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Short communication

Circadian rhythm of the B_{max} of [^{3}H]-imipramine binding in rabbit platelets

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Summary. [^{3}H]-imipramine binding was measured in rabbit blood platelet membranes on a 24 h cycle. Animals were kept on a 14 h light (L) 10 h dark (D) schedule, and blood samples were collected at L + 2, L + 8, D + 2, D + 8 and L + 2 h on a following cycle. Significant differences were found for B_{max} values of [^{3}H]-imipramine binding, with highest values during the dark phase and lowest during the light phase. No significant differences were found in K_d values. These results suggest the existence of a circadian rhythm for the B_{max} of [^{3}H]-imipramine binding in blood platelets.

Key words: [^{3}H]-imipramine binding – Rabbit platelets – Circadian rhythm

Introduction

It is now well established that [^{3}H]-imipramine labels with high affinity a site which is associated with the serotonin transporter in brain and platelets (for reviews, see Langer et al. 1986; 1987). It has been reported by several laboratories that the B_{max} of [^{3}H]-imipramine in blood platelets is reduced in untreated depressed patients, and the use of platelet [^{3}H]-imipramine binding as a biological marker in depression represents a potentially useful tool (for review, see Poirier et al. 1986; Langer et al. 1987).

It is well established that serotonin (5HT) uptake in blood platelets of depressed patients is significantly decreased when compared to controls (Tuomisto and Tukiainen 1976; Coppen et al. 1978; Born et al. 1980). However, for both [^{3}H]-imipramine binding and [^{3}H]-5HT uptake in platelets of depressed patients, conflicting results have been reported, either the absence of differences or an increase of these parameters when compared with control values (Poirier et al. 1986; 1987 for discussion). One of the hypotheses taking into account these discrepancies is the existence of circadian variations for [^{3}H]-imipramine binding or [^{3}H]-5HT uptake. In fact, a circadian rhythm of 5HT uptake in the blood platelets of normal controls has been reported (Wirz-Justice and Richter 1979) and slight differences for the V_{max} of uptake between 10.00 AM and 4.00 PM samples were also described (Arora et al. 1984). In a more recent study, a modest decrease in the V_{max} values for [^{3}H]-5HT uptake into blood platelets at 2.00 PM was found (Modai et al. 1986).

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To our knowledge, there is at present no information on the existence of a circadian rhythm for [^{3}H]-imipramine binding in blood platelets. Therefore, we decided to study the variations of the binding of this ligand in rabbits because blood samples can be obtained from the same animals at different times of the day. Such an experimental approach reduces the interindividual variations which could mask the occurrence of a circadian rhythm.

Methods

Nine male albino rabbits (3–5 kg) were kept at 22° C, with food and water ad libitum, under a light:dark (LD) cycle of 14:10 h with lights on at 7.00 h. Blood samples of 15–20 ml were taken from the ear artery, at 9.00–10.00 h and 15.00–16.00 h (L + 2 and L + 8 respectively). Once all the light samples had been obtained, the rabbits were put on an reversed LD cycle, with lights off at 9.00 h, and were kept for three weeks in order to achieve resynchronization of their endogenous rhythms. On the fourth week of reversed LD cycle, blood samples were taken from the ear artery under a dim red light, at 11.00 h and 17.00 h (D + 2 and D + 8 respectively). For a group of 5 rabbits, a normal LD cycle was established, and after three additional weeks, blood samples were taken at 9.00 h (L + 2, second cycle). Membranes from rabbit platelets were prepared according to the method described by Langer et al. (1986) for human platelets. Blood was withdrawn into plastic tubes containing Na-citrate (0.38% final concentration). Platelet rich plasma was obtained by centrifuging blood samples twice at room temperature (100 × g for 20 min), and the platelet pellet was prepared by further centrifugation at 16,000 × g for 10 min at 4° C. Platelets were washed twice with buffer (5 mmol/l Tris-HCl, 20 mmol/l Na_2 EDTA, 150 mmol/l NaCl, pH 7.5) at 4° C, and exposed to hypotonic lysis in 5 mmol/l Tris-HCl containing 5 mmol/l EDTA (pH 7.5). After homogenisation (glass teflon Potter) and centrifugation at 39,000 × g for 10 min, the pellet was washed with 50 mmol/l Tris-HCl (pH 7.4) and finally resuspended in 50 mmol/l Tris-HCl buffer (pH 7.4) containing 120 mmol/l NaCl and 3 mmol/l KCl, at a concentration of 0.25–0.40 mg protein/ml. Aliquots of this membrane suspension (100 µl) were then incubated with [^{3}H]-imipramine (specific activity 888 GBq/mmol; Amersham, International Limited, Amersham, UK) at eight concentrations in duplicate (0.25–5 nmol/l) in a final volume of 300 µl. Following incubation for 60 min at 4° C, the samples were diluted, rapidly filtered over Whatman GF/F glass fiber filters and the radioactivity retained was

Table 1. B_{max} and K_d values of [^{3}H]-imipramine binding in rabbit blood platelets during a light-dark cycle. Shown are individual B_{max} and K_d values. [^{3}H]-imipramine binding was measured using 8 concentrations of [^{3}H]-imipramine between 0.25 and 5 nmol/l, each point determined in duplicate. Non specific binding was determined in the presence of 100 µmol/l desipramine. Rabbits were submitted to a 14 h – 10 h light-dark cycle (LD)

Rabbit	B_{max} (fmol/mg protein)				K_d (nmol/l)			
	L + 2	L + 8	D + 2	D + 8	L + 2	L + 8	D + 2	D + 8
1	3861	6077	4430	4858	1.37	1.87	2.90	1.36
2	2766	3475	4832	5829	1.73	0.82	1.06	1.23
3	3000	5145	3835	4915	3.17	2.28	2.03	2.43
4	2077	3495	4210	5859	2.71	2.24	4.78	2.00
5	4185	6013	6539	5925	1.41	2.13	2.56	2.61
6	4405	6444	7651	8532	2.42	1.70	3.66	2.10
7	6937	3399	7320	6318	2.18	1.73	2.78	1.78
8	3268	3798	5560	6173	1.73	1.01	1.15	0.84
9	5324	4875	6627	6265	2.06	1.57	1.02	1.65
x ± SEM	3980 ± 491	4747 ± 414	5667 ± 472*	6075* ± 355	2.09 ± 0.20	1.71 ± 0.17	2.44 ± 0.43	1.78 ± 0.19

* $p < 0.01$ when compared with respective values at L + 2 (Duncan test)

counted. Non specific binding was defined as residual binding observed in the presence of 100 µmol/l desipramine (desipramine-HCl, Ciba Geigy, Basel, Switzerland). Binding parameters were obtained by Scatchard analysis. Specific binding represented 70% of the total binding at the level of the K_d for [^{3}H]-imipramine binding. Protein was estimated by the method of Peterson (1977) using bovine serum albumin as the standard.

The parametric Duncan test (randomized blocks) was used to compare mean B_{max} values, and the non-parametric Kruskal-Wallis test to compare K_d values.

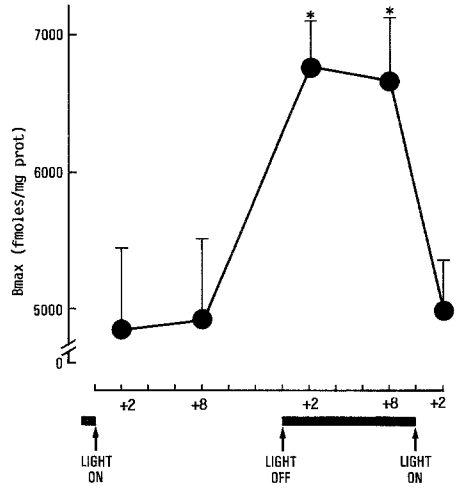

Fig. 1. Changes in B_{max} of [^{3}H]-imipramine binding in platelet membranes from 5 rabbits on a 24 h cycle. Shown are mean ± SEM (*vertical bars*) of B_{max} values of [^{3}H]-imipramine binding obtained by Scatchard analysis with 8 concentrations of [^{3}H]-imipramine between 0.25 and 5 nmol/l. * $p < 0.05$ when compared with the values at L + 2 and L + 8 (Duncan test)

Results

Using the experimental protocol described in Methods, a single class of high affinity [^{3}H]-imipramine binding sites could be demonstrated in rabbit platelets (data not shown).

The maximal number of binding sites (B_{max}) showed significant variations during a 24 h cycle, with low B_{max} values during the light period and high B_{max} values during the dark period (Table 1). The B_{max} values at D + 8 represent a 53% increase in density of [^{3}H]-imipramine binding sites when compared with the B_{max} measured at L + 2. Values of B_{max} of [^{3}H]-imipramine binding obtained within the light period (L + 2 and L + 8) or within the dark period (D + 2 and D + 8) were not significantly different. In contrast with the pronounced effect of sampling time on the B_{max} values of [^{3}H]-imipramine binding, no significant differences were found for the K_d values (Table 1). In a subgroup of 5 rabbits, an additional blood sample was obtained at L + 2, at the beginning of a second cycle, three weeks later. As shown in Fig. 1, there was no significant difference between the B_{max} values obtained at L + 2 on two consecutive cycles, and these values were significantly lower than those obtained during the dark periods.

Discussion

We found that the B_{max} values of [^{3}H]-imipramine binding in rabbit blood platelets follow a light-dark rhythmicity, with a maximum difference of 53%, while the K_d values were not significantly modified. Such differences could be attributed to changes at the level of the platelets or to the influence of plasmatic factors. There is no indication in the literature of important changes in the number of platelets or their size in human blood platelets (Modai et al. 1986), which could be causally related to the differences reported here in the B_{max} of [^{3}H]-imipramine binding. The mean half-life for platelets (4 – 5 days) is also too long to account for such circadian variations. The possible involvement of plasmatic factors appears more likely (Abraham et al. 1987). It has been reported that plasma from depressed and normal subjects inhibited the binding of [^{3}H]-imipramine to rat cerebral membranes, and that this inhibition was associated with plasma proteins (Barkai et al. 1986a). The inhibitory factor was tentatively identified as a soluble α_1-acid-glycoprotein, which competed with platelet membranes for [^{3}H]-imipramine binding (Barkai et al. 1986b). Similarly, the

recent findings of Abraham et al. (1987) suggest that the α_1-acid-glycoprotein enhances serotonin uptake while it inhibits [^{3}H]-imipramine binding in platelets through a competitive mechanism, an effect consistent with the observed circadian rhythm in B_{max} of [^{3}H]-imipramine binding. However, this type of competitive inhibition resulted in an increase of the K_d values without modification of B_{max} of [^{3}H]-imipramine binding, which may not explain our present observations of changes in B_{max} values during the LD cycle (Barkai et al. 1986b; Abraham et al. 1987).

Accumulating evidence indicates that the [^{3}H]-imipramine binding site and the 5HT uptake site, although closely linked, are not identical. Moreover, the site labelled with [^{3}H]-imipramine appears to be coupled allosterically to the 5HT recognition site of the carrier (Segonzac et al. 1985; Meyerson et al. 1987).

Therefore, the existence of an endogenous ligand different from 5HT and acting on the [^{3}H]-imipramine binding site has been postulated by several laboratories (Langer et al. 1986, for review). One of the possible candidates for the endocoid with a chemical structure close to the 5-methoxy substituted tryptoline has also been proposed (Langer et al. 1986). However, there is at present no formal identification of such a ligand, and methodological difficulties as well as the presence of possible artefacts in active fractions isolated from the rat brain have been recently emphasized (Lee et al. 1987). Nevertheless, our results do not exclude the existence of a circulating plasmatic factor, the levels of which could fluctuate during a 24 h cycle and thereby modulate the activity of the 5HT transporter through the site labelled with [^{3}H]-imipramine. The existence of a circadian rhythm of [^{3}H]-imipramine binding in the rat suprachiasmatic nuclei has been described (Wirz-Justice et al. 1983) and these findings were recently extended in other regions of the rat brain, namely occipital cortex, caudate putamen, raphe nuclei and lateral hypothalamus (Kraeuchi et al. 1986). The existence of a circadian rhythm for the B_{max} of [^{3}H]-imipramine binding in human blood platelets, and the comparison of this phenomenon between control and depressed patients would be of great interest in the understanding of the pathogenesis of this disease. In addition, such studies in human platelets may help to clarify the controversy concerning changes in B_{max} of platelet [^{3}H]-imipramine binding between control volunteers and depressed patients (Poirier et al. 1986; Langer et al. 1987).

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