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Circadian variations in melatonin-binding sites in discrete areas of the male rat brain

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The binding of ^{125}I-melatonin to synaptosomes prepared from whole brains of male rats of the CD strain and from the brain, hypothalamus and striatum of male rats of the Sabra-Wistar strain was assessed throughout a 24 h period. The animals were maintained under a daily schedule of 14 h light (05:00–19:00 h) and 10 h darkness. In whole brain preparations the density of binding sites at 18:00 h was higher by about 70% than at 02:00 h with no variations in apparent affinity of the binding sites throughout the daily period. Specific binding of ^{125}I-melatonin was found in both hypothalamus and striatum of the male rat with a distinct diurnal variation in binding site density in the hypothalamus only. The density of ^{125}I-melatonin-binding sites in the hypothalamus was maximal between 10:00 and 18:00 h and dropped sharply after the lights went off. The apparent ^{125}I-melatonin-binding affinities in these regions were constant and very similar to those in whole brain preparations. The daily variations in densities of ^{125}I-melatonin-binding sites in discrete brain areas may represent a diurnal rhythmicity in the responsiveness of the neuroendocrine axis to melatonin.

Melatonin; Iodomelatonin; Binding site; Circadian rhythm; (Rat brain)

1. INTRODUCTION

The nocturnal synthesis and secretion of melatonin by the pineal gland of mammals results in a distinct light-dark rhythm of melatonin in the blood and other biological fluids (reviews [1,2]). Not only does the daily melatonin level show a cyclic fluctuation, but there is also a pronounced diurnal change in biological response to melatonin. Exogenously administered melatonin exhibits gonadal effects only during certain periods of the day in hamsters [3], white-footed mice [4] and rats [5]. An antigonadotrophic effect is manifested late in the photophase and a counter-antigonadotrophic response is noted in early photophase [6]. The latter coincides with a peak inhibitory effect of melatonin on dopamine release in vitro from the male rat hypothalamus [7]. Hence, the rhythmicity in responsiveness to melatonin does not coincide with the maximal level of melatonin production and secretion [8]. The aim of the present study was to determine whether there is a correlation between melatonin-binding capacity in discrete areas of the brain and the biological responsiveness of the rat to melatonin.

Previous [^{3}H]melatonin-binding studies on membrane preparations from whole rat and hamster brains indicated that there is a diurnal rhythm of melatonin-binding sites, with more sites available near the end of the photophase [9]. These studies could not be replicated or extended however, even by the authors themselves (Cardinali, P., personal communication). We have recently introduced the use of the potent melatonin analog [2-^{125}I]iodomelatonin (^{125}I-melatonin) [10,11] as a melatonin receptor probe [10,12–14]. Here, we have employed ^{125}I-melatonin to investigate the properties of melatonin-binding sites in the male rat brain throughout a 24 h period.

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2. MATERIALS AND METHODS

Male rats of the CD strain (Levinstein's farm, Yokneam, Israel) and of the Sabra-Wistar strain (animal facilities, Hebrew University, Jerusalem) were housed under controlled temperature conditions (24 ± 2°C) and maintained on a daily schedule of 14 h light (cool-white fluorescent illumination; 05:00–17:00 h) and 10 h darkness. Food and water were supplied ad libitum. All rats were 3–4 months old, weighing 190–250 g at the time of experiments. After an acclimatization period of at least 4 weeks, rats were decapitated at 4-h intervals and the brains were rapidly removed. Brain areas were dissected after identification with the aid of the De Groot atlas of the brain [15]. Tissue sections were suspended in 10 ml/g ice-cold 0.32 M sucrose. Crude synaptosomal pellets were prepared as described [10] and suspended in 2 vols of 50 mM Tris-HCl buffer (pH 7.4) containing 4 mM $CaCl_2$. Protein content was determined [16] with bovine serum albumin (Sigma) as a standard.

[2-^{125}I]Iodomelatonin (^{125}I-melatonin) was prepared by iodination of melatonin (Sigma) with Na^{125}I (Amersham) [17]. Aliquots of the synaptosomal preparations (50 μg protein/20 μl) were incubated with 40 μl Tris buffer containing 20–100 nM ^{125}I-melatonin (20 Ci/mmol) for 30 min at 37°C in a shaking water bath, in the absence or presence of unlabeled melatonin (50 μM). Membranes were then collected by vacuum filtration using GF/C glass fiber filters and assayed for radioactivity as in [10]. All samples were run in triplicate and each experiment was repeated 3 times. Specific binding was defined as that displaced by 50 μM non-radioactive melatonin and ranged from 50 to 60% of the total binding at 50 nM ^{125}I-melatonin. Scatchard analysis of the equilibrium binding data was employed to obtain the apparent dissociation constants (K_d) and the binding site densities (B_{max}). The data obtained from each experiment were analyzed separately and means and SD values of K_d and B_{max} obtained are presented (n = 3). The binding parameters were compared by an analysis of variance followed by Student's t-test for differences among multiple means. Significance was determined at $p < 0.05$.

3. RESULTS

The concentration dependency of ^{125}I-melatonin binding was assessed in synaptosomal preparations from whole brains of male rats of the CD strain (fig.1). Specific binding at equilibrium increased greatly at low and slightly at higher concentrations of ^{125}I-melatonin approaching saturation (fig.1A). Scatchard plots of the data obtained in the equilibrium experiments (fig.1A, inset) were compatible with the simplest model of a single class of

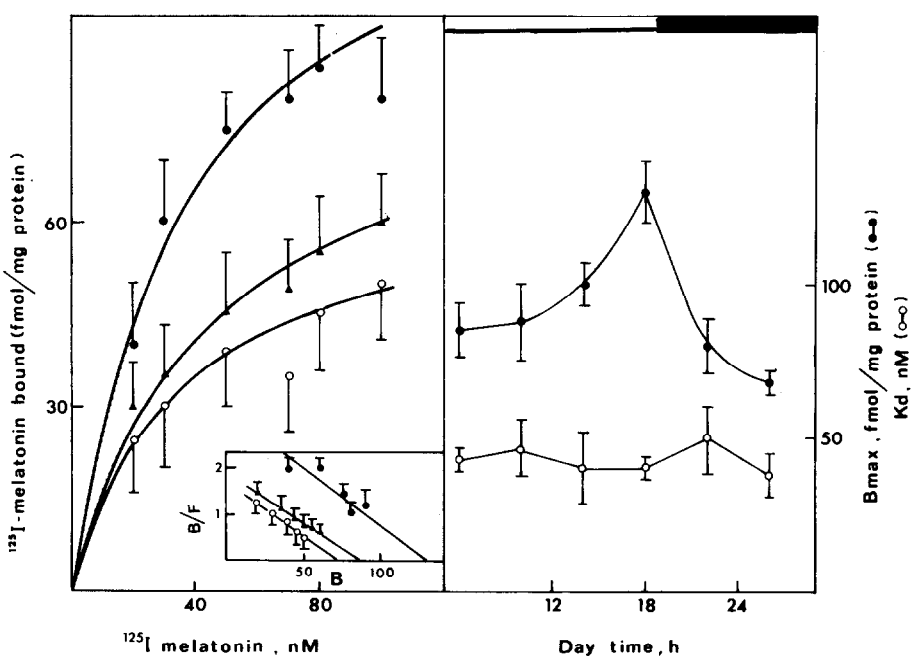

Fig.1. ^{125}I-melatonin binding to synaptosomal preparations from rat brain as a function of ^{125}I-melatonin concentration. Synaptosomes were prepared from the brains of male rats of the CD strain at different hours of the day. (Left) Specific binding at 02:00 (○), 10:00 (▲) and 18:00 h (●). The solid curves are theoretical curves reconstructed from the K_d and B_{max} values obtained from the Scatchard plots (inset) presenting the ratio of bound/free (B/F) ligand as a function of bound (B) ligand. (Right) Equilibrium binding parameters of ^{125}I-melatonin-binding sites at different hours of the day. The parameters were obtained by Scatchard analyses as in (A). The periods during which the animals were kept in light and darkness over the 24 h cycle are indicated.

binding sites for ^{125}I-melatonin with mean dissociation constants (K_d) around 40 nM which did not change significantly at the different hours (fig.1B). The maximal ^{125}I-melatonin-binding capacity varied significantly throughout the day, reaching its peak at 18:00 h. Similar results were obtained with synaptosomes from whole brains of male rats of the Sabra-Wistar strain (not shown). The mean dissociation constant of the ^{125}I-melatonin-binding sites in the Sabra-Wistar rats (84 nM, figs 2,3) was somewhat higher than that observed in the CD strain (fig.1A, inset) presumably due to subtle strain-specific differences in circulating hormones [12,13].

We have furthermore assessed ^{125}I-melatonin binding in synaptosomal preparations from the hypothalamus and striatum of the male Sabra-Wistar rats at 4-h intervals (figs 2,3). The binding of ^{125}I-melatonin to synaptosomes from the hypothalamus (fig.2A) increased with increasing ligand concentration, approaching saturation. Scatchard plots of the data obtained in the equilibrium experiments were compatible with the simplest model of a single class of binding sites for ^{125}I-melatonin with mean dissociation constants (K_d) around 80 nM which did not change significantly at the different hours (figs 2B,3).

Maximal binding capacity in the hypothalamus varied significantly over the 24 h period: the densi-

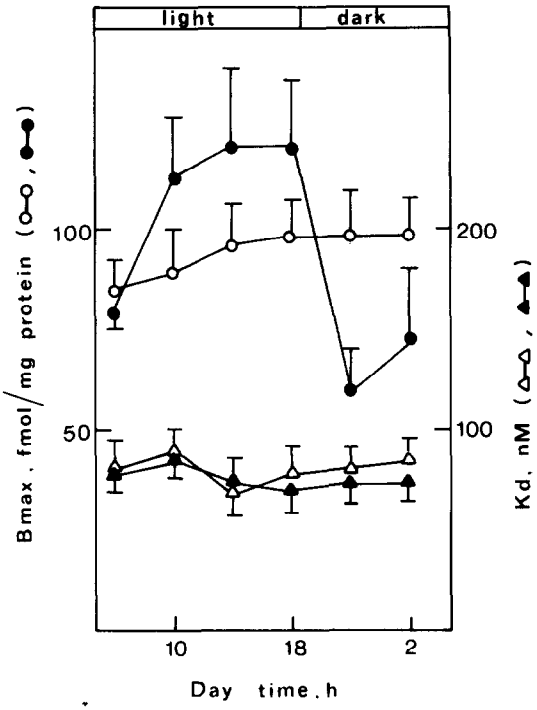

Fig.3. Equilibrium binding parameters of ^{125}I-melatonin-binding sites at different hours of the day. Specific binding of ^{125}I-melatonin to synaptosomes prepared from the hypothalamus (●,▲) and striatum (○,△) of male rats of the Sabra-Wistar strain, at different times of the day, was assessed. The K_d and B_{max} values were obtained by Scatchard analyses of the data. Means ± SD values obtained from 3 repetitive experiments are presented.

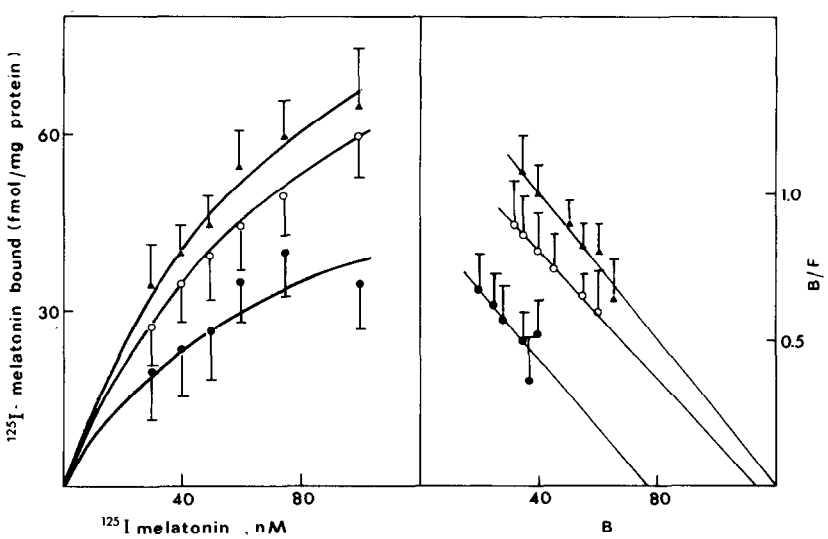

Fig.2. ^{125}I-melatonin binding to synaptosomal preparations from the hypothalamus of male rats of the Sabra-Wistar strain as a function of ^{125}I-melatonin concentration. (Left) Specific binding at 02:00 (●), 10:00 (○) and 18:00 h (▲). The solid lines are theoretical curves reconstructed from the K_d and B_{max} values obtained from Scatchard analysis of the same data (right).

ty of binding sites (B_{max}, fmol/mg protein) was maximal between 10:00 (113 ± 10) and 18:00 h (120 ± 23) and dropped after lights-off, reaching minimal values at 22:00 h (59 ± 11) which persisted throughout the dark period (fig.3). These variations were not accompanied by changes in the apparent K_d values (fig.3). ^{125}I-melatonin-binding sites were also found in the striatum. The densities and apparent affinities of these sites did not vary significantly over the 24 h period (fig.3).

4. DISCUSSION

The data demonstrate the presence of ^{125}I-melatonin-binding sites in the hypothalamus and striatum of the male as previously found in the female rat [12], thus qualitatively confirming the results obtained with [^{3}H]melatonin [9,18]. The presence of melatonin-binding sites in the hypothalamus may have a physiological relevance, since a number of studies have implicated the hypothalamus as the main site of melatonin's neuroendocrine activities [1,2]. The role of the striatal ^{125}I-melatonin-binding sites is still an enigma.

The daily variations in melatonin-binding sites in the hypothalamus might be the consequence of changes in the endogenous content of the hormone or of down-regulation of melatonin-binding sites by the elevated nocturnal melatonin. The regional specificity (e.g. hypothalamus but not striatum) of the rhythms and the fact that the binding site density in the hypothalamus declines 5–7 h before the circulating melatonin level reportedly reaches its nocturnal peak [2] contradict this possibility. Circadian rhythms have been observed in a number of hormone and neurotransmitter receptors in the rat brain [19,20]. In accordance with the results obtained in this study, such rhythms very frequently represented changes in the apparent number and not in affinity of the receptors. There is a considerable difference in wave form and in peak phase position of the melatonin-binding sites in the hypothalamus and in whole brain preparations: while the density in the hypothalamus is maximal between 10:00 and 18:00 h the value in the whole brain is maximal only between 14:00 and 18:00 h. Hence, the density of melatonin-binding sites in some brain besides the hypothalamus probably increases towards the end of the photophase.

The diurnal variations in the density of melatonin-binding sites at specific brain regions may underlie the physiological rhythms in the response of the neuroendocrine system to the hormone. The broad peak in the density of sites in the hypothalamus overlapped the phase of maximal antigonadal response to exogenously injected melatonin (i.e. late in the photophase [3–5]) and of a counter-antigonadal effect (i.e. early in the photophase [6]) which coincides with the phase of maximal sensitivity of the dopamine-releasing system in the hypothalamus to melatonin observed in vitro [7]. The similarity in apparent K_d values of the melatonin-binding sites in the hypothalamus at 10:00 and 18:00 h may indicate that the same type of sites is responsible for the antigonadotrophic and counter-antigonadotrophic effects and for the activity of melatonin on the hypothalamic neurosecretory system. Further experimental effort will be required in order to localize within the hypothalamus the sites involved in the different functions of melatonin.

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REFERENCES

[1] Reiter, R.J. (1980) Endocr. Rev. 1, 109–130.
[2] Tamarkin, L., Baird, C.J. and Almeida, O.F.X. (1985) Science 227, 714–720.
[3] Tamarkin, L., Westrom, W.K., Hamill, A.L. and Goldman, B.D. (1976) Endocrinology 99, 1534–1541.
[4] Glass, J.D. and Lynch, G.R. (1982) Neuroendocrinology 35, 117–122.
[5] Reiter, R.J., Petterborg, L.J., Trakulrungsi, C. and Trakulrungsi, W.K. (1980) J. Exp. Zool. 212, 47–52.
[6] Chen, H.J., Brainard, G.C. and Reiter, R.J. (1980) Neuroendocrinology 31, 129–132.
[7] Zisapel, N., Egozi, Y. and Laudon, M. (1985) Neuroendocrinology 40, 102–108.
[8] Reiter, R.J., Blask, D.E., Johnson, Y.L., Rudeen, P.K., Vaughan, M.K. and Waring, P.J. (1976) Neuroendocrinology 22, 107–116.
[9] Vacas, M.I. and Cardinali, D.P. (1979) Neurosci. Lett. 15, 259–263.
[10] Laudon, M. and Zisapel, N. (1986) FEBS Lett. 197, 9–12.
[11] Weaver, D.R., Namboodiri, M.A.A. and Reppert, S.M. (1988) FEBS Lett. 228, 123–127.
[12] Laudon, M. and Zisapel, N. (1987) Brain Res. 402, 146–150.

[13] Zisapel, N., Shaharabani, M. and Laudon, M. (1987) Neuroendocrinology 46, 207–216.
[14] Laudon, M., Yaron, Z. and Zisapel, N. (1988) J. Endocrinol. 116, 43–53.
[15] De Groot, J. (1972) Verh. K. Ned. Akad. Wet. Ser. II, 52.
[16] Markwell, M.A.K., Haas, S.M., Bieber, L.C. and Talbert, N.E. (1978) Anal. Biochem. 87, 206–210.
[17] Vakkuri, O., Lamsa, E., Rahkamaa, E., Ruotsalainen, H. and Leppaluoto, J. (1984) Anal. Biochem. 142, 284–289.
[18] Niles, L.P., Wong, Y.W., Mishra, R.K. and Brown, G.M. (1979) Eur. J. Pharmacol. 55, 219–225.
[19] Wilson, M.A., Clark, A.S., Clyde, V. and Roy, E.J. (1983) Neuroendocrinology 37, 14–22.
[20] Wirz-Justice, A. (1987) Prog. Neurobiol. 29, 219–259.