SEASONAL AND CIRCADIAN RHTHMS OF SEROTONIN CONTENT AND METABOLISM IN THE MOUSE BRAIN

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Many workers have demonstrated circadian rhythms of the serotonin (5-HT) level in the brain of laboratory animals [4-6, 8, 10]. There has been less research into seasonal rhythms of 5-HT and the rate of its metabolism in the rodent brain [2, 7]. One reason for this may be the fact that seasonal changes require investigations to continue for at least 5 years [13, 14].

This paper describes the results of an investigation of the content of 5-HT and its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the whole mouse brain over a number of years.

EXPERIMENTAL METHOD

To the earlier material (1972-1976) were added the results of systematic experiments (from 1978 through 1980) on albino mice of both sexes weighing 20-23 g. For the 2-4 weeks before the biochemical tests the animals were kept in cages with 25 to 50 mice per cage at a constant temperature of 20 ± 2°C and with alternation of 12 h daylight and 12 h darkness (light from 7 h 5 min a.m. to 7 h 5 min p.m.); access to food and water was unrestricted. The cages were cleaned and animals fed every morning between 9 a.m. and noon. Both seasonal and circadian rhythms of 5-HT content and of this metabolic rate were determined. The mice were decapitated and the brain quickly removed, frozen in liquid nitrogen, and weighed, after which material, wrapped in tinfoil, was kept at −20°C until analysis. To determine circadian rhythms the material was taken from 6-10 mice each time at 6 a.m., noon, 6 p.m., and midnight, respectively. During the dark period the mice were decapitated in the dark red light of a photographic lamp. To determine seasonal rhythms 6-8 mice were investigated in each group (always at 10 a. m.), twice a month on definite days.

5-HT and 5-HIAA were determined by a spectrophotofluorometric method [1] using a fluorescence spectrometer from Hitachi (Japan). The content of the test compounds was expressed in μg/g wet weight of brain. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The mean annual 5-HT content in whole mouse brain was 0.65 ± 0.06 μg/g, but in the course of the year the content both of 5-HT itself and of its metabolite changed significantly. A peak of the 5-HT content was discovered [7] during the summer months. In the present experiments peaks of the 5-HT content in the course of the year occurred from May to June and in December. The increase in 5-HT concentration was accompanied by fluctuation of the 5-HIAA concentration (Fig. 1).

A circadian rhythm of the 5-HT and 5-HIAA concentrations also was found. The 5-HT concentration was higher during daylight and lower during darkness. The fluctuations in the 5-HT concentration during the 24-h period did not exceed 25-35% of the mean daily concentration, depending on the season of the year. Circadian changes in the 5-HIAA concentration were much greater (Table 1). The results are in agreement with data in the literature [4, 10], even in absolute values.

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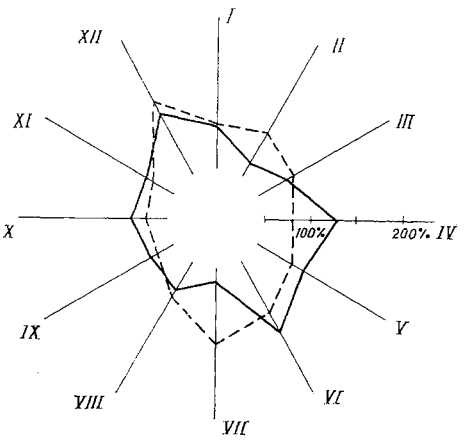

Fig. 1. Changes in 5-HT (continuous line) and 5-HIAA (broken line) content in whole mouse brain in the course of the year (in % of annual mean). Annual mean 5-HT concentration 0.65 ± 0.6 μg/g, 5-HIAA 0.53 ± 0.06 μg/g.

TABLE 1. Concentrations of 5-HT and 5-HIAA (in μg/g) in Whole Mouse Brain, Measured at 6-Hourly Intervals during the 24-h Period in April

Time of day	5-H	5-HIAA
6a.m.	0,55±0,04	0,51±0,04
12noon	0,71±0,03†	0,20±0,04*
18 p.m.	0,67±0,03†	0,30±0,03
24 midnight	0,57±0,02	0,42±0,009

* $P \leqslant 0.05$ compared with control.

† $P < 0.01$ compared with control.

Comparison of the phases of the rhythms of 5-HT concentration with phases of the rhythm of typical motor activity of rodents and insects [9, 11] shows that the peak of the 5-HT level in the brain corresponds to rest and a minimum of motor activity. The amplitude of rhythmic fluctuations of the 5-HT content in the rodent epiphysis reaches 900% during daylight [12], but rhythmic changes in 5-HT in both brain and epiphysis are identical in phase. This fact confirms that the 5-HT content is positively synchronized with the conditions of illumination, despite the fact that in the epiphysis 5-HT plays the rule of a precursor of melanotonin, whereas in the brain it performs a neuromediator function.

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FIBRONECTIN AND ITS RECEPTORS ON THE SURFACE OF POLYMORPHS

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The surface of monocytes has receptors for fibronectin [2], a fact that is closely linked with its function as a nonspecific opsonin. There is reason to suppose that fibronectin can perform the role of opsonin also during phagocytosis of disintegrated tissues, collagen, and fibrin by polymorphonuclear neutrophils. However, the role of fibronectin in the regulation of neutrophilic phagocytosis has not yet been studied and, in particular, there is no information on the existence of receptors for fibronectin on the surface of polymorphs.

The object of this investigation was to study interaction between fibronectin and polymorphs, and so to obtain evidence of the existence of receptors for fibronectin and fibronectin itself on their surface.

EXPERIMENTAL METHOD

Since fibronectin binds with cells only when in an immobilized state [4], as fibronectin-covered matrix we used fibronectin–gelatin–Sepharose (FGS). Gelatin–Sepharose (GS) was obtained by the method in [6] by attaching gelatin (from Kepf, West Germany) to CnBr-activated Sepharose 4B (from Pharmacia, Sweden). After equilibration of the column with GS (2·5 cm) with buffered physiological saline (BPS), pH 7.4, containing 0.1 M sodium citrate, 150 ml of fresh pooled human citrated blood plasma was passed through it. After the column had been rinsed with BPS and 1 M urea in BPS to remove plasma (verified at E_{280}), the column was equilibrated with Hanks' solution containing (20 units/ml) or not containing heparin. As a result, pure (95%) fibronectin was found in the absorbed state on the GS column, as was confirmed by the results of electrophoresis in polyacrylamide gel in the presence of sodium dodecylsulfate after elution of the protein with 4 M urea.

Human polymorphs were obtained from heparinized blood (20 units/ml) by centrifugation in a Ficoll–Verografin mixture (ρ = 1.114 g/ml). In this way a suspension of viable (as regards ability to phagocytose trypan blue) polymorphs of 98% purity was obtained.

A suspension of FGS granules (0.1 ml) was mixed with a suspension of polymorphs with a concentration of $2 \cdot 10^6$ cells/ml in 2% albumin solution in buffered Hanks' solution with heparin (20 units/ml) or without it. The mixture was incubated for 30 min at 37°C with mixing, after which it was examined under the microscope. GS granules and pure Sepharose 4B, treated in the same way, were used for the control.

In some experiments EDTA was added to the reaction mixture in a final concentrattion of 0.05 M.

The polymorphs were trypsinized by incubating them in Hanks' solution containing trypsin (from Spofa, Czechoslovakia) in a concentration of 1 mg/ml, at 37°C for 30 min, followed by rinsing to remove the enzyme.

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