A CIRCADIAN RHYTHM IN 5-HYDROXYTRYPTOPHAN DECARBOXYLASE?

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A 24 hour variation in brain concentrations of 5-hydroxytryptamine (5-HT) is well recognised and disruption of this rhythmic variation has been implicated in the underlying biochemistry of endogenous depression. The mechanisms responsible for regulating 5-HT concentrations over 24 hours are less clearly understood although most available evidence suggests that in the rat brain either enzymatic hydroxylation or uptake of tryptophan (TRY) provides the endogenous regulation.

Preliminary findings in our laboratory indicated a 24 h rhythm in 5-hydroxy-tryptophan (5-HTP) decarboxylase activity (Hillier & Redfern 1976). Kinetic studies on 5-HTP decarboxylation were therefore undertaken. Brains were taken from rats killed at two clock hours corresponding to the peak and trough of the decarboxylase activity obtained in the previous work. Brains were homogenized in a glass homogenizer (clearance .01") to form a synaptosome suspension. Homogenates were centrifuged at 1500g for 10 min, the supernatant collected and diluted (1 vol + 3 vols 0.32M sucrose), and 1ml of this preparation was made up to 2.2ml to form the final incubation mixture. This contained 0.45×10^{-4}M pargyline and 1.82×10^{-5}M pyridoxal phosphate adjusted to pH 8.0 with phosphate buffer. The mixture was preincubated for 10 min at 37°C before addition of the 5-HTP. The incubation was then allowed to proceed for 15 min and stopped with 0.1ml of 4N sulphuric acid. 5-HT was extracted and assayed by adapting the method described by Snyder & others (1965). This involved an organic solvent extraction followed by fluorimetric determination of 5-HT. Any small amount of substrate that might be extracted does not interfere as it yields only 2% of the fluorescence of 5-HT.

The experimental data were displayed in the traditional form of Lineweaver-Burk plots for the two clock hours and were also analysed statistically by fitting rectangular hyperbolae using a maximum likelihood computer program (MLP, Ross and others) The Km and Vmax values are shown in Table 1. The statistical analysis showed a highly significant separation between the lines but no significant difference between their slopes. The functional significance of this observed difference for the rate of 5-HT synthesis in rat brain can only be assessed after measurement of tryptophan 5-hydroxylase activity under similar circumstances.

Table 1.

Clock hour	Vmax	Km
05.00	0.510 µmol $g^{-1}h^{-1}$	1.39×10^{-5}M
17.00	0.465 µmol $g^{-1}h^{-1}$	1.28×10^{-5}M

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