} for the high- and low-affinity sites of 5-HT-1 receptors showed a circadian rhythm. Most reports on circadian rhythm of the intracerebral 5-HT level in rats are in agreement with the finding that the peak is situated in the light period, with the trough in the dark period. As far as the rhythm in the cerebral cortex is concerned, the peak is in the middle light period (9, 14, 17). Héry *et al.* suggested that the synthesis of 5-HT in the rat brain is maximal during the light period and release is activated during the dark period (5). Thus, the B_{max} for the 5-HT-1 receptor is maximal during the time that the synthesis of 5-HT is activated and minimal during the time that the release of 5-HT is maximal in the cerebral cortex of rats. O'Donnell and Allen reported that the high-affinity receptors for 5-HT in the rat hippocampus, which are of the 5-HT-1 subtype, may be subject to agonist-induced desensitization (11). Therefore, the circadian rhythm of the B_{max} for the 5-HT-1 receptors may be regulated by the release of 5-HT.

The result from Wesemann *et al.* (18) and our group were inverse, i.e., their main peak was in the middle of the dark period and ours in the middle of the light period. The differences in the results may be caused by three experimental variants where the peak affect occurs. They are the animals' prior training, the receptor binding assay, and the time of year during which the experiment was performed. Thus, their rats were subjected to a training program for 1 week to keep unspecific stress at a minimum. We had no such procedure which might disturb the rhythm, in order to confirm the presence or absence of original rhythm. Secondly, they measured only the specific binding, whereas we measured the B_{max} and K_d, factors that are considered to be more important than the specific binding for observation of receptor function. Finally, the time of year of the experiment may cause the conflicting results. Kafka *et al.* reported that both alpha and beta receptor have a circannual rhythms (6). We performed the present experiment in October. The time of year of Wesemann *et al.* experiment is not mentioned in their publication.

Further studies are underway to determine whether this phenomenon means the existence of a cause and effect relation between the two rhythms or the existence of only one common oscillator to regulate the two rhythms (the B_{max} for the 5-HT-1 receptors and the release of 5-HT). It is of interest that the ratios of B_{max} for the high- and low-affinity sites of the 5-HT-1 receptors, and the ratios of 5-HT-1A and 5-HT-1B receptors are constant, despite the existence of circadian rhythm in each component. These results suggest that the B_{max} for the 5-HT-1 receptors may have the same circadian rhythm as high- and low-affinity sites and the B_{max} of the 5-HT-1A and 5-HT-1B receptors may also have circadian rhythm.

ACKNOWLEDGEMENT

This study was supported by Grant No. 84-14 from the National Center for Nervous, Mental and Muscular Disorders (NCNMMD) of the Ministry of Health and Welfare, Japan.

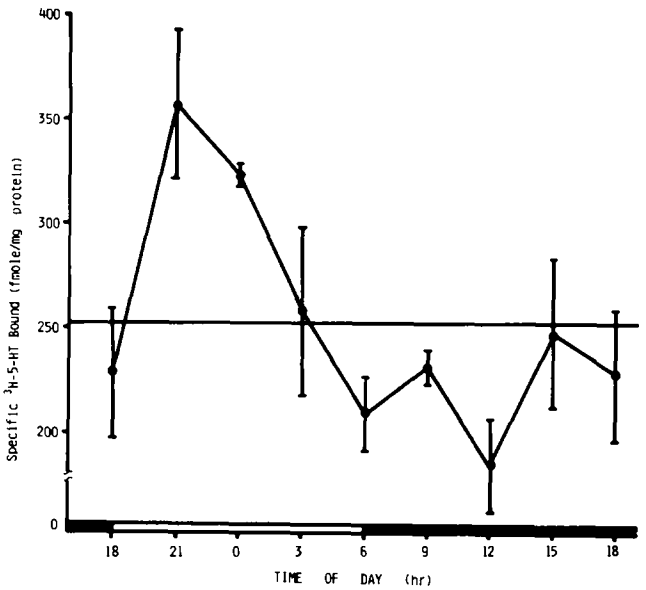

FIG. 3. Circadian rhythm of B_{max} for the low-affinity sites of the 5-HT-1 receptor. The light period is 18:00–06:00 and the dark period, 06:00–18:00. F(7,18)=3.387 by one-way ANOVA ($p<0.05$).

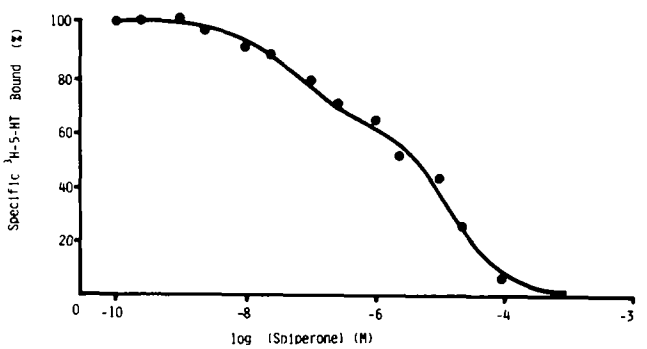

FIG. 4. Spiperone inhibition of specific [³H]-5-HT binding. The curve is theoretical plots derived from a nonlinear regression analysis based on two sites.

REFERENCES

1. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254; 1976.
2. Ehlert, F. J.; Roeske, W. R.; Rosenberger, L. B.; Yamamura, H. I. The influence of guanyl-5'-YL imidodiphosphate and sodium on muscarinic receptor binding in the rat brain and longitudinal muscle of the rat ileum. Life Sci. 26:245–252; 1980.
3. Fleisher, L. N.; Simon, J. R.; Aprison, M. H. A biochemical-behavioral model for studying serotonergic supersensitivity in brain. J. Neurochem. 32:1613–1619; 1979.
4. Glowinski, J.; Iversen, L. L. Regional studies of catecholamines in the rat brain. J. Neurochem. 13:665–669; 1966.
5. Héry, F.; Rouer, E.; Glowinski, J. Daily variations of serotonin metabolism in the rat brain. Brain Res. 43:445–465; 1972.
6. Kafka, M. S.; Wirz-Justice, A.; Naber, D. Circadian and seasonal rhythm in α- and β-adrenergic receptors in the rat brain. Brain Res. 207:409–419; 1981.
7. Kafka, M. S.; Wirz-Justice, A.; Naber, D.; Wehr, T. A. Circadian acetylcholine receptor rhythm in rat brain and its modification by imipramine. Neuropharmacology 20:421–442; 1981.
8. Marsden, C. A.; Martin, K. F.; Webb, A. J. Diurnal variation in 5-HT 1 receptor function. Br. J. Pharmacol. 86:398; 1985.
9. Morgan, W. W.; Pfeil, K. A.; Reiter, R. J.; Gonzales, E. Comparison of changes in tryptophan and serotonin in regions of the hamster and the rat brain over a twenty-four hour period. Brain Res. 117:77–84; 1976.
10. Naber, D.; Wirz-Justice, A.; Kafka, M. S. Circadian rhythm in rat brain opiate receptor. Neurosci. Lett. 21:45–50; 1981.
11. O'Donnel, J.; Allen, M. M. Rapid desensitization of 5-HT-1 receptors by incubation of rat hippocampal slices with agonist in vitro. IRCS Med. Sci. 13:53–54; 1985.
12. Pedigo, N. N.; Yamamura, H. I.; Nelson, D. L. Discrimination of multiple [³H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. J. Neurochem. 36:220–226; 1981.
13. Peroutka, S. J.; Snyder, S. H. Multiple serotonin receptors: differential binding of [³H]serotonin, [³H]lysergic acid diethylamide and [³H]spiroperidol. Mol. Pharmacol. 16:687–699; 1979.
14. Quay, W. B. Regional and circadian differences in cerebral cortical serotonin concentrations. Life Sci. 4:379–384; 1965.
15. Romero, J. A.; Zatz, M.; Kebabian, J. W.; Axelrod, J. Circadian cycles in binding of ³H-alprenolol to β-adrenergic receptor sites in rat pineal. Nature 258:435–436; 1975.
16. Scatchard, G. The attractions of protein for small molecules and ions. Ann. NY Acad. Sci. 51:660–672; 1949.
17. Scheving, L. E.; Harrison, W. H.; Gorden, P.; Pauly, J. E. Daily fluctuation (circadian and ultradian) in biogenic amines of the rat brain. Am. J. Physiol. 214:166–173; 1968.
18. Wesemann, W. W.; Weiner, N.; Rotsch, M.; Schulz, E. Serotonin binding in rat brain: circadian rhythm and effect of sleep deprivation. J. Neural Transm. 18:287–294; 1983.