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DAILY AND SEASONAL RHYTHMS OF INDOLEAMINE AND CATECHOLAMINE CONTENT IN MOUSE BRAIN

Numerous investigators have demonstrated the presence of diurnal rhythmic changes in dopamine, noradrenaline and 5-hydroxytryptamine (serotonin) content in the rat brain (Dixit, Buckley, 1967; Schewing et al., 1968; Lew, Quay, 1973; Asano, Morgi, 1974; Walker, 1974; Henry et al., 1977; Lemmer, Berger, 1978a, b). Less is known about seasonal variation in the monoamine content in the brains of rodents (Beauvallet et al., 1968; Андреев, Кобкова, 1970; Голиков, Голиков, 1973; Оттер, Нурманд, 1980). There are, however, few reports on biochemically determined rhythmical variations. One can mention papers dealing with 5-hydroxytryptamine (5-HT) concentration variation in the mouse brain (Albrecht et al., 1956; Morgan, Yndo, 1973). The present report makes an attempt at summarizing the results of recent experiments (1978—1980) performed in our laboratory to demonstrate the presence of daily and seasonal rhythms in mouse brain catechol and indoleamine content.

Material and methods

Male and female white mice averaging 25 g in weight were used. Four weeks prior to each study, 25—50 animals were kept in a cage at a nearly constant temperature (20 °C). The mice were given food and water *ad libitum* and they were exposed to 12 : 12 hr regimen of alternating dark and artificial light. The light phase ran daily from 07.00 to 19.00. The room was entered for cage cleaning and replenishing of food and water at 9.00—11.00 every day. In order to better characterize the rhythm phenomena of the whole brain and the relationship of the rhythms to the functional processes of the brain as well as to provide information essential to neurochemical studies involving the time variables, the daily rhythm of the important biogenic amines of the brain — serotonin, dopamine (DA), noradrenaline (NA) and their metabolites, 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA), were determined. Great care was taken to minimize any disturbance of the animals during the transfer. The animals were decapitated, the brains were immediately removed, frozen in liquid nitrogen, weighed, wrapped in aluminium foil and storaged in deep freeze at —12° until analysis. The entire procedure took from 30 to 40 min, depending on the number of animals. To determine circadian rhythms, the brains of 6—10 animals were collected at 06.00, 12.00, 18.00 and 24.00 respectively. During the dark period the animals were decapitated in the dark red photolamp light. To establish seasonal variations, 6—8 mice per group were decapitated invariably at 10.00 on certain days twice a month.

Chemical assay

Each tissue sample was homogenized in 0.4 N perchloracetic acid for determining catecholamine, or in acidified butanol for serotonin determination. The contents of dopamine and noradrenaline in the whole brain tissue of the experimental animals were analyzed by fluorimetric measurement of their trihydroxyindole derivatives after partial purification by adsorption and elution from Al_2O_3. The fluorescent derivatives of dopamine and noradrenaline were obtained by iodine oxidation followed by alkalization, subsequent acidification and modified heating at 100° according to the method of Shellenberger and Gordon (1971); the derivative of homovanillic acid was obtained by ferricyanic oxidation. Serotonin content was determined by the micromethod of Curzon and Green in our modification (Оттер, 1978). All fluorometric measurements were made with a Hitachi fluorescence spectrophotometer. Tissue concentration data are presented as microgrammes of amine per gramme of wet weight of the brain tissue.

Statistical significance of the difference between the two mean values was determined by Student's *t* test.

Results and discussion

Literature presents few data on the seasonal rhythmical variations in amine contents of rodent brain. Andreyev and Kobkova (Андреев, Кобкова, 1970) report a summer and autumn increase of noradrenaline content in the rat brain (in winter 0.19 ± 0.02, in spring 0.20 ± 0.02, in summer 0.26 ± 0.02 and in autumn 0.36 ± 0.06 μg/g). However, some other investigators (Beauvallet et al., 1968) have, on the contrary, stated the highest content of noradrenaline in the whole rat brain in December (0.30 μg/g) and the lowest in April (0.13 μg/g). Garattini and Walzelli reported on serotonin and 5-HIAA content variations in the mouse brain tissue with peaks during the summer months (Walker, 1974). Some of this inconsistency between the reported data may be attributed to the use of experimental animals of different genetic strains and sexes or it may be put down to differences in keeping conditions. To some extent, the rhythmical variations in the monoamine content may depend on the sex of the rodents, the main trends for both sexes being essentially the same. There are differences only in the absolute values of the content. In females they may be somewhat higher.

Our investigations were carried out twice a month in the course of two years. The individual plotted monthly data represent the percentage of yearly averages measured over a fortnight period in two years. Expressing the monthly data by means of a fortnight average eliminates some monthly fluctuations that seem to be of inconsiderable significance for the overall seasonal pattern; besides, the basic trends over the annual period are better illustrated. This facilitates a comparison of the basic trends, especially when there is a difference in the absolute values of the experiments to be compared. The two-year mean value for serotonin was 0.68, for noradrenaline 0.46 and for dopamine 1.50 μg/g, respectively. The data presented in this report confirm the seasonal nature of the biogenic amine rhythms in the whole brain in mice. While the annual mean value of 5-HT was 0.68 μg/g, the two significant peaks in the serotonin content over a 12-month period coincide with June (147%) and December (128%). The increase in the serotonin content begins in

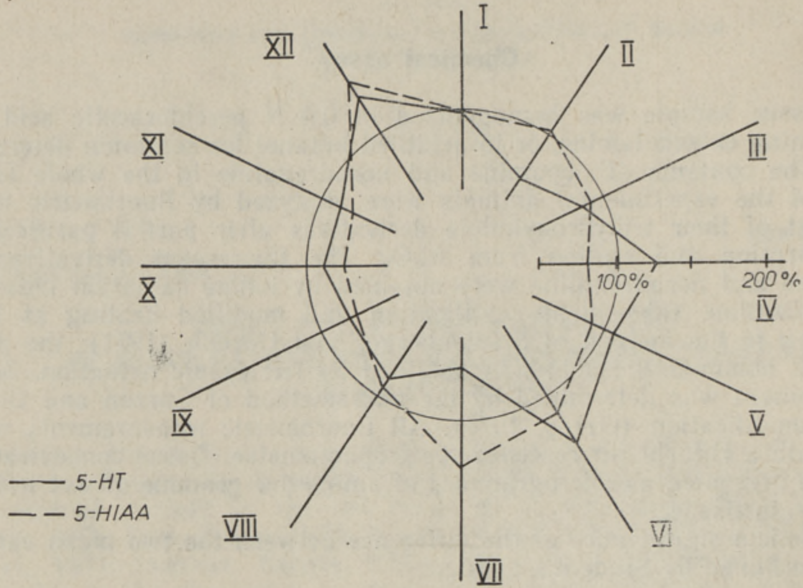

Fig. 1. Annual changes in the contents of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) of mouse brain (% of the annual mean values).

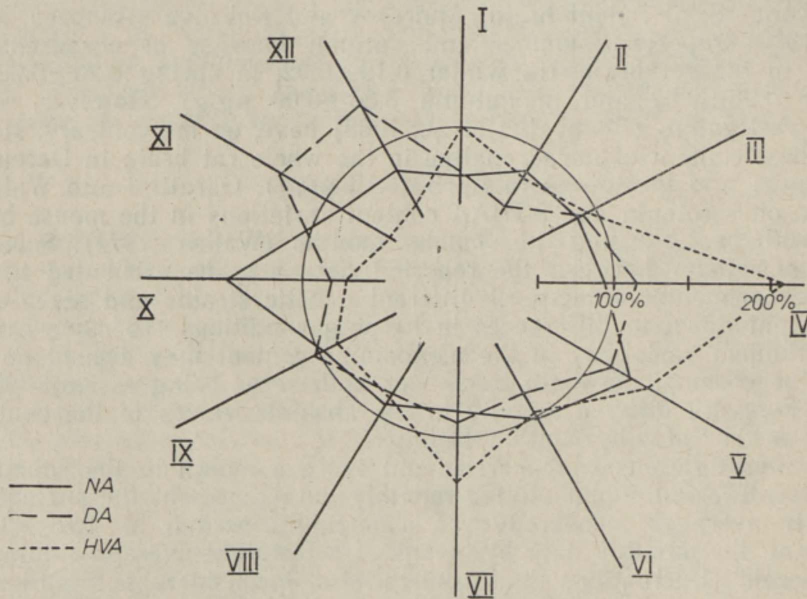

Fig. 2. Annual changes in the contents of noradrenaline (NA), dopamine (DA) and homovanillic acid (HVA) of mouse brain (% of the annual mean values).

April and remains high throughout the spring. Another increase occurs in December, being in good correlation with the fluctuation of 5-HT levels in the rat brain (Оттер, 1980). The metabolism of serotonin, as shown by the increase of the 5-HIAA content, is heightened during the summer months and in December (Fig. 1).

As for 5-HT, a clearly manifested rhythm in the catecholamine content can be established for a 12-month period. The content of dopamine is at its highest in April-May (120%) and November-December (140%). The metabolism of DA via HVA is increased during the spring months. The monthly values indicate that the major peaks for the noradrenaline content occur in May (133%) and October (159%), with minimum values in the winter months (Fig. 2).

These data of our experiments on seasonal variation of the biogenic amine content in mouse brain are in good correlation with the information concerning the activation of the metabolic processes in rodents in general and in spring in particular (Голиков, Голиков, 1973). Our data seem to be in good accordance with some other seasonal variations in the physiological functions in animals, such as sexual activity (Голиков, Голиков, 1973), cardiovascular function, sensitivity to drugs, their degree of toxicity in particular (Walker, 1974), etc.

A marked rhythm could be observed in the levels of serotonin content within the 24-hour period (Table). The mean values indicate that the serotonin content is higher during the period of light and lower during the dark phase. The comparison of peak values during the 24-hour period (0.71 μg/g at 12.00) reveals a 26% flux in the serotonin levels. The peak value for the 5-HIAA content is threefold higher than the mean, indicating a very active serotonin metabolism during the daytime. A comparison of the highest and lowest levels of the serotonin content with the overall 24-hour mean (0.63 μg/g) indicates statistically significant differences, P being <0.02 for the highest and lowest levels, respectively. The comparison of the phasing of the serotonin rhythm with the typical motor activity rhythm of rodents reveals that the maximum serotonin levels correspond to the animals being at rest and the minimal levels to their activity (Schewing et al., 1968; Lemmer, Berger, 1978a, b).

The available information on periodicity phenomena in the brain in connection with serotonin is apparently limited to the papers by Albrecht et al. (1956), and Morgan, Yndo (1973) who stated a time-dependent fluctuation in the levels of 5-HT in the mouse brain. Comparing the results of the present study with those of Albrecht et al. (1956), a similarity can be established even in the absolute values of serotonin.

As mentioned above, the amplitude of the serotonin rhythm fluctuations in the brain is small; it is only a minor fraction of that in the pineal gland where serotonin demonstrates a 900% flux. But the rhythms of serotonin content in the whole brain tissue as demonstrated by our data and in the pineal gland as demonstrated by Quay (1964) are identical in phasing and pattern. Similar serotonin fluctuation phasing patterns in the brain and the pineal gland suggest a strong synchronization in the serotonin content according to light-dark alterations even

Biogenic amines and their metabolites (microgrammes/g) measured in the whole mouse brain at 6-hour intervals over a 24-hour period in April

Hour	Noradrenaline	Dopamine	HVA	5-HT	5-HIAA
06.00	0.35±0.02	1.07±0.06	0.20±0.03	0.55±0.04	0.51±0.04**
12.00	0.49±0.01**	1.33±0.10*	0.22±0.04	0.71±0.03**	0.20+0.04
18.00	0.47±0.02**	1.28±0.08*	0.21±0.03	0.67±0.03**	0.30±0.03
24.00	0.43±0.02	1.15±0.04	0.27±0.02	0.57±0.02	0.42±0.009**

* $P \leq 0.05$.
** $P \leq 0.02$.

though serotonin has presumably a melantonin precursor function in the pineal gland and a neuronal function in the brain.

There are no investigations available on circadian rhythms in noradrenaline and dopamine levels in the mouse brain. In this experiment the level of dopamine was significantly higher during the light period, and the metabolism of dopamine via HVA was greater at midnight. The levels of noradrenaline in the whole brain of mice over the 24-hour period showed a 40% increase at 12.00 in comparison with the lowest figures at 6.00. The increase was statistically significant ($P<0.02$). When the daily mean was compared with the overall 24-hour mean, the differences were statistically significant ($P<0.02$).

Our data are somewhat similar to those reported by Lemmer and Berger who observed, during a light-dark cycle of 12 : 12 hr, a daily rhythm in the accumulation of catecholamines synthesized from circulating ^{3}H-tyrosine in the rat brain. In the middle of the light period, the accumulation of ^{3}H-catecholamines was significantly higher than in the middle of the dark period (Lemmer, Berger, 1978a, b).

The speed of 5-HT metabolism seems to be inversely proportional to that of dopamine. During the daytime the content of 5-HIAA in the mouse brain was low, and the content of HVA unchanged or high. Schewing et al. (1968) have found serotonin levels to be highest in the rat brain during the normal sleep time of the colony. Norepinephrine secretion in the brain, on the other hand, has been suggested as a contributor to the triggering of paradoxal sleep, which is associated with eye movement, muscle atony, and EEG patterns of arousal, and in the human with dreaming.

Certain parallels may be drawn between the daily variations of various parameters of monoamine metabolism in the rodent brain and the other biochemical and physiological processes, such as plasma corticosterone levels (Asano, Morgi, 1974), efficiency of drug toxicity (Walker, 1974), etc.

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HIIRE AJU INDOOL- JA KATEHOOLAMIINIDE SISALDUSE ÖÖPÄEVANE JA AASTARINGNE RÜTMIKA

Artiklis on esitatud aastail 1978—1980 korraldatud süstemaatiliste biokeemiliste katsete tulemused hiire kogu aju indool- ja katehoolamiinide sisalduse kohta. Kahe aasta keskmisena oli aju toorkaalus serotoniini 0,68, noradrenaliini 0,46 ja dofamiini 1,50 µg/g. Biogeensete monoamiinide sisalduses ja nende metabolismi kiiruses toimuvad aasta kestel märkimisväärsed kõikumised. Serotoniinisisalduse kõrgpunktid on juunis (147% aasta keskmisest) ja detsembris (128%), ka selle ainevahetus on suvekuudel ja detsembris kiirem (otsustades peamise metaboliidi, 5-hüdroksüindooläädikhappe sisalduse tõusu järgi). Dofamiinisisaldus on kõrgeim aprillis-mais (120%) ja novembris-detsembris (140%), katabolism kiire kevadkuudel, millest annab tunnistust suur HVH-sisaldus. Noradrenaliini tippsisaldused esinevad mais (133%) ja oktoobris (159%), minimaalsed talvekuudel.

Ööpäeva lõikes on serotoniinisisaldus kõrge ja ainevahetus kiire valgusperioodi vältel. Noradrenaliini- ja dofamiinisisaldus on ka keskpäeval kõrge (võrreldes ööpäevase keskmisega), kuid ringkäik on kiireim südaöõl.

Маргареете ОТТЕР

СУТОЧНАЯ И СЕЗОННАЯ РИТМИЧНОСТЬ СОДЕРЖАНИЯ ИНДОЛ- И КАТЕХОЛАМИНОВ В МОЗГУ БЕЛЫХ КРЫС

В результате систематических опытов, проведенных с 1978 по 1980 г., выяснилось, что годовое среднее содержание серотонина составляет 0,68, норадреналина 0,46 и дофамина 1,50 *мкг* на 1 *г* веса мозга. Однако в содержании биогенных моноаминов и в скорости их метаболизма происходят заметные колебания. Содержание серотонина наивысшее в июне (147% от годового среднего) и в декабре (128%), и метаболизм его ускорен в летние месяцы и в декабре, судя по высокому содержанию его главного метаболита 5-ОИУК. Содержание дофамина высокое в апреле—мае (120%) и в ноябре—декабре (140%), катаболизм же ускорен в весенние месяцы, о чем говорит высокое содержание ГВК. Пик содержания норадреналина наблюдается в мае (133%) и в октябре (159%), минимум в зимние месяцы.

В течение суток содержание серотонина и его метаболизм изменяются в зависимости от светового периода (днем выше; метаболизм ускорен). Содержание норадреналина и дофамина высокое в полдень, а метаболизм, наоборот, ускорен в полночь.