Possible Circadian and Seasonal Rhythmicity in an in vitro Model: Monoamine Uptake in Rat Brain Slices[1]

Circadian rhythms have been demonstrated for many biochemical parameters in the rat[2], as well as for regional brain fluctuations in endogenous concentrations of noradrenaline (NA) or serotonin (5HT)[3–5], histamine[4], dopamine (DA)[3], GABA[6], glycine[7], and acetylcholine[8]. The regulation of the endogenous concentration may be determined by circadian variations in the rate of synthesis[9–11], liberation from nerve endings[11], or inactivation, dependent or not on an external Zeitgeber such as light. While it is of great interest to know which aspects of monoamine metabolism are susceptible to daily fluctuations, we investigated the specific uptake mechanisms with selective high affinities which have been found for several putative neurotransmitters[12].

Experimental. Male or female (ovariectomized 3–6 weeks previously) Wistar rats were housed for at least 3 weeks 3 to a cage in the animal room kept at 22 ± 2 °C, with a light/dark schedule 06.00 h to 18.00 h. Particular care was taken to ensure conditions of minimal handling and stress, the rats not being disturbed for cage cleaning for at least 3 days before the experiment. Rats were killed at 08.00 h and 20.00 h, the times of minimum and maximum activity after onset or extinction of light, brain regions dissected and slices prepared for incubation using methods previously described[13,14]. In the female group, uptake of ^{3}H-DA and ^{3}H-5HT (1×10^{-7} *M*) was measured after 10 min incubation (linearity of uptake) in Krebs-Ringer bicarbonate glucose-supplemented medium containing nialamide (1.25×10^{-5} *M*) to prevent metabolism of the amines, with slices from cortex, hypothalamus, thalamus, midbrain, and striatum. In the male group, hippocampus and pons and medulla were also investigated; in addition, uptake of ^{3}H-NA and ^{3}H-GABA (1×10^{-7} *M*) as well as DA and 5HT was measured. TLC of the perchloric acid extract in butan-1-ol: acetic acid: water, (12 : 3 : 5 by volume) indicated > 90% original unmetabolized amine after incubation. The Wilcoxon test for matched pairs[15] was used to analyze the data, and the T/M ratios expressed in terms of the median instead of the mean (due to left-skew distribution of the uptake data).

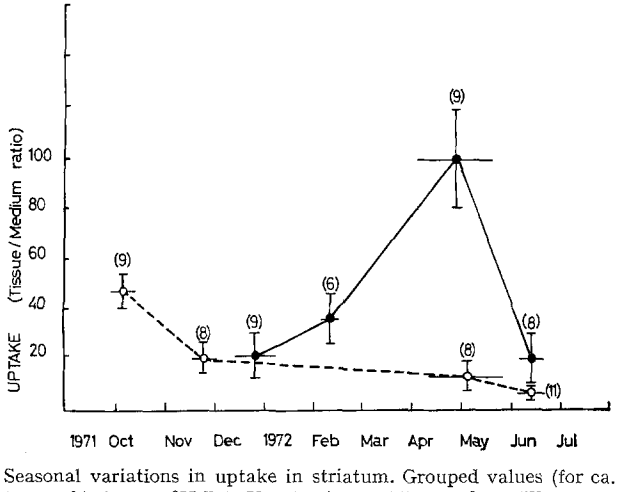
Results. A significantly higher uptake at 08.00 h than 20.00 h was found (Table) in male rats for 5HT in the hippocampus, NA in the hypothalamus, and DA in the hypothalamus and striatum, whereas GABA uptake did not vary with time in any region, and was found for DA in cortex, striatum and midbrain of ovariectomized rats.

Chronological arrangement of the tissue/medium (T/M) data from a similar series using ovariectomized rats[14], where the experiments were carried out over a longer period of 7 months, resulted in apparent subgroups of data (the radioactive substrates were regularly checked as before for purity by TLC, and new batches, kept at −70 °C, used every 8 weeks). The same seasonal pattern was found in all brain regions, but most noticeably in the striatum (Figure), DA uptake in March-April being 3 times as high as the average yearly value, whereas in contrast, 5HT uptake was twice as high in October than at other times of the year. The opposing pattern for the 2 monoamines indicate that this observation is not an artefact of a longitudinal study.

While this seasonal data is not susceptible to statistical analysis (requiring data obtained with identical methods over several years), it is worth noting as a possible source of variation in long-term studies.

Discussion. The higher uptake at 08.00 h than at 20.00 h could be interpreted as nonspecific were it not for the regional differences found where the postulated neurotransmitter has an important role. Re-uptake may therefore play a role in controlling cerebral monoamine levels, in this case, the daily fluctuations.

The annual rhythmicity of monoamine metabolism has been less extensively studied. Endogenous levels of NA have been noted to fluctuate widely within each rat species, with a low in early spring almost $^1/_3$ of the average yearly NA concentrations[16]; similarly, mouse brain 5HT levels have been recorded with a peak in winter 25% above the lowest summer value[17]. Interpretation of a possible seasonal variation in uptake is difficult but intriguing. Studies on monoamine levels in the frog[18] have shown that the opposite seasonal brain



Seasonal variations in uptake in striatum. Grouped values (for ca. 1 month) for ●, ^{3}H-DA Uptake (n = 32); ○, ^{3}H-5HT uptake (n = 36).

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Significant differences in neurotransmitter uptake at 08.00 h and 20.00 h

Neurotransmitter	Region	Uptake (as median tissue/medium ratio) 08.00 h	20.00 h	Significance[a] (p)
Male rats (n = 14)				
DA	Hypothalamus	17.6	15.8	< 0.05
DA	Striatum	65.2	52.2	< 0.01
NA	Hypothalamus	19.6	17.3	< 0.05
5HT	Hippocampus	16.8	13.8	< 0.05
Ovariectomized female rats (n = 14)				
DA	Cortex	16.0	10.3	< 0.05
DA	Striatum	62.5	52.5	< 0.01
DA	Midbrain	14.5	13.0	< 0.05

Regions investigated: Cortex, hypothalamus, thalamus, midbrain, striatum, hippocampus, pons and medulla. [a] Wilcoxon test for paired values.

5HT and NA maxima are not simple effects of the external temperature, although this is contributory. Seasonal rhythms occur in many vertebrates primarily in relation to endocrine functions. Such a seasonal adaptive capacity might be expected to have an important selective value which is retained even in laboratory animals kept under controlled conditions.

Rhythmic phenomena in the metabolism of the monoamines may have far-reaching consequences. For example, periodicity is a well-established characteristic of manic-depressive illness, whether it be its cyclic nature, whether the circadian mood changes often associated with alterations of sleep-waking patterns and corticosteroid rhythms, or the seasonal increase in depressions in autumn and early spring[19]. Metabolic rhythmicity of the amines thought to be involved in the aetiology of depression[20] may be one of the factors causing periods of increased susceptibility.

Zusammenfassung. Eine signifikante höhere Neurotransmitter-Aufnahme um 08.00 h als um 20.00 h wurde für Dopamin im Striatum und Hypothalamus, Noradrenalin im Hypothalamus, und Serotonin im Hippocampus von männlichen Ratten gefunden, aber keine Veränderung der GABA-Aufnahme. Ähnliche Unterschiede wurden für Dopamin im Cortex, Striatum und Mittelhirn von ovarektomierten Ratten gefunden. Ausserdem wurde eine gegenläufige jahresrhythmische Veränderung der Dopamin- und Serotonin-Aufnahme im Striatum beobachtet.

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Plasma Enzyme Activities in Rats with Diet-Induced Alterations in Liver Enzyme Activities[1]

A variety of intracellular enzymes can be found in minute quantities in the blood[2]. The plasma level of an enzyme clearly depends upon the rate of its loss from tissues and the rate of its clearance from plasma. However, a lack of specific information on this topic and the variety of organs possessing a particular enzyme, make it difficult to estimate the basic determinants of the activity of an enzyme in the plasma.

Table I. Diet composition (% by weight)

	20 P	45 P
Casein	20.0	45.0
Sucrose	63.7	38.7
Corn Oil	10.0	10.0
Salts Mix	3.8	3.8
Vitamin Mix	2.5	2.5
Caloric density	4.11 cal/g	4.11 cal/g

Physical training leads to an increase in the resting level of lactic dehydrogenase (LDH) in the plasma of man[3], and of glutamic-oxalacetic transaminase (GOT) in the plasma, and skeletal and cardiac muscle of rats[4]. The increased resting levels of plasma enzyme activity following training could result possibly from either an increase in the level of their activity in certain tissues, or the repeated loss of tissue enzyme during the exercise comprizing the physical training. This latter mechanism seems unlikely since HUNTER and CRITZ[3] have found that in man the elevation of plasma LDH occurring during maximal exercise was eliminated by physical training. This suggests then, that the amount of tissue activity of an enzyme could be an important determinant of the plasma activity of that enzyme.

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