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Daily variations in in vivo tryptophan hydroxylation and in the contents of serotonin and 5-hydroxyindoleacetic acid in discrete brain areas of the rat

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Summary. The in vivo rate of brain tryptophan hydroxylation was determined through 5-hydroxytryptophan accumulation (5-HTPacc) following the administration of NSD 1015, a L-aromatic amino-acid decarboxylase inhibitor. This measurement was performed every 4 h throughout a 24 h hour period in 10 discrete brain areas of rats maintained on a regular 12 h/12 h light-dark cycle. The concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were also determined in untreated rats. Daily variations in 5-HTPacc were found in all the areas studied, the 5-HTPacc being higher during the dark period in most structures. These results strongly suggest that tryptophan hydroxylation is involved in the control of the 5-HT biosynthesis circadian rhythm. However, various patterns of 5-HT and 5-HIAA daily variations were observed, suggesting that the circadian factors affecting serotonin metabolism can be different among brain areas.

Keywords: Tryptophan hydroxylase, serotonin, 5-hydroxyindoleacetic acid, circadian rhythms, brain.

Introduction

Experimental studies have suggested the involvement of central serotoninergic neurons in the modulation of circadian rhythms. First, the suprachiasmatic nuclei (SCN), which are one of the major circadian pacemakers (Meijer and Rietveld, 1989), receive a serotoninergic innervation which originates from the midbrain raphe nuclei (Azmitia and Segal, 1978; Moore et al., 1978; Van de Kar and Lorens, 1979). Second, electrolytic lesions of the midbrain raphe nuclei, as well as the destruction of the central serotoninergic system by the neurotoxin 5,7 dihydroxytryptamine, alters the circadian expression of locomotor activity, sleep-wake cycle, plasma ACTH and corticosterone levels (Szafarczyk et al.,

1981; Levine et al., 1986; Louis-Coindet, 1980; Banky et al., 1988; Smale et al., 1990). Furthermore, serotoninergic agonists can shift the circadian rhythm of spontaneous SCN electrical activity in the rat in vitro (Prosser et al., 1990; Medanic and Gillette, 1992).

Circadian variations in several biochemical parameters of central serotoninergic neurons have been reported (Martin, 1991). Circadian rhythms of serotonin (5-HT) levels have been described in brain regions of rats housed on a regular light-dark cycle (Quay, 1968; Héry et al., 1972, 1977; Agren et al., 1986). These rhythms of 5-HT levels may be related to changes in 5-HT biosynthesis since the in vivo rate of formation of [^{3}H]5-HT from [^{3}H]tryptophan exhibits daily variations (Héry et al., 1972). Such circadian rhythms in 5-HT biosynthesis could result from fluctuations in tryptophan hydroxylation which is the first and rate-limiting step in 5-HT biosynthesis and represents an optimal site for the regulation of serotoninergic neurons (Hamon and Glowinski, 1974).

Indeed, circadian variations in brain tryptophan hydroxylase (TrH) activity, the enzyme catalyzing the conversion of tryptophan to 5-hydroxytryptophan (5-HTP), have been reported (Kan et al., 1977; Cahill and Ehret, 1981). Other studies, on the contrary, have failed to demonstrate any rhythmicity in the activity of this enzyme (McLennan and Lees, 1978; Brown et al., 1982). Although, the reasons for these discrepancies are unclear, the activity of tryptophan hydroxylase in all of these studies was determined in vitro, under optimal concentrations of substrate and cofactors and therefore may not fully reflect the actual in vivo rate of tryptophan hydroxylation (Meek and Lofstrandh, 1976; Bourgoin et al., 1980). DiRaddo and Kellogg (1975), on the other hand, reported a trend for an increased in vivo rate of tryptophan hydroxylation in the rat brainstem and diencephalon during the dark period of the nycthemer. These variations, however, were not significant. This study involved large brain regions and would have missed local changes, since it has been shown that the rhythms of in vitro TrH activity can be different among brain nuclei (Kan et al., 1977). It is thus presently difficult to state the involvement of the tryptophan hydroxylation step and the role of TrH in the modulation of the 5-HT biosynthesis circadian rhythm.

The present study was undertaken to determine whether the in vivo rate of tryptophan hydroxylation undergoes daily variation and therefore contributes to the circadian rhythm of 5-HT biosynthesis. The rate of tryptophan hydroxylation was determined by measuring the in vivo accumulation of 5-HTP after pharmacological inhibition of L-aromatic amino acid decarboxylase (the enzyme converting 5-HTP to 5-HT) (Carlsson et al., 1972; Tappaz and Pujol, 1980). This measurement was made at several times during the nycthemer in discrete brain areas of rats maintained on a regular 12 h/12 h light-dark cycle. In order to further investigate the rhythmicity of 5-HT metabolism, the levels of 5-HT and of its metabolite, 5 hydroxyindoleacetic acid (5-HIAA), were also measured.

Materials and methods

Animals

Male OFA rats (IFFA CREDO, Saint-Germain-sur-l'Arbresle, France) weighing approximately 250 g each were used for experimentation after three weeks of habituation in the laboratory environment. They were housed 10 per cage in a sound-proof room at a constant temperature (23 ± 1 °C) with a 12 h light/dark cycle (lights on at 05.00 h, off at 17.00 h). They received normal rat chow and water ad libitum.

Dissection

Groups of ten rats were sacrificed by decapitation at specific times of the day (08.00, 12.00, 16.00, 20.00, 24.00, 04.00 h). In each group five animals (treated) received an intraperitoneal injection of an L-aromatic amino-acid decarboxylase inhibitor (3-hydroxybenzyl hydrazine dihydrochloride, NSD 1015, Aldrich; 50 mg/kg, 0.5 ml) 30 minutes before decapitation. Under such conditions, the decarboxylase is completely inhibited and 5-HTP accumulation occurs linearly (Carlsson et al., 1972; Tappaz and Pujol, 1980). The other animals (controls) were injected with 0.5 ml of vehicle saline (NaCl 0,9%). In the dark period the injections were made under a dim red lamp.

After decapitation brains were quickly removed and sectioned in a vertical plane at the mesencephalic level. The anterior and the posterior parts were frozen on a block of metal cooled by liquid nitrogen. The anterior and posterior parts of the brain were cut in frontal sections (thickness 400 µm and 500 µm respectively) and the brain microareas were punched out from these sections (Palkovits, 1973). As previously described (Morin et al., 1991), the ventrolateral medulla (VLM, corresponding to the A 1-C 1 catecholaminergic region), dorsomedial medulla (DMM, A 2-C 2 region), locus coeruleus area (LC), raphe dorsalis (RD), raphe centralis (RC), and raphe pallidus (RP) were punched out with a 0.9 mm i.d. hollow needle. The periaqueductal gray (PAG) was removed with a 1.5 mm i.d. hollow needle from the same sections as the RD and RC. The suprachiasmatic (SCN) and paraventricular (PVN) nuclei of the hypothalamus were dissected with a needle of 0.6 mm i.d.. The cortex (CX) samples were dissected out with a small scalpel blade. Tissue samples were placed in Eppendorf tubes and stored at −80 °C.

Biochemical assays

The tissues were homogenized by ultrasound in a solution of 0.4 N $HClO_4$, containing 0.1 µM Nω-methyl-5-hydroxytryptamine as the internal standard (Sigma, Saint-Louis). The homogenization volume was 70 µl for the LC, 200 µl for the CX, and 50 µl for the other brain structures. After centrifugation (11,000 g, 15 min, +4 °C), the supernatant was diluted with an equal volume of water and was analyzed by high performance liquid chromatography with electrochemical detection (HPLC-ED). Each sample was assayed in duplicate. The injection volume was 30 µl. Preliminary data have shown that the samples were stable for 20 h while maintained at +4 °C.

The HPLC-ED system consisted of a 420 pump (Kontron Instruments), a WISP 712 autosampler with a cooling module set at +4 °C (Waters), a guard column (Spheri 5, RP 18, 30 × 4.6 mm), an analytical column (Spheri 5, RP 18, 220 × 4.6 mm; both from Brownlee Lab.) and a Waters 460 electrochemical detector with a carbon working electrode. Detection of 5-hydroxyindole compounds was made at a potential of 0.6 V. A Shimadzu C-R6A integrator was used to quantify detected peaks. The mobile phase was composed of 0.1 M citric acid monohydrate, 0.1 M potassium hydrogen phosphate, 0.27 mM disodium ethylenediamine tetraacetate (Merck), and 12% methanol (Carlo Erba). The flow rate was set to 1 ml/min. 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), Nω-methyl-5-

hydroxytryptamine (M 5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were separated in less than 15 min without interference with catecholamines. The limit of detection was 50 fmoles for 5-HTP, 5-HT, and 5-HIAA.

The protein pellets obtained after centrifugation of the homogenates were dissolved in NaOH 0.1 N and total proteins were estimated according to Bradford (1976) using bovine serum albumin as a standard.

Expression of results and statistical analysis

5-HT and 5-HIAA concentrations were only determined in control animals. The concentration of 5-HTP was determined in both treated and control animals. 5-HTP accumulation (5-HTPacc) was expressed as the difference in 5-HTP between treated and control animals.

As significant daily variations in total protein levels were observed in some brain areas (NPV, RD, LC, and CX; data not shown), 5-HTPacc, 5-HT, and 5-HIAA concentrations were expressed by structure and not by mg of total protein. Daily variations in total protein levels of discrete rat brain nuclei have already been described (Kan et al., 1977; Morin et al., 1991), but their mechanism remains unknown.

5-HTPacc, 5-HT, and 5-HIAA concentrations at the different time-points were compared by one way analysis of variance (ANOVA). If a significant difference appeared, they were subjected to a statistical analysis by the single Cosinor procedure (Halberg et al., 1967). This analysis uses the least squares method, yielding information on the probability that the data follow sinusoidal fluctuations with a 24 h period and giving parameters of the best fitting sinusoidal function. Two parameters are reported: (a) the amplitude (A) which is equal to one half the total extent of the sinusoid and which is expressed as a percentage relative to the daily mean value and (b) the acrophase (Φ) or time of the sinusoid maximum (expressed in hours). In order to test the significance of the calculated theoretical curve, an associated confidence interval with a 5% risk ($R_{0.05}$) for A was determined. If $R_{0.05} \geqslant A$, the theoretical sinusoidal rhythm was not significant. Only significant sinusoidal variations are reported.

Results

Daily variations in 5-HTP accumulation

As shown in Fig. 1, the 5-HTPacc exhibited significant daily variations ($P < 0.001$ by ANOVA) in the nuclei raphe dorsalis (RD), raphe centralis (RC), and raphe pallidus (RP). In the RD, the lowest value of 5-HTPacc was at the beginning of the dark period, while the maximum (+ 43,9% of the daily mean value) was in the second part of the dark period. A similar but slightly delayed pattern of variation was found in the RC, with a maximum (+ 48.7%) at the end of the dark period. In the RP, the highest 5-HTPacc was also observed at the end of the dark period (+ 47.1%), while two minima were found during the dark and light period respectively. Similar daily variations were also found in the pariaqueductal gray (PAG, $P < 0.001$), but the maximum (+ 14.3%) was lower than in the raphe nuclei (Fig. 1).

Daily variations ($P < 0.001$) in 5-HTPacc were also observed in the dorsomedian (DMM) and ventrolateral (VLM) parts of the medulla oblongata (Fig. 2). In the VLM, the maximum was at the end of the dark period (+ 42.3%), whereas in the DMM (+ 25.9%), it was close to the middle of the light period. In both of these structures, the 5-HTPacc followed sinusoidal fluctuations.

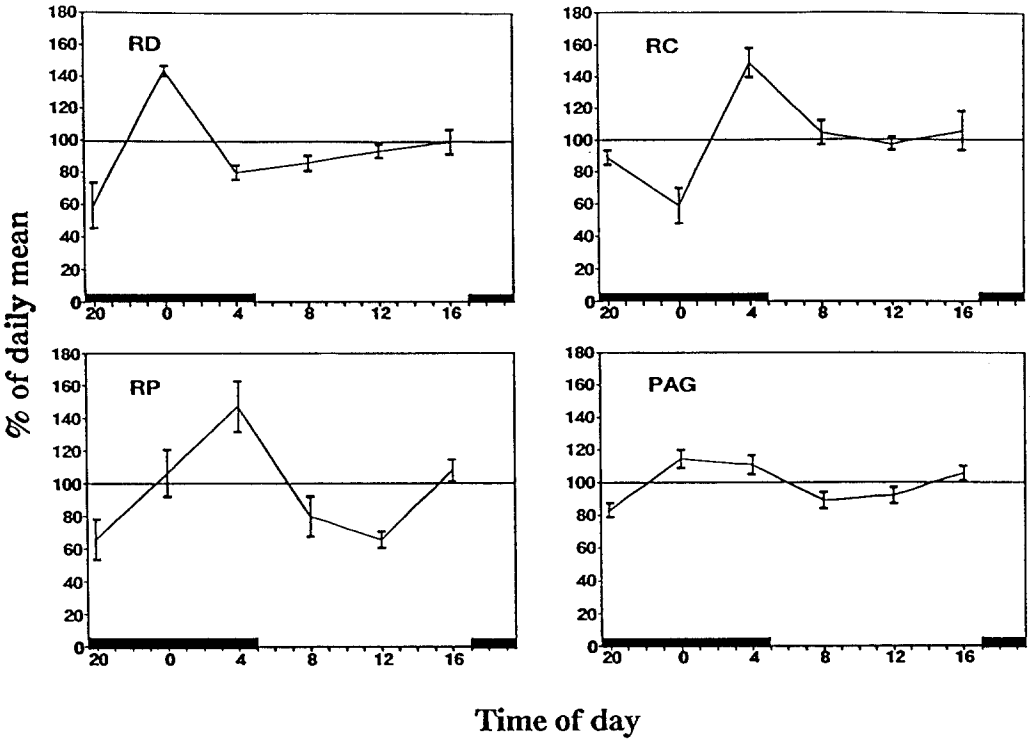

Fig. 1. Daily variations in 5-HTP accumulation (5-HTPacc) in the raphe dorsalis (RD), the raphe centralis (RC), the raphe pallidus (RP), and the periaqueductal gray (PAG). The tissue course of 5-HTPacc is represented by the dark line and expressed per structure as a percentage (± SEM as vertical bars) of the daily mean value (100%). Number of animals at each time: 10. Daily mean 5-HTPacc (pmoles of 5-HTP per structure and per 30 min ± SEM): RD; 14.63 ± 0.58; RC; 10.09 ± 0.50; RP; 1.35 ± 0.08; PAG; 10.69 ± 0.25

In the locus coeruleus (Fig. 2), the 5-HTPacc also showed significant ($P < 0.01$) daily variations with a maximum (+ 17.5%) at the end of the dark period. Significant ($P < 0.001$) daily variations in 5-HTPacc with a maximum close to the middle of the dark period were observed in the cortex (CX, + 48.0%), the suprachiasmatic (SCN, + 37.4%) and the paraventricular (PVN, + 35.1%) nuclei. The fluctuations in 5-HTPacc followed a sinusoidal function in these three structures.

Daily variations in 5-HT concentration

Significant daily variations in 5-HT concentration were observed in several brainstem structures (Fig. 3). In the RD ($P < 0.001$), the maximum was near the middle of the dark period (+ 47.1%) and the variations followed a sinusoidal function. The RC also exhibited significant ($P < 0.05$) daily variations in 5-HT concentration, but, in contrast to the RD, lower values were mainly observed during the dark period.

Moderate variations in 5-HT content were found in the medulla oblongata

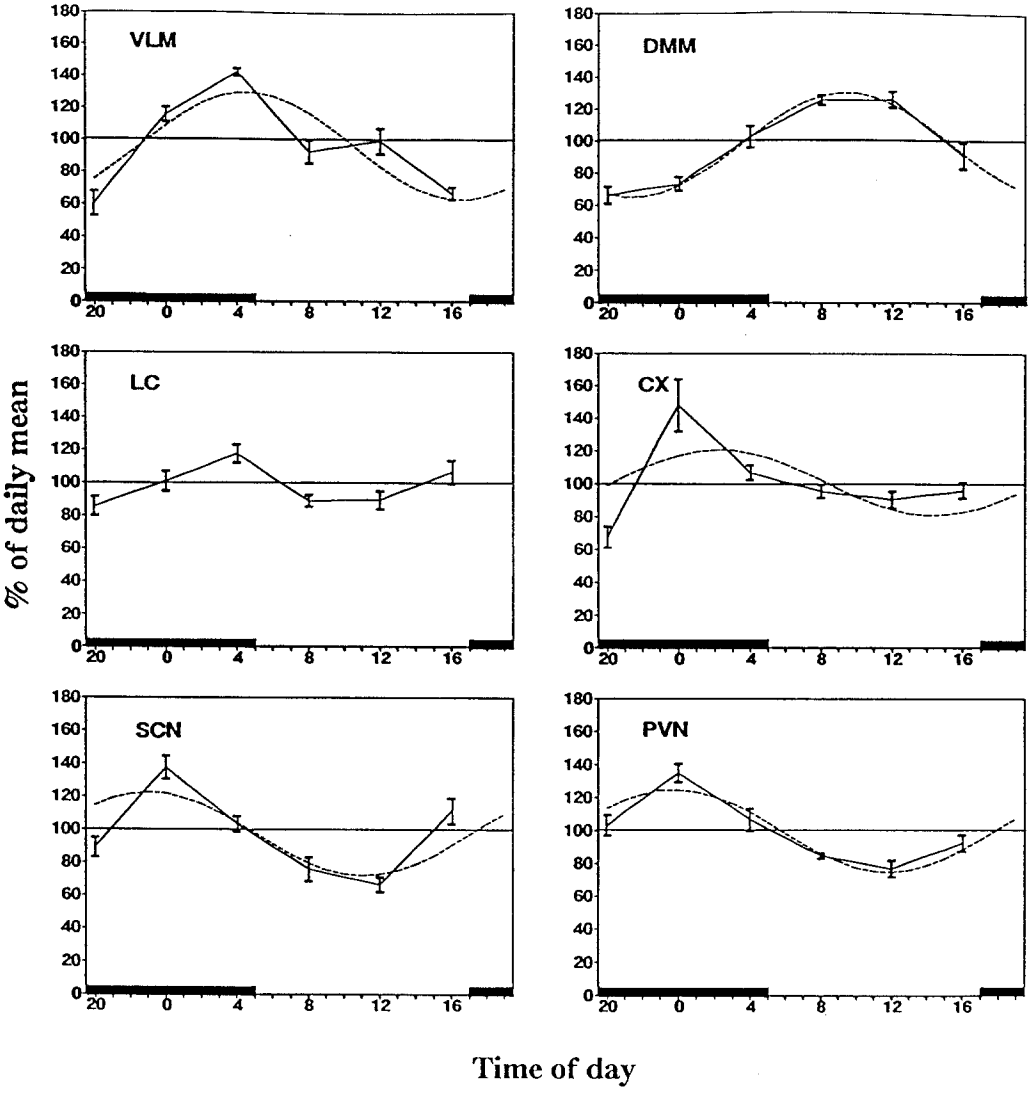

Fig. 2. Daily variations in 5-HTPacc in the ventrolateral medulla (VLM), the dorsomedial medulla (DMM), the locus coeruleus (LC), the cortex (CX), the suprachiasmatic nuclei (SCN), and the paraventricular nuclei (PVN). SEM as vertical bars of the daily mean value (100%). Same representation as in Fig. 1. Number of animals at each time: 10. Daily mean 5-HTPacc (pmoles of 5-HTP per structure and per 30 min ± SEM): VLM; 2.30 ± 0.09; DMM; 2.96 ± 0.10; LC; 2.98 ± 0.08; CX; 8.90 ± 0.34; SCN; 0.68 ± 0.03; PVN; 0.41 ± 0.01. The dotted curve represents the fitted theoretical curve calculated by the cosinor procedure with a fundamental period T = 24 h (only for structures in which the 5-HTPacc follows sinusoidal fluctuations). A, amplitude (% of daily mean value); $R_{0.05}$, associated confidence interval with a risk of 5%; and Φ, acrophase (hour.min) of the theoretical curves are reported: VLM; A = + 33.6, $R_{0.05}$ = 11.74, Φ = 04.30 ± 1.20; DMM; A = + 32.7, $R_{0.05}$ = 11.42, Φ = 09.20 ± 1.20; CX; A = + 18.9, $R_{0.05}$ = 10.45, Φ = 02.00 ± 2.10; SCN; A = + 25.2, $R_{0.05}$ = 13.42, Φ = 23.00 ± 2.10; PVN; A = + 24.9, $R_{0.05}$ = 10.64, Φ = 23.50 ± 1.40

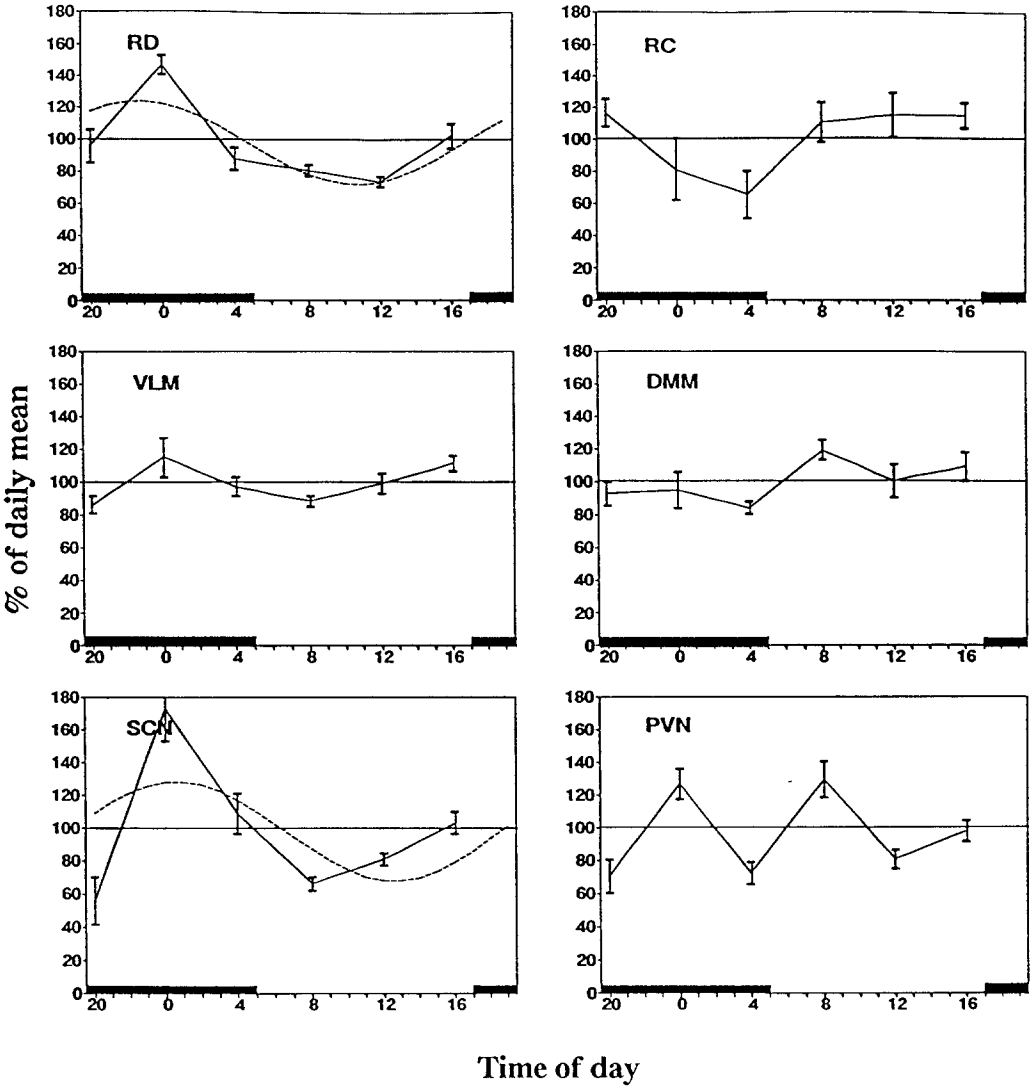

Fig. 3. Daily variations in the concentration of serotonin (5-HT) in the raphe dorsalis (RD), the raphe centralis (RC), the ventrolateral medulla (VLM), the dorsomedial medulla (DMM), the suprachiasmatic nuclei (SCN), and the paraventricular nuclei (PVN). Same representation as in Fig. 2. Daily mean concentration of 5-HT (pmoles of 5-HT per structure ± SEM): RD; 12.43 ± 0.53; RC; 5.44 ± 0.32; VLM; 4.64 ± 0.18; DMM; 5.62 ± 0.21; SCN; 0.85 ± 0.05; PVN; 0.34 ± 0.02. Parameters of theoretical curves: RD; A = + 26.1, $R_{0.05}$ = 13.22, Φ = 22.40 ± 2.00; SCN; A = + 30.3, $R_{0.05}$ = 18.64, Φ = 00.30 ± 2.30

($P < 0.05$). In the VLM, the maximum (+ 15.2%) was in the middle of the dark period, whereas, in the DMM, (+ 19.2%) it was at the beginning of the light period.

In the hypothalamus (Fig. 3), the SCN exhibited significant ($P < 0.001$) daily variations in 5-HT concentration with a maximum (+ 72.9%) close to the middle of the dark period; these variations followed a sinusoidal function. In

the PVN ($P < 0.0001$), complex fluctuations were observed with two maxima during nycthemer.

Finally, the 5-HT concentration did not change throughout the nycthemer in the following structures (data not shown): RP (daily mean concentration ± SEM: 0.59 ± 0.03 pmoles per structure), PAG (6.05 ± 0.24), LC (2.44 ± 0.09), and CX (16.05 ± 0.68).

Daily variations in 5-HIAA concentration

In the RD and RC, the 5-HIAA concentration followed significant ($P < 0.001$) daily variations (Fig. 4). In the RD, the maximum (+ 20.7%) was near the middle of the dark period; on the contrary, the maximum in 5-HIAA concentration of the RC (+ 22.0%) was during the light period. In both structures, these variations followed a sinusoidal function. As shown in Fig. 4, the RP and the PAG exhibited significant ($P < 0.05$) daily variations in 5-HIAA concentration with a maximum at the end of the dark period (+ 22.7% and + 18.9%, respectively).

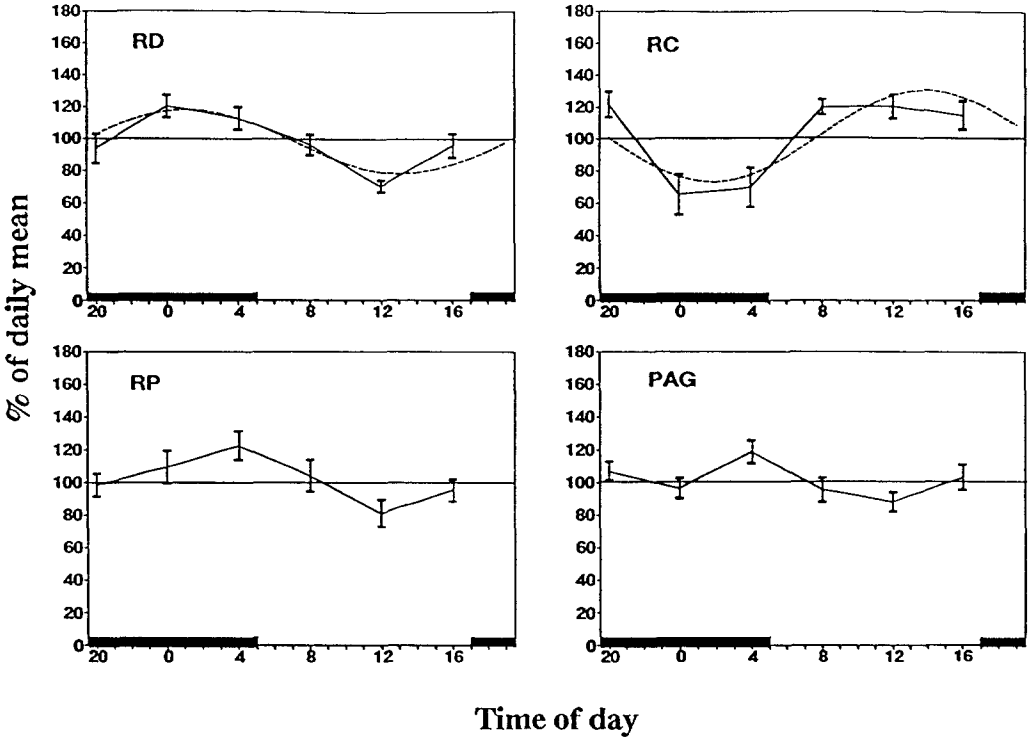

Fig. 4. Daily variations in the concentration of 5-hydroxyindoleacetic acid (5-HIAA) in the raphe dorsalis (RD), the raphe centralis (RC), the raphe pallidus (RP), and the periaqueductal gray (PAG). Same representation as in Fig. 2. Daily mean concentration of 5-HIAA (pmoles of 5-HIAA per structure): RD; 16.35 ± 0.57; RC; 10.35 ± 0.51; RP; 1.92 ± 0.07; PAG; 11.88 ± 0.35. Parameters of theoretical curves: RD; A = + 19.9, $R_{0.05}$ = 14.45, Φ = 01.10 ± 3.10; RC; A = + 28.9, $R_{0.05}$ = 18.05, Φ = 13.50 ± 2.30

Daily variations in 5-HIAA concentration were found in the VLM, but they did not reach significance. However, the moderate fluctuations in 5-HIAA of the DMM were significant ($P < 0.01$) (Fig. 5). The 5-HIAA content of the LC was stable throughout most of the nycthemer, except at 16.00 h where it was found to be significantly higher (+ 23.0%; $P < 0.05$) (Fig. 5).

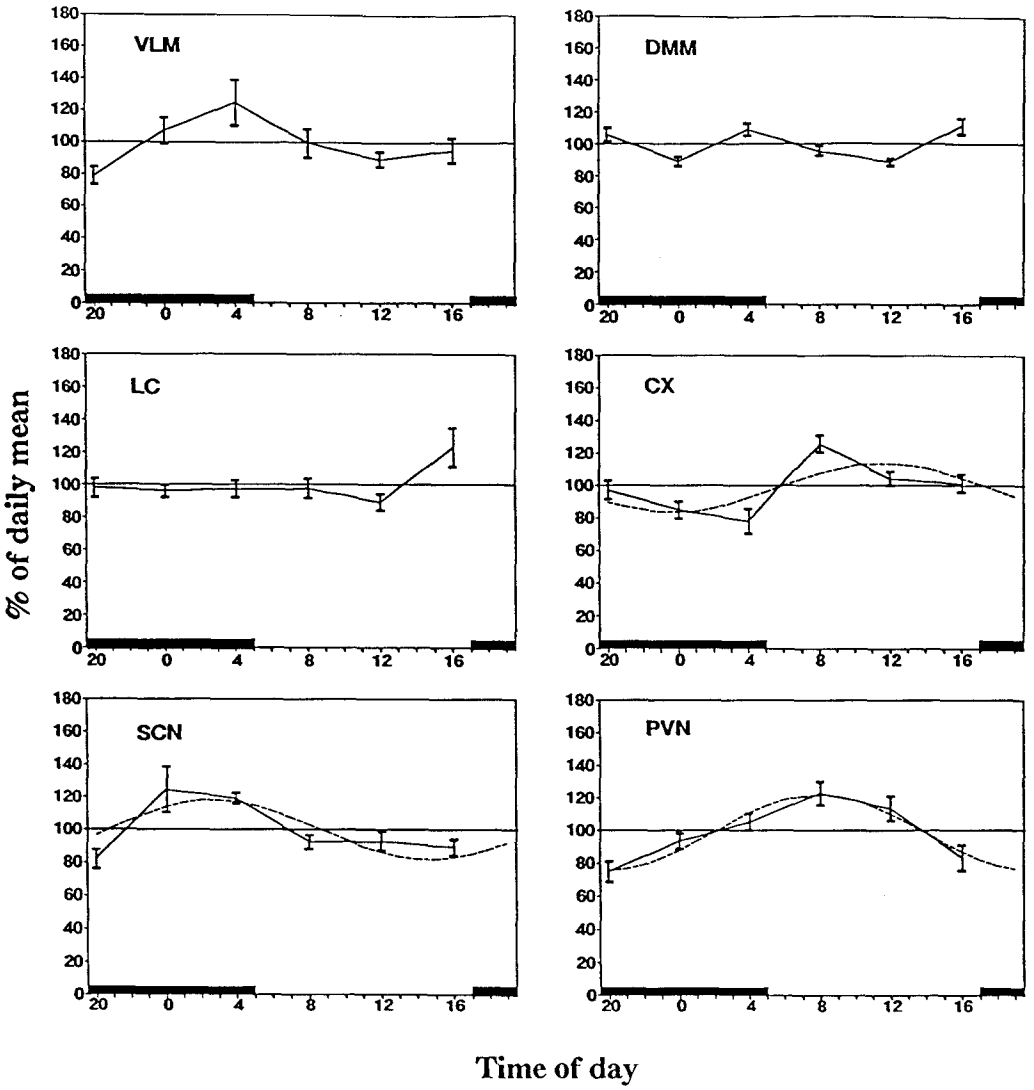

Fig. 5. Daily variations in the concentration of 5-hydroxyindoleacetic acid (5-HIAA) in the ventrolateral medulla (VLM), the dorsomedial medulla (DMM), the locus coeruleus (LC), the cortex (CX), the suprachiasmatic nuclei (SCN), and the paraventricular nuclei (PVN). Same representation as in Fig. 2. Daily mean concentration of 5-HIAA (pmoles of 5-HIAA per structure): VLM; 5.57 ± 0.2; DMM; 6.43 ± 0.13; LC; 5.29 ± 0.16; CX; 27.03 ± 0.71; SCN; 0.93 ± 0.03; PVN; 0.64 ± 0.03. Parameters of theoretical curves: CX; A = + 21.7, $R_{0.05}$ = 11.48, Φ = 11.30 ± 2.10; SCN; A = + 18.1, $R_{0.05}$ = 11.97, Φ = 02.40 ± 2.50; PVN; A = + 23.1, $R_{0.05}$ = 12.61, Φ = 07.50 ± 2.10

As shown in Fig. 5, the 5-HIAA concentration of the CX, SCN, and PVN exhibited significant ($P < 0.01$) daily variations. In both CX and PVN, the maximum was observed during the light period (+ 31.8% and + 23.0% respectively), whereas it was near the middle of the dark period in the SCN (+ 24.4%). In these three structures, the 5-HIAA concentration followed sinusoidal fluctuations.

Discussion

The 5-HTP accumulation (5-HTPacc) after inhibition of L-aromatic amino acid decarboxylase was determined at different times of the nycthemer in discrete brain areas (dissected as "punches") of rats maintained on a regular 12 h/12 h light-dark cycle. Daily variations in 5-HTPacc with a maximum during the dark period were found in all brain areas studied except in the DMM where the maximum was near the middle of the light phase. Some brain areas (RD, PAG, CX, SCN, and PVN) exhibited a maximum in 5-HTPacc near the middle of the dark period, while others (RC, RP, LC, and VLM) showed a maximum at the end of the dark phase.

These results show that the rate of tryptophan hydroxylation undergoes daily rhythmicity and, therefore, is likely to be involved in the circadian rhythm of 5-HT biosynthesis. Furthermore, the circadian rhythm of 5-HT biosynthesis should be parallel to the time course of tryptophan hydroxylation since it is unlikely that 5-HTP decarboxylation influences the circadian rhythm of 5-HT biosynthesis (Martin, 1991). Therefore, our data suggest that in the brain areas studied, 5-HT biosynthesis is activated during the dark phase, except in the DMM where it was found to be higher during the light phase. This is in contrast with the previous work of Héry et al. (1972) reporting that the rate of synthesis of [^{3}H]5-HT from [^{3}H]tryptophan is increased during the light period in the cerebral cortex, brainstem, and hypothalamus of the rat. Differences in experimental methodology could explain this discrepancy. Results of Héry et al. (1972) are complicated by the fact that the measurements were not performed on a complete 24 h cycle, but during two 6 h successive periods of light and darkness, and, as remarked by McLennan and Lees (1978), by the fact that [^{3}H]tryptophan uptake is less at night.

Under normal conditions, the enzyme TrH is not saturated by tryptophan and fluctuations in the local concentration of this amino acid trigger parallel modifications in the rate of tryptophan hydroxylation (Hamon and Glowinski, 1974). The daily variations in the rate of tryptophan hydroxylation could be due to changes either in the efficiency or quantitiy of TrH or to fluctuations in tryptophan level at the site of hydroxylation. Although the present data do not allow one to separate these hypotheses, we nevertheless suggest that changes in the TrH level should be more important than fluctuations in tryptophan availability. The local concentration of tryptophan depends on both brain tryptophan level and neuronal uptake of this amino acid. Although brain tryptophan content was not measured in the present study, one would assume that

it should be higher during the middle of the dark period, following the nocturnal peak in food intake in the rat (Héry et al., 1977). Nevertheless, the rate and affinity of the neuronal uptake of tryptophan seem to undergo daily changes which could buffer the rhythm in extracellular tryptophan concentration (Loizou and Redfern, 1986) and should prevent, in this way, any influence on the rate of tryptophan hydroxylation. The daily variations in brain tryptophan content, on the other hand, are likely to have similar time-courses throughout brain regions (Morgan et al., 1975), and, if they influence the rate of tryptophan hydroxylation, this effect should occur at the same time in all the brain areas studied. This does not seem to be the case, since the phase of the daily variations in 5-HTPacc was different and even opposite between brain areas (see for example SCN and DMM). It is thus unlikely that tryptophan availability is an important factor in controlling circadian variations in the rate of tryptophan hydroxylation. This is in agreement with previous work which did not support any involvement of tryptophan availability in the circadian rhythm of 5-HT biosynthesis and 5-HT level (Loizou and Redfern, 1986; Martin, 1991).

It is likely, on the other hand, that at least in the RD and the RC the daily variations in the rate of tryptophan hydroxylation are mainly due to fluctuations in TrH activity since 5-HTPacc is maximum during the dark period as is the in vitro TrH activity determined in homogenates of brainstem or raphe nuclei (Kan et al., 1977; Cahill and Ehret, 1981). Further studies are needed to determine whether fluctuations in TrH activity result from changes in TrH concentration or in the kinetic characteristics of this enzyme. This latter possibility should be considered since day-night changes in the Km for pteridin cofactor have been described for rat brain TrH (Mc Lennan and Lees, 1978).

The measurement of 5-HTP accumulation after inhibition of L-aromatic amino acid decarboxylase is considered a valid index of 5-HT turnover and could be related to neuronal activity since the 5-HTPacc is increased after electrical stimulation of serotoninergic pathways (Boadle-Biber et al., 1983; Duda and Moore, 1985). In addition, 5-HTPacc in most of the structures studied is minimal during the light phase, a period when sleep principally occurs in rats and when 5-HT neurons should be less active, given that the axonal activity of serotoninergic neurons is thought to be nearly or totally inhibited during the different states of sleep (Cespuglio et al., 1990). Furthermore, at least in the SCN, the daily variations in 5-HTPacc seem to follow a pattern similar to the rhythm of extracellular 5-HIAA concentration, an index of 5-HT release determined by in vivo voltammetry (Faradji et al., 1983) or by push-pull cannula (Ramirez et al., 1987).

In the present study, the content of 5-HT and of its metabolite 5-HIAA were also determined. Daily variations in 5-HT and 5-HIAA levels were found in most of the brain areas studied. In the RD and in the SCN, the 5-HT and 5-HIAA contents exhibited fluctuations which have similar time courses to the 5-HTPacc rhythm i.e., with a maximum in the middle of the dark phase and a trough in the middle of the light period. It is interesting to note that this

similarity occurs in both structures which seem to be implicated in regulation of the circadian clock. In contrast, the relative phase and amplitude of the respective daily variations in 5-HT, 5-HIAA, and 5-HTPacc can be very different in other brain areas. For example, the RC, PVN, and CX exhibit daily variations in 5-HIAA content which are delayed compared to the rhythm in 5-HTPacc, since the maximum in 5-HIAA content was found during the light period. In addition, these three structures exhibit different rhythms in 5-HT content. The RC 5-HT peak occurs during the light period, whereas the PVN exhibits a complex bimodal rhythm and the cortical 5-HT content remains stable throughout the nycthemer. The RP, VLM, and DMM, in which variations in 5-HTPacc are marked, exhibit low amplitude daily variations in 5-HT and 5-HIAA contents.

The circadian rhythms of 5-HT and 5-HIAA levels do not have a unique pattern and can vary from one brain area to another. This may be due to the fact that both 5-HT and 5-HIAA levels in a particular structure depend on several factors. Indeed, 5-HT level depends on 5-HT biosynthesis, release, uptake, and catabolism; whereas the rate of HIAA formation depends on 5-HT level, monoamine oxidase activity and active transport of 5-HIAA from the brain. Most of these factors seem to exhibit daily variation (Meyer and Quay, 1976; Kan et al., 1977; Chevillard et al., 1981; Wirz-Justice et al., 1983; Blier et al., 1989; Martin, 1991), and therefore are likely to control the intrinsic circadian variations in 5-HT and 5-HIAA contents. Consequently, the determination of the rhythmicity of all these factors in discrete brain areas of rats is needed to understand the origin of the circadian variations in 5-HT and 5-HIAA levels. This point illustrates the complexity of circadian mechanisms in central serotoninergic neurons and shows that further studies are needed to approach the mechanisms of daily change in serotoninergic metabolism.

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