Studies on the serotonin transporter in platelets

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Summary. [^{3}H]-Imipramine and [^{3}H]-paroxetine label with high affinity a recognition site which is associated with the serotonergic transporter in blood platelets. The pharmacological profile of [^{3}H]-imipramine and [^{3}H]-paroxetine binding is highly correlated with the potency of drugs to inhibit the uptake of serotonin. Dissociation kinetic experiments suggest that the substrate recognition site for serotonin may be different from the modulatory site which is labeled with [^{3}H]-imipramine or [^{3}H]-paroxetine. The existence of an endocoid acting on the imipramine receptor to modulate the serotonin transporter has been proposed by several laboratories. In clinical studies most laboratories have reported a decrease in B_{max} of [^{3}H]-imipramine binding in platelets from depressed untreated patients when compared with matched healthy volunteers. The B_{max} of [^{3}H]-imipramine binding in platelets appears to be a state-dependent biological marker in depression.
Key words. [^{3}H]-imipramine binding; [^{3}H]-paroxetine binding; serotonin transporter; blood platelets; depression.

Introduction

Tricyclic antidepressant drugs represent the most widely prescribed therapy for affective disorders. In spite of their widespread use, their mechanism of action in depression has still not been completely elucidated. The clinical manifestation of therapeutic effects of antidepressant drugs generally appears after one to two weeks of treatment, thus implying that a degree of neuronal adaptation under the effect of antidepressants is required for clinical efficacy. Radioligand binding studies using mostly [^{3}H]-labeled antidepressants have been used extensively in recent years in attempts to identify the sites of action of these drugs. [^{3}H]-Imipramine was among the first antidepressants for which a high affinity binding site could be demonstrated, and evidence has accumulated to indicate that the high affinity [^{3}H]-imipramine binding site identified in the brain and platelets of several species, including humans, is associated with the neuronal uptake of serotonin[19–22, 42]. Current evidence suggests that the [^{3}H]-imipramine binding site may not be identical with the substrate recognition site of the serotonin transporter, but may represent a novel presynaptic receptor that functions to modulate serotonin uptake[14, 16, 26, 28, 30]. Furthermore, high affinity binding sites for other tricyclic ([^{3}H]-cyanoimipramine) and non-tricyclic ([^{3}H]-paroxetine, [^{3}H]-indalpine, [^{3}H]-norzimelidine) antidepressants were shown to be associated with the neuronal serotonin transporter.

Methods

Membranes from human and rabbit platelets were prepared according to the methods described by Langer et al.[20] and Raisman et al.[43]. [^{3}H]-Imipramine binding was measured according to the methodology described by Raisman et al.[43]. Briefly, blood was withdrawn into plastic tubes containing Na-citrate. Platelet rich plasma was obtained by centrifuging blood samples twice at room temperature (100 × g for 20 min) and platelets pellet was prepared by further centrifugation at 16.000 × g for 10 min at 4 °C. Platelets were then washed twice with buffer (5 mM Tris-HCl, 20 mM Na_2 EDTA, 150 mM Tris-HCl containing 5 mM EDTA (pH 7.5)). After homogenization (glass Teflon Potter) and centrifugation at 39,000 × g for 10 min, the pellet was washed with 50 mM Tris-HCl (pH 7.4) and finally resuspended in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 3 mM KCl, at a concentration of 0.25–0.40 mg protein/ml (rabbit) or 0.5–0.75 mg protein/ml (human).

Results and discussion

Using the methodology described above, high affinity [^{3}H]-imipramine binding sites have been demonstrated in platelets[11, 20]. The dissociation constant (K_d) of [^{3}H]-imipramine for its binding site in platelets of different species is usually between 0.6 and 3 nM, depending on the experimental conditions used. Among the factors contributing to this relatively large variation of the affinity of [^{3}H]-imipramine, a heterogeneity of [^{3}H]-imipramine binding sites has been reported by several authors[4–6]. The K_d of [^{3}H]-imipramine, at least in human platelet membranes, depends also to a certain extent, on the membraneous protein concentrations[4, 7]. The maximal binding density (B_{max}) of [^{3}H]-imipramine in human platelet membranes is generally estimated to be approximately 1000 fmol/mg protein as determined by the 'rapid filtration' technique[20], although this value can be slightly overestimated as suggested by recent results obtained with 10 μM fluoxetine to define non-specific binding. Platelet [^{3}H]-imipramine B_{max} values also appear to be species-dependent. Thus, as compared to human platelets, higher B_{max} values are observed in rabbit[15] and cat platelets[13]. The high affinity [^{3}H]-imipramine recognition site possesses most of the properties of a pharmacological receptor[17].

The high affinity binding of [^{3}H]-imipramine is inhibited with high affinity by tricyclic antidepressants and by non-tricyclic inhibitors of the neuronal uptake of serotonin, with IC_{50} values in the nanomolar range[52] (table 1), while serotonin, the only neurotransmitter that inhibits [^{3}H]-imipramine binding, has an IC_{50} in the low micromolar range (table 1). [^{3}H]-Imipramine binding to membranes from blood platelets, follows a pharmacological profile identical to that established for the inhibition of [^{3}H]-serotonin uptake into platelets[51]. The discrepancies reported for several drugs between their ability to affect [^{3}H]-imipramine binding and [^{3}H]-serotonin uptake can be explained through the thermodynamics of both experimental protocols[47, 51], since [^{3}H]-imipramine binding is carried out at 0 °C, while serotonin transport is performed at 37 °C. Radioligand binding studies to the serotoninergic transporter at 37 °C have now become possible, using a new selective non-tricyclic serotonin uptake inhibitor, [^{3}H]-paroxetine[34, 37, 51]. The pharmacological profile of [^{3}H]-paroxetine binding to human platelet membranes at 37 °C is significantly correlated with that of [^{3}H]-serotonin uptake inhibition. The correlation of K_i values for drug inhibition of [^{3}H]-serotonin uptake with K_i values obtained on [^{3}H]-paroxetine binding at 37 °C is significantly better than that obtained with drug K_i values measured at 0 °C using [^{3}H]-imipramine as the radioligand[51] (table 1).

These results support the view that [^{3}H]-imipramine binding sites are associated with the transporter for the serotonin uptake system. Moreover, there is evidence in favor of the hypothesis that the [^{3}H]-imipramine and the substrate recognition sites of the transporter are not identical. In platelets,

Table 1. Drug affinity for inhibition of radioligand binding to and [^{3}H]-5 HT uptake by the serotonergic transporter in human platelets

Drugs	[^{3}H]-imipramine binding (0 °C) K_i (nM)	[^{3}H]-paroxetine binding (37 °C) K_i (nM)	[^{3}H]-5 HT uptake (37 °C) K_i (nM)
Chlorimipramine	3.2	2.7	2.3
Desmethylchlorimipramine	2.9	22.7	41.0
Imipramine	3.0	31.5	29.4
Desipramine	31.1	229	238
5-Methoxytryptoline	24.7	865	333
Paroxetine	2.0	1.7	1.8
Citalopram	6.7	22.0	12.9
Femoxetine	26.3	63.3	43.1
Indalpine	1.1	13.9	8.2
Fluoxetine	4.5	16.8	28.1
5 HT	2000	3300	–
Tryptamine	2000	7300	–

The pharmacological profile of the 5 HT transporter in human platelets was studied using [^{3}H]-imipramine binding to washed membranes from outdated platelet-rich plasma at a radioligand concentration of 0.6 nM and an incubation temperature of 0 °C [50]. Similarly, radioligand binding to the 5 HT transporter was studied at 37 °C using [^{3}H]-paroxetine at a concentration of 0.1 nM [51].

serotonin allosterically affects [^{3}H]-imipramine binding, and in particular, produces a pronounced decrease in the rate of dissociation of [^{3}H]-imipramine from its receptor, as compared to the rate of [^{3}H]-imipramine dissociation in the presence of fluoxetine [38, 46, 49, 57]. This property of serotonin is shared by tryptamine, a substrate for the serotonin transporter in rabbit and human platelets [48, 49]. Thus, the imipramine recognition site might be a novel type of receptor that modulates the serotonin transporter in platelets. Recently, a similar interaction of serotonin with the [^{3}H]-paroxetine binding site in platelet membranes has been reported [38]. It follows from this concept that an endogenous ligand for the [^{3}H]-imipramine labeled modulatory site may exist, which is different from serotonin. A factor extracted from the rat brain that inhibits both [^{3}H]-imipramine binding and [^{3}H]-serotonin uptake, was reported by Barbaccia et al. [5, 6] and Rehavi et al. [45]. However, the biological significance of this factor extracted from the rat brain has been recently questioned [31]. Similarly, a fraction from human plasma and rat serum which inhibits both [^{3}H]-serotonin uptake and [^{3}H]-imipramine binding in rat synaptosomes and human platelets has been described [2, 3]. It has also been recently reported that plasma from normal as well as depressed subjects inhibited the binding of [^{3}H]-imipramine to rat cerebral membranes, and that this inhibition was associated with plasma proteins [8]. The inhibitory factor was tentatively identified as a soluble α_1-acid-glycoprotein, which inhibits [^{3}H]-imipramine binding while enhancing serotonin uptake in platelets [1, 9]. However, these findings do not exclude the existence of low molecular weight factors that could inhibit simultaneously [^{3}H]-imipramine binding and [^{3}H]-serotonin uptake. Of special interest are several tryptoline derivatives substituted in the 5 position [24, 25]. These compounds are linked to the metabolism of tryptophan and result from the condensation of indoleamines with formaldehyde [32] or from a one-carbon substituted tetrahydrofolate. As 5-methoxytryptoline potently inhibits both serotonin uptake and [^{3}H]-imipramine binding in the brain and in blood platelets, it has been suggested that this tricyclic indoleamine derivative or a closely related analogue may be the endogenous modulator of serotonin uptake. However, several questions remain to be clarified, such as the occurrence of 5-methoxytryptoline in tissues, the existence of biochemical pathways for synthesis and inactivation of the compound, and finally the pharmacological and physiological profile of 5-methoxytryptoline in addition to the inhibition of serotonin uptake and [^{3}H]-imipramine binding [26].

It is of interest to note that a circadian rhythm for serotonin uptake in human blood platelets has been recently reported [36]. In blood platelets from rabbits maintained on a long photoperiod (14 h light – 10 h dark), higher B_{max} values of

Table 2. B_{max} and K_d values of [^{3}H]-imipramine binding in rabbit blood platelets during a light-dark cycle

Time of sampling	n	B_{max} (fmol/mg prot)	K_d (nM)
L + 2	9	3980 ± 491	2.09 ± 0.20
L + 8	9	4747 ± 414	1.71 ± 0.17
D + 2	9	5667 ± 472*	2.44 ± 0.43
D + 8	9	6075 ± 355*	1.78 ± 0.19

Shown are mean values ± SEM · [^{3}H]-imipramine binding was measured using 8 concentrations of [^{3}H]-imipramine between 0.25 and 5 nM, each point determined in duplicate. Non-specific binding was determined in the presence of 100 µM desipramine. Binding parameters were obtained by Scatchard analysis. Rabbits were kept in a 14 h–10 h light-dark cycle (LD). * $p < 0.01$ when compared with respective values at L + 2 (Duncan test).

[^{3}H]-imipramine binding were found during the dark period than during the light period [15] (table 2). In contrast, the K_d values were not significantly modified. In human blood platelets, preliminary results obtained during winter do not indicate the existence of a circadian rhythm of the B_{max} values of [^{3}H]-imipramine binding (Galzin, BenHadjAli, Schoemaker, Sechter, Galinovski, Poirier, Loo and Langer; unpublished observations). Nevertheless, a similar study in control volunteers during summer is essential to validate the conclusion that [^{3}H]-imipramine binding in human platelets does not show circadian variations. This question is of special interest in view of the hypothesis that internal desynchronization of circadian rhythms might be causally related to depression [56].

Although it was demonstrated that several tritiated antidepressants label the serotonin transporter complex, clinical research has centered almost exclusively on [^{3}H]-imipramine binding to platelets as a potential biological marker in depression [12, 17, 18, 22, 23, 43, 44].

Studies in healthy volunteers were designed mainly in an attempt to evaluate the effect of repeated antidepressant administration on platelet [^{3}H]-imipramine binding. It was found that the B_{max} values of [^{3}H]-imipramine binding in platelets were decreased by 63% following one week administration of a low dose of chlorimipramine (50 mg/day), a tricyclic antidepressant which is more potent at inhibiting serotonin than noradrenaline uptake [35, 39, 40]. This decrease in B_{max} of platelet [^{3}H]-imipramine binding was maintained throughout the first week of drug washout. The B_{max} values of platelet [^{3}H]-imipramine binding remained decreased as compared to pretreatment values for up to three weeks after the end of the administration of chlorimipramine. Serotonin uptake into platelets was completely inhibited during the week of chlorimipramine treatment, but was fully recovered after one week of washout [39]. Similar 1-week treatments of healthy volunteers with maprotiline, an antidepressant in-

hibiting noradrenaline uptake, and amineptine, which acts on dopaminergic mechanisms, did not significantly affect the parameters of [^{3}H]-serotonin uptake or [^{3}H]-imipramine binding in platelets[40]. A similar lack of effect on platelet [^{3}H]-imipramine binding was reported with chronic imipramine in healthy volunteers[55].

Taken together, these results support the view that a washout period of 4 weeks may be required for studies on [^{3}H]-imipramine binding in platelets from depressed patients, if they were previously medicated with chlorimipramine or drugs affecting serotonin uptake[39].

We have recently found that a 20-day administration of lithium to healthy volunteers, while significantly reducing the V_{max} values of [^{3}H]-serotonin uptake in blood platelets, did not modify the binding of [^{3}H]-imipramine[41]. These results confirm that [^{3}H]-serotonin uptake and [^{3}H]-imipramine binding can be regulated independently.

In 16 out of the 26 studies published to date on platelet [^{3}H]-imipramine binding in depression, a significant decrease in B_{max} values was reported in platelets from untreated depressed patients when compared with control healthy volunteers, while there were not significant changes in the K_d values[29]. In one study[33], a small but significant increase in the B_{max} of [^{3}H]-imipramine binding in the platelets of depressed manic melancholic patients was described, while nine studies failed to detect any differences in B_{max} of platelet [^{3}H]-imipramine binding between depressed untreated patients and control volunteers. Problems in methodology used for [^{3}H]-imipramine binding[46] or clinical factors involving the selection of depressed patients, could explain these discrepancies. Additional studies are needed to further clarify the value of platelet [^{3}H]-imipramine binding in depression.

In our first longitudinal study, the low density of [^{3}H]-imipramine binding in human platelets was found to remain unaffected during treatment with tricyclic antidepressant drugs, in spite of a major improvement in the psychiatric parameters[10, 43]. However, in more recent studies, it appears that it takes two to three additional months following the clinical improvement of depressed patients for the normalization of the [^{3}H]-imipramine binding in platelets. In support of this view, the decreased values of [^{3}H]-imipramine binding in depressed patients were reported to return to normal levels after at least 2 months of full remission[53, 54]. In addition, we recently reported that after at least six sessions of electroconvulsive shock therapy, severely endogenously depressed patients showed a persistent decrease in the B_{max} of platelet [^{3}H]-imipramine binding, in spite of their clinical improvement[27]. However, 12 to 18 months after discharge from the hospital, the B_{max} of [^{3}H]-imipramine binding in six euthymic but formerly depressed patients had returned to the range of control values[27]. These results suggest that the B_{max} of [^{3}H]-imipramine binding in platelets may represent a state-dependent marker in depression, which normalizes several weeks or months after the clinical improvement, indicating perhaps, the consolidation of the remission period. This suggestion remains to be validated by additional and extensive studies, which are needed to confirm that [^{3}H]-imipramine binding is a valid and useful biochemical marker for the study of depressive disorders in man. A limited number of studies have examined [^{3}H]-imipramine binding and [^{3}H]-5 HT uptake in human platelets, in relation to several diseases[29]. The results suggest that the site of [^{3}H]-imipramine binding and the site of [^{3}H]-5 HT uptake, although associated, are not identical, and can be regulated independently. It is therefore possible to find changes in 5 HT uptake with no concomitant changes of [^{3}H]-imipramine binding[29]. The evidence available so far supports the view that depression is characterized by decreases in both the density of [^{3}H]-imipramine binding and the V_{max} of [^{3}H]-5 HT uptake in platelets, although the existence of concomitant changes in these parameters in the brain remains an open question.

Conclusions

The high affinity binding sites for [^{3}H]-imipramine are associated with the serotonin transporter complex, and may be different from the substrate recognition site of the transporter. The hypothesis that the [^{3}H]-imipramine recognition site is a novel modulatory receptor which regulates serotonin transport, suggests that an endocoid for this receptor could exist, although this possibility is still highly speculative. The density of the imipramine recognition sites in human platelets is decreased in untreated depressed patients, and this decrease appears to be a state-dependent marker in depression. To further evaluate the value of [^{3}H]-imipramine binding as a biochemical marker of depression, there is a need for additional studies on platelet [^{3}H]-imipramine and [^{3}H]-paroxetine binding in depression and in other psychiatric and non-psychiatric illnesses which involve the serotonergic system.

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