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Daily Changes in 5-Hydroxytryptamine Concentration in Mouse Brain.*† (22586)

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The status of our knowledge on 24-hour periodicity has been reviewed from neurophysiologic(1) and endocrinologic points of view(2). Almost certainly, 24-hour periodic body function involves a nervous-endocrine-metabolic sequence(3), and in this connection the behavior of neurohumors around the 24-hour time scale appears to be of interest. Earlier work from this laboratory has dealt with the effects of 5-hydroxy-tryptamine (5-HTA) upon the number of circulating eosinophils(4), and thus, perhaps, with the effects of this neurohumoral agent upon adrenal mechanisms of physiologic 24-hour periodicity. This report is concerned with the daily changes in the concentration in mouse brain of 5-HTA itself. From thorough reviews of the literature on 5-HTA, made by Erspamer (5,6), it would appear that this problem has not previously been considered.

Materials and methods. Seven series of determinations were made on ZBC‡ mice, male or female, 5 or 7 weeks of age. Purina Fox Chow and tap water were available to all of the mice since weaning and until sacrifice. For at least 7 days prior to sacrifice, the mice were singly housed in a room maintained at 78 ± 2°F, and illuminated by artificial light only. A clock controlled switch turned the lights on at 06:00 and off at 18:00.

In a given series, groups composed each of 25 mice were used during certain periods of day (specified under results). During any one period of day, individual mice were decapitated, a mid-line incision was then made through the skull with small, pointed scissors, the skull divided, and the entire brain was removed and weighed to the nearest milligram on a torsion balance. Immediately after weighing, the brain was transferred to a previously cooled mortar containing 95% acetone (20 ml/g, as suggested by Amin *et al.* (7)) and crushed with a pestle. The time elapsed between decapitation and the beginning of extraction did not exceed 1½ minutes.

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‡ ZBC mice are obtained by mating F_1 hybrid females of several stocks to a male of the Z or Zb stock.

After a minimum of 24 hours, the acetone extract was filtered off, and the brain residue was re-extracted with an equal volume of cold 95% acetone. The 2 extracts were combined and evaporated to dryness at 37°C *in vacuo*. The dry residue was taken up in 3 ml of 0.1 N HCl, and its 5-HTA content determined spectrophotometrically according to the method of Udenfriend *et al.*(8), with only slight changes made in order to adapt the procedure to available equipment. Each of the values for brain 5-HTA concentration here discussed is based upon the pooled extract of 25 mouse brains. The specificity of the method has been discussed by Udenfriend (8), and the possible interference by materials other than 5-HTA has not been evaluated herein. Methodologically, a check of sensitivity was first undertaken. Quantities of 6 μg of synthetic 5-HTA in water solution could reliably be determined, in close agreement with Udenfriend's report. Next, pools of mouse brains were studied in order to obtain 5-HTA values of comparable magnitude. The choice of as many as 25 mouse brains as source material for one extract was made in view of earlier work on brains from several species(7,9,10), in the light of which the necessity of such a pool was anticipated. Third, pools with and without added 5-HTA were extracted and analyzed, and in two such studies about 90% of the added 5-HTA was recovered.

Results. Among 7 brain pools, obtained in an exploratory series at 4-hour intervals from 08:30 of one day to 08:30 of the next, the highest 5-HTA value was found at 12:30 and the lowest at 16:30. Subsequently, the study was focused upon the detection of changes which may occur around these time points. Pertinent data from 6 series are shown in Table I, which reveals that, on the average, the concentration of 5-HTA in brain was higher before and around noon than at or after 16:30. The corresponding difference of .12 ± .04 was analyzed by the use of Student's t and was significant at 2% ($t = 2.76$; $P = .011$). It also is pertinent that similar daily changes may be seen in Table I for males of 2 age groups (series 1-5) and for females (series 6). But this change in 5-HTA concentration as a function of time of day is more readily apparent from Fig. 1; the daily change stands out clearly in data expressed as per cent of series mean. For comparison of 5-HTA concentrations at several ages, data are available in this study on males 5, 7, and 16 weeks of age. The mean 5-HTA concentration of 5 pools describing mice 5 weeks of age was .74 μg/g (with SE = .01 μg/g), while the corresponding values of 2 pools describing mice 7 weeks of age were .65 and .74, and that for one pool describing mice 16 weeks of age was .82. Further observations will be needed to clarify the question of possible age trends in brain 5-HTA concentration.

TABLE I. 5-HTA Concentration in Mouse Brain* at Certain Times of Day.

	5-HTA, μg/g (wet wt)						
	Series No.						Mean
Time of day	1	2	3	4	5	6	± S.E.
8:30- 9:30			.76	.65		.89	.81 ± .03
9:30-10:30						.82	
10:30-11:30	.73	.70			.89	1.05	
12:00-13:00		.72	.78	.74	.90	.90	
16:30-17:30			.62	.55	.87	.86	.69 ± .03
17:30-18:30						.74	
18:30-19:30	.53	.59				.73	
19:30-20:30		.61			.80	.72	
20:30-21:30			.60	.73			

* Values describe pools consisting each of 25 brains from ZBC-Bittner mice.

Males were used for series 1-5, females for series 6; mice 5 wk of age were used for series 1-3, mice 7 wk of age were used for series 4-6.

Table II compares the 5-HTA content in brain pools from mice of the two sexes, but of roughly comparable age and stock. Without generalizing beyond the scope of the limited sample size employed, it would appear that the concentration of 5-HTA may be slightly higher in females than in males. The analysis of the sex difference of .15 ± .07 yielded a $t = 2.03$ and a P of .07.

Discussion. Previous work on brain 5-HTA dealt with the central nervous system as a whole, as well as with defined parts thereof. By means of bioassay, Twarog and Page(9) have reported concentrations of .1 and .36 μg/g for whole dog brain, and a concentration of .24 μg/g in rat brain. Also by bioassay, Amin, Crawford, and Gaddum(7) have thor-

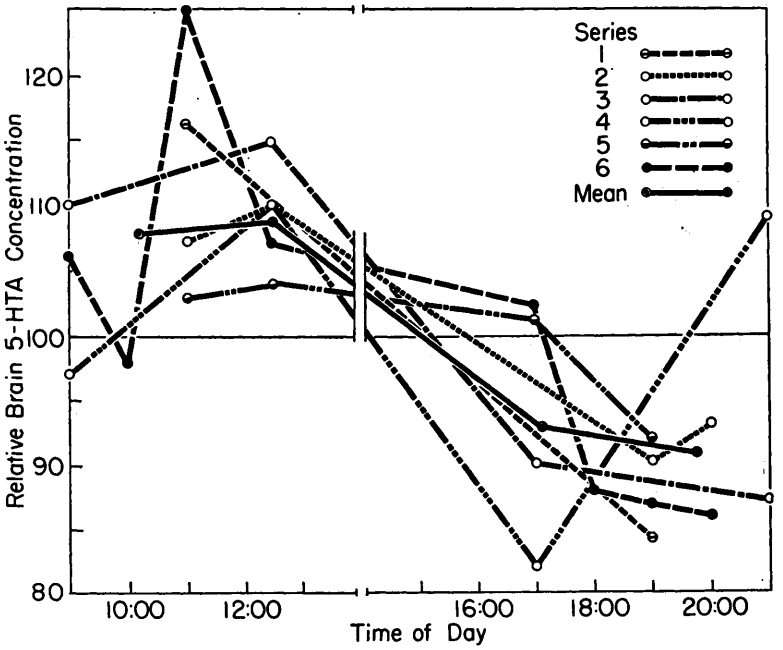

FIG. 1. Daily changes in 5-HTA concentration in mouse brain expressed as % of mean for each series.

oughly analyzed the 5-HTA content in defined parts of the brain of the dog. More recently, Pletscher, Shore, and Brodie(10), using a chemical method, have reported a "normal average" of .54 μg/g in rabbit brain. Using the same chemical method, Bogdanski and Udenfriend(11) have reported concentrations of between .07 and 1.75 μg/g for various parts of dog brain, and between .30 and 1.78 μg/g for various parts of cat brain. In both instances, the highest concentrations were found in the hypothalamus. The data of this report on mouse brains are roughly of the same order of magnitude. Moreover, if, as seems likely, the apparent 5-HTA in brain determined chemically in this study is identical with authentic 5-HTA, several suggestions can be made on the basis of the data presented herein.

TABLE II. Sex Comparison* of Brain 5-HTA in ZBC Mice, 5 and 7 Weeks of Age.

Sex of mice	No. of pools†	5-HTA, μg/g, mean ± S.E.
♂	6	.78 ± .05
♀	5	.93 ± .04

* Based upon data obtained during daily time period of apparent peak concentration (Table I and text).

† Each pool consisting of 25 mouse brains.

1. It would appear that the 5-HTA concentration in mouse brain constitutes a 24-hour periodic variable. This finding is clearly visualized in Fig. 1, in which the data of each time series are expressed as % of series mean, *i.e.*, as relative 5-HTA concentrations. Even though the absolute values at the time of daily peak concentration appear to exhibit a sex difference (being higher in females than in males studied at the same time of day, Table II), both sexes exhibit similar "within day" changes (compare series 6 with series 1-5, Fig. 1).

2. The temporal placement within the 24-hour period of the daily decrease in brain 5-HTA concentration is of added interest. Under the conditions of this study, this decrease precedes the usual period of onset of increased body activity, which in the nocturnal mouse occurs around the beginning of the dark period(12). It may be pertinent that daily adrenal activation in mice does occur during roughly the same period(2,13) and also that Bertelli, Cantone, and Martini have implicated 5-HTA as a mechanism of ACTH

release from the anterior pituitary(14).

3. (And also apparent from Fig. 1) the extent of daily change in brain 5-HTA concentration is relatively small as compared with the amplitude of 24-hour changes observed under standardized circumstances for some other body functions of rodents(2,15). The suggestion by Pletscher, Shore, and Brodie(10) that 5-HTA exists mainly in a bound form comes to mind in this connection. Before this assumption is validated, the possibility that those changes noted herein may reflect primarily changes in free 5-HTA can hardly be discussed. It also must be remembered that 5-HTA behavior of the entire brain was studied herein. Thus, drastic changes in certain parts of the brain, like the hypothalamus, may be obscured if other parts of the brain do not vary at the same time in the same direction. Analysis of these problems must await the study with more sensitive procedures(8) of the behavior along the 24-hour time scale of 5-HTA concentration in various areas of the brain.

Summary. ZBC mice, male or female, 5 or 7 weeks of age, were studied under conditions standardized for evaluation of 24-hour periodicity. A small but significant decrease in 5-HTA concentration of brain pools was noted prior to usual time of arousal. A sex difference in brain 5-HTA concentration also was recorded, the females exhibiting slightly higher values.

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Metal Chelates as Therapeutic and Detoxifying Agents in Gas Gangrene.* (22587)

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The ability of ethylenediamine tetraacetic acid (EDTA) to protect mice against a lethal dose of *Clostridium perfringens* Type A toxin has been noted(1). It was shown that this protection was due to the metal chelating ability of EDTA inasmuch as it was reversed by Zn^{++}, Co^{++} and Mn^{++}. In those experiments the toxin and EDTA were mixed together before injection but it appeared that EDTA could also protect when given independently of the toxin. In relatively large doses EDTA is toxic for animals because it induces hypocalcemia, and thus large amounts cannot be administered safely. Although Ca^{++} activates the principal lethal component of this toxin, a lecithinase, it was demonstrated that it did not reverse the protective effect of EDTA. This observation suggested that the calcium chelate of EDTA (Ca-

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