CIRCADIAN VARIATION IN UPTAKE OF TRYPTOPHAN BY SYNAPTOSOMES FROM THE RAT CORTEX

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The concentration of 5-HT in the brain varies rhythmically during each 24-hour cycle. This variation which results from circadian changes in both the rate of synthesis in and the rate of transmitter release from 5-HT containing neurones can be shown in turn to have a significant influence on the efficacy of drugs e.g. antidepressants, which act predominantly on these cells. Hydroxylation of tryptophan (TRY) is believed to be the rate-limiting step in the synthetic process, but since the hydroxylase enzyme is rarely saturated with its substrate the availability of TRY could also be important in modulating the rate of synthesis. We have therefore measured the circadian variation in the high-affinity TRY uptake process in the rat cortex.

Synaptosomes were prepared by homogenising cortical tissue, freshly obtained from male Wistar rats, in sufficient ice-cold 0.32M sucrose pH 7.2 (Tris-HCl buffer 0.05M) to give a 10% $^{w}/_{v}$ homogenate. The homogenate was centrifuged at 1000rpm for 10 min at 4°, and the supernatant removed and centrifuged at 35,000g for 30 min. The final pellet was resuspended in Krebs-bicarbonate buffer to produce a 10% $^{w}/_{v}$ suspension.

Uptake of TRY was measured by incubating 200 µl of the synaptosomal suspension with 600 µl Krebs containing 0.2 µl L-(5-^{3}H)-TRY and sufficient unlabelled TRY to give a final concentration in the range of 0.01-0.1mMol. Incubations were terminated by rapid filtration through Whatman GF/F filters. The accumulated TRY was measured by liquid scintillation counting. Estimates of Km and Vmax were carried out using the direct linear plot of Eisenthal and Cornish-Bowden (1974), and statistical significance was assessed by analysis of variance.

Table 1 summarises the characteristics of the uptake process measured at 3-hourly intervals throughout the 12:12 light-dark cycle.

Table 1 - Uptake of TRY in synaptosomes of the rat cortex. (mean ± sem, n = 10)

Time (Hours after lights-on)	3 LIGHT	6	9	11.5	15 DARK	18	21	23.5
Km ($x10^{-5}$)	5.8±0.6	5.0±0.5	4.8±0.4	6.4±0.7	4.3±0.4	4.2±0.4	5.6±0.4	6.5±0.9
Vmax ($x10^{-9}$moles.mg^{-1}.5min^{-1})	8.1±1.6	5.1±0.5	6.8±0.6	6.7±0.8	4.8±0.5	5.1±0.4	6.4±0.5	10.4±2.3

There is significant variation with time in both Km [$F(9,87)=2.36, p<0.05$] and Vmax [$F(9,87)=2.53, p<0.05$]. Most estimates suggest that the extraneuronal concentration of TRY lies in the region of 1-10 µM. (Knowles & Pogson, 1984). Calculation of the relative affinity (Km/Vmax ratio) shows that within this range there is little evidence of circadian variation in TRY uptake. It appears rather that the system is designed to maintain a constant rate of entry of TRY in the face of circadian or ultradian fluctuations in extracellular TRY levels. Only in the presence of much higher extracellular TRY concentrations, as might be produced by drug intervention, are changes in the uptake process likely to influence the circadian variation in the rate of 5-HT synthesis.

Eisenthal R. & Cornish-Bowden A. Biochem. J. 1974, 139: 715 - 720.
Knowles R. G. & Pogson C. I. J. Neurochem. 1984, 42: 679 - 684.