VARIATION WITH TIME OF DAY IN SPECIFIC BINDING TO 5-HT_1 RECEPTORS IN RAT CORTEX

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It is now clear that 5-HT receptors can be divided into two types, designated 5-HT_1 and 5-HT_2 on the basis of binding studies by Peroutka & Snyder (1976). Behaviours evoked in rodents by stimulation of the 5-HT_2 receptor display a marked circadian variation (Moser & Redfern, 1984), whereas the results of stimulation of the 5-HT_1 receptor are apparently immune to circadian influence (Moser & Redfern, 1985). It seemed likely that lack of a circadian rhythm in 5-HT_1-mediated behaviours reflected a similar stability in receptor sensitivity. We have therefore investigated the binding characteristics of 5-HT_1 receptors in rat cortex at mid-dark and mid-light, time points corresponding to the peak and trough in the rhythm of presynaptic 5-HT release.

Homogenates of cerebral cortex in 10 volumes ice-cold 0.32 M sucrose were prepared from male Wistar rats housed under a 12:12 light:dark cycle for at least 14 days before experiment. The homogenate was centifuged at 1,000 g for 10 min, and the resulting supernatant at 50,000 g for 10 min. The pellet from this second centrifugation was resuspended in 10 volumes 50 mM Tris-HCl buffer (pH 7.5) before a 10 min incubation at 37°, to destroy endogenous 5-HT. After a further centrifugation at 50,000 g, the pellet was resuspended in standard assay buffer (50μ M tris-HCl buffer, pH 7.7, 10μ M pargyline, 0.1% ascorbic acid, 4 mM $CaCl_2$, 0.1% BSA). Incubation mixtures consisted of 0.8 ml of the membrane preparation (10 $mg.ml^{-1}$) prewarmed to 37°, and 0.1 ml of varying dilutions of ^{3}H-5-HT (specific activity 14 $Ci.ml^{-1}$) in 10^{-5}M 5-HT or 0.1 ml standard assay buffer. Incubation was stopped after 10 min by rapid filtration under vacuum through Whatman GF/B filters. After washing, and extraction for at least 18 h, radioactivity was measured by liqud scintillation counting. Under these conditions, specific binding accounted for up to 80% of total. Displacement with unlabelled 5-HT gave an IC_{50} value of 15 μM; Scatchard plots indicated that the binding sites constituted a single population.

TABLE 1. ^{3}H-5HT binding in rat cerebral cortex Mid-light and Mid-dark.

Kd (nm)		Bmax (pmoles g^{-1})	
Mid-light	Mid-dark	Mid-light	Mid-dark
3.3	10.3	7	12
3.8	21	8	11.5
3.2	11.4	9	13
5.3	8	13.5	11.5
3.3	12.9	7	6.5

Table 1 summarizes the results of 5 separate experiments in which the kinetics of binding were compared at mid-light and mid-dark. There is a highly significant difference between the Kd values measured mid-light and mid-dark; Bmax values were not significantly different. Thus, contrary to our expectation, it is possible to demonstrate a time-of-day related variation in the Kd of 5-HT_1 receptors. Whether this relates to a circadian change in receptor conformation, or whether binding is influenced by the presence of some modulating substance which persists in the membrane preparation remains to be elucidated. If, however, this finding is confirmed, it means that we must look elsewhere for the site of the mechanism which ensures that the rhythmic variation in 5-HT release is not reflected in a similar variation in 5-HT_1-receptor mediated behaviours.

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