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Interaction of D-LSD with Blood Platelets of Rabbits: Shape Change and Specific Binding[1]

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ABSTRACT

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In blood platelets of rabbits, the shape change-inducing effect of D-lysergic acid diethylamide (D-LSD) has been compared with the D-LSD-binding. D-LSD, but not L-LSD, caused a shape change reaction (EC_{50} 1.3×10^{-9} M) which was inhibited by various 5-HT antagonists (methergoline and neuroleptic drugs), butaclamol showing marked stereospecificity. Strong inhibitors of 5 HT uptake were only weak in counteracting the D-LSD-induced shape change. Furthermore, D-[^{3}H]LSD bound to platelets at a single saturable, high affinity, stereoselective site [K_d = $(31.1 \pm 3.3) \times 10^{-9}$ M; B_{max} = 28.9 ± 2.7 fmols per 10^8 platelets]. This binding was strongly antagonized by D-LSD and methergoline and less by the hallucinogenic drugs, psilocin, bufotenin and N'N'-dimethyltryptamine. L-LSD and 5 HT, inhibitors of 5 HT uptake and neuroleptics, especially spiroperidol and butaclamol, had only a weak antagonistic effect. The latter showed stereospecificity. It is concluded that 1) the shape change reaction caused by D-LSD in platelets is mediated by the specific 5 HT-receptor, 2) the sites mediating the D-LSD-induced shape change reaction are not identical with those responsible for D-[^{3}H]LSD-binding and both these sites are different from the 5 HT-transport sites and 3) with respect to D-LSD-binding sites, platelets and neurons are not exactly identical.

Blood platelets have certain similarities with monoaminergic neurons, for instance in the carrier-mediated uptake of 5-hydroxytryptamine (5 HT) at the plasma membrane and its inhibition by drugs and in the intracellular storage, liberation and metabolism of monoamines, especially 5 HT. In addition, platelets possess high affinity binding sites for 5 HT at the plasma membrane suggesting the presence of specific 5 HT-receptors. These probably also mediate the 5 HT-induced shape change reaction in which the normal discoid shape of the platelets becomes spheroid with the extrusion of blebs and pseudopods (Born *et al.*, 1978; Graf and Pletscher, 1979). It has indeed been shown that there is close correlation between the potencies of 5 HT antagonists in inhibiting high affinity binding of [^{3}H]5 HT and their potencies in counteracting the 5 HT-induced shape change (Drummond, 1976).

Other 5-hydroxytryptaminergic drugs, *e.g.* the potent hallucinogen D-lysergic acid diethylamide (D-LSD), also caused a shape change reaction in platelets (Graf and Pletscher, 1979). In addition, D-LSD showed saturable, high affinity, stereospecific binding to membranes of brain neurons (Bennett and Snyder, 1976; Leysen *et al.*, 1978; Peroutka and Snyder, 1979). It was of interest therefore to find out if D-LSD also binds to platelets and if there is a relation between binding and functional changes, *e.g.* the shape change reaction.

The present work indicates that D-LSD shows saturable, high affinity, stereospecific binding to the plasma membrane of rabbit platelets. Among the compounds which are specific agonists and antagonists of the shape change reaction induced by D-LSD, only ergoline derivatives specifically inhibited D-LSD binding, neuroleptics and 5 HT exerting little activity.

Materials and Methods

Materials. D-[^{3}H]LSD (13.3 Ci mmol^{-1}) was obtained from the Radiochemical Center, Amersham, England. The source of the other compounds has been indicated earlier (Graf and Pletscher, 1979).

Isolation of platelets. Rabbits (Swiss, either sex, 2–4 kg) fasted for 16 hr, were bled under light ether anesthesia through a polyethylene cannula inserted into a carotid artery. Blood was collected in a plastic bottle containing 0.38% Na-citrate (final concentration). Platelet-rich plasma (PRP) was separated by centrifugation of the whole blood at 400 × *g* for 10 min at 22°C. Platelets were then isolated from PRP by means of a discontinuous dextran gradient and resuspended in tris-buffer, containing 0.14 M NaCl, 0.0076 M KCl, 0.0076 M tris(hydroxymethylamino)methane and 0.0129 M tri-sodium-citrate · $2H_2O$, pH 7.4, as previously described (Graf *et al.*, 1979). In some experiments, platelets were isolated by centrifugation of PRP at 6000 × *g* and washed three times in tris-buffer, each washing being followed by centrifugation at 6000 × *g* for 10 min at 4°C. For most of the binding studies, the second washing was carried out with tris-buffer containing 3.5% butanol (at 0°C for 15 min) (Noll *et al.*, 1979). This procedure removes super-

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ficial proteins from the platelets, mostly plasma proteins as shown by sodium dodecyl sulfate-acrylamide gel electrophoresis of the supernatant. Electronmicroscopy showed that the membranes of the butanol-treated platelets were still intact, although there was a decrease of osmophilic structures in the interior, especially α- and 5 HT-granules.

Shape change experiments. Platelets isolated by a dextran gradient were used for these experiments (Graf and Pletscher, 1979). Aliquots of the final platelet suspension (500 μl, final concentration of platelets 10^5 μl^{-1} in tris-buffer) were preincubated for 30 min at 37°C. D-LSD and antagonists were then added in a volume of 2.5 to 7.5 μl of H_2O, the antagonist 1 min before the D-LSD. Control experiments using solvent only were also carried out. The shape change was determined with an Elvi 840 Aggregometer by measuring the maximal light absorption of the platelet suspensions which occurred within 5 min. The samples were stirred at 900 rpm with a magnetic stirrer during measurement. The light absorption was recorded on a Rikadenki B-361 recorder with a full scale deflection of 500 mV. The reference point O mV was obtained from suspensions of normal platelets stirred as indicated above. Increasing millivolts means increasing light absorption due to shape change.

Binding experiments. Blood platelets (5×10^8, determined in a Coulter counter) were incubated for 10 min at 37°C in 500 μl of tris-buffer with D-[^{3}H]LSD either alone to determine total binding or in the presence of a 1000-fold excess of the unlabeled compound to determine nonspecific binding. In some experiments the compounds listed in table 2 were also added to the incubation mixture. Subsequently, the samples were rapidly filtered through a Whatman GF/C filter and washed twice with 5 ml of cold tris-buffer. The radioactivity on the filters was measured in toluene containing 2,5-diphenyloxazole-1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene as scintillator. The effect of varying the platelet concentration of the incubation mixture was also determined. Specific binding of D-[^{3}H]LSD was calculated as the difference between total binding and nonspecific binding. Totally bound D-[^{3}H]LSD did not exceed 1% of the D-[^{3}H]LSD initially present in the incubation medium.

Calculations. The molar concentrations of the compounds inducing half the maximal effect (EC_{50}) and those causing half-maximal inhibition (IC_{50}) were determined graphically. The binding of D-[^{3}H]LSD to platelets was analyzed graphically by the method of Scatchard (1949). Statistical analysis was performed with Student's t test.

Results

Shape change. The shape change reaction (measured by the increase in the light absorption of the platelet suspensions) induced by D-LSD differed in its time course from that caused by 5 HT (fig. 1). With D-LSD, the shape change reached its maximum after 5 to 10 min and remained at this level for at least 30 min. In contrast, the shape change induced by 5 HT was maximal after about 1 min and then it decreased continuously to control level in about 60 min (Graf and Pletscher, 1979). The light absorption increased with rising concentrations of D-LSD, maximal values being reached at about 3×10^{-9} M (fig. 2). The EC_{50} of D-LSD was $(1.3 \pm 0.4) \times 10^{-9}$ M, thus considerably lower than that previously reported for 5 HT (2.1×10^{-7}) (Graf and Pletscher, 1979). The maximum shape change obtained with D-LSD (98 ± 20 mV) was about 20% of that induced by 10^{-6} M 5 HT (500 mV).

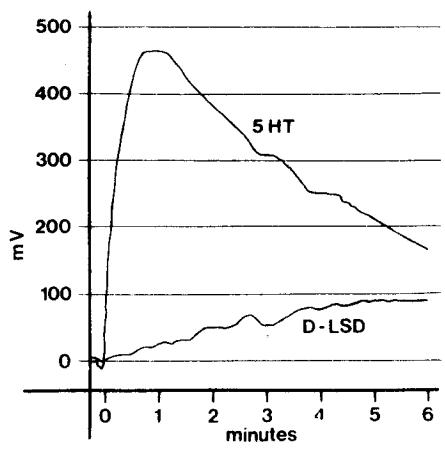

Fig. 1. Shape change reaction induced by 10^{-8} M D-LSD and 10^{-6} M 5 HT in rabbit platelets. The ordinate indicates the light absorption in millivolts of the platelet suspensions. Typical experiments.

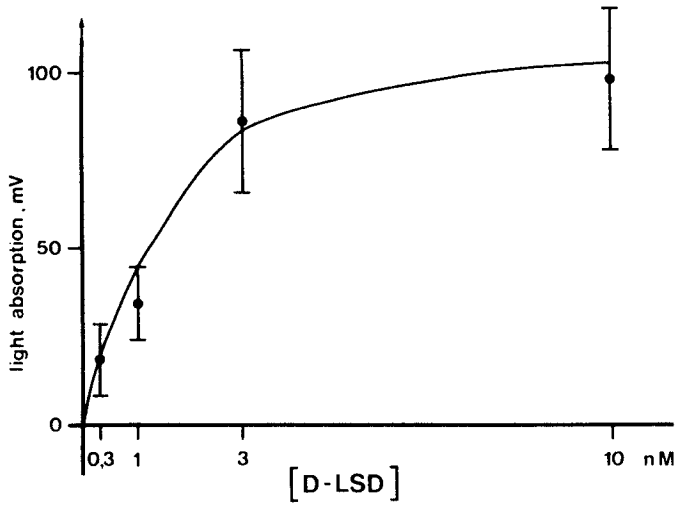

Fig. 2. Shape change reaction of rabbit platelets induced by various molar concentrations of D-LSD. Each point is an average with S.E.M. of four experiments.

Effect of drugs on shape change. The shape change induced by 10^{-8} M D-LSD was potently antagonized by methergoline and neuroleptic drugs, including D-butaclamol (IC_{50} values between 10^{-9} and 10^{-8} M). There was marked stereospecificity, since D-LSD, which did not cause any shape change reaction in concentrations as high as 10^{-5} M (Graf and Pletscher, 1979), and L-butaclamol were virtually inactive as antagonists. Chlorimipramine and imipramine which are potent inhibitors of 5 HT uptake were up to 3 orders less potent than methergoline and the active neuroleptics (table 1).

Binding. D-LSD bound specifically to platelets, the binding being maximal after an incubation time of 1 min and remaining unchanged for at least 30 min. Specifically bound D-[^{3}H]LSD was displaced from the platelets by incubation with a 1000-fold excess of unlabeled D-LSD after the binding reaction had taken place. The amount of bound D-[^{3}H]LSD was proportional to the concentration of the platelets. A plot of specific binding as a function of D-LSD-concentration is shown in figure 3. Analysis of this curve according to Scatchard (1949) indicates the presence of one class of binding site with a K_d of $(31.1 \pm 3.3) \times 10^{-9}$ M and B_{max} (maximal binding), of 28.9 ± 2.7 fmols/10^8 platelets, equivalent to 174 ± 15 binding sites per platelet (fig. 3, inset). There was no significant difference ($P > .05$) in the K_d or B_{max} values between the platelets which had been isolated by a dextran gradient and those isolated by centrifugation of the PRP followed by washing either with tris-buffer alone or with tris-buffer and 3.5% butanol. However, the butanol procedure was mainly used in the present experiments because it considerably decreased the nonspecific binding (table 2).

Effects of drugs on binding. Unlabeled D-LSD showed

TABLE 1

IC_{50} (molar concentration causing a 50% inhibition of the maximal effect) of various compounds for the shape change induced by 10^{-8} M D-LSD in rabbit platelets, for specific binding of D-[^{3}H]LSD (10^{-8} M) to rabbit platelets and for specific binding of D-[^{3}H]LSD to rat brain membranes.

The values for platelets are averages with S.E.M. of three to five experiments. The values for rat brain membranes have been taken from the literature (Bennett and Snyder, 1976; Leysen *et al.*, 1978; Peroutka and Snyder, 1979; Oegren *et al.*, 1979); the concentrations of D-[^{3}H]LSD were 2 to 4 × 10^{-9} M.

Drug	IC_{50}		
	Platelets		Brain membrane
	Shape change	Binding	Binding
D-LSD[a]		(3.4 ± 0.8) × 10^{-8}	5–8 × 10^{-9}
L-LSD	>10^{-5}	(2.4 ± 0.5) × 10^{-6}	2 × 10^{-5}
Methergoline	(1.8 ± 0.7) × 10^{-9}	(3.5 ± 1.2) × 10^{-8}	0.8–10 × 10^{-8}
Methiothepine	(1.3 ± 0.4) × 10^{-9}	(7.2 ± 0.7) × 10^{-7}	~10^{-8}
Spiroperidol	(1.7 ± 0.7) × 10^{-9}	(2.7 ± 0.6) × 10^{-6}	6–11 × 10^{-9}
D-Butaclamol	(6.1 ± 0.3) × 10^{-9}	(2.9 ± 0.9) × 10^{-6}	4–7 × 10^{-8}
L-Butaclamol	>10^{-5}	(3.0 ± 0.3) × 10^{-6}	7 × 10^{-6}
Bufotenin[a]		(2.0 ± 0.6) × 10^{-7}	2–3 × 10^{-7}
Psilocin[a]		(6.6 ± 1.3) × 10^{-7}	10^{-6}
N',N''dimethyltryptamine[a]		(7.5 ± 1.2) × 10^{-7}	2 × 10^{-6}
5 HT[a]		(3.7 ± 1.2) × 10^{-5}	0.1–7 × 10^{-7}
Chlorimipramine	(2.4 ± 0.1) × 10^{-7}	(3.3 ± 0.7) × 10^{-6}	2.1 × 10^{-6}
Imipramine	(3.2 ± 1.0) × 10^{-6}	(1.5 ± 0.4) × 10^{-5}	1.5 × 10^{-6}

[a] 5-HT agonists inducing a shape change reaction.

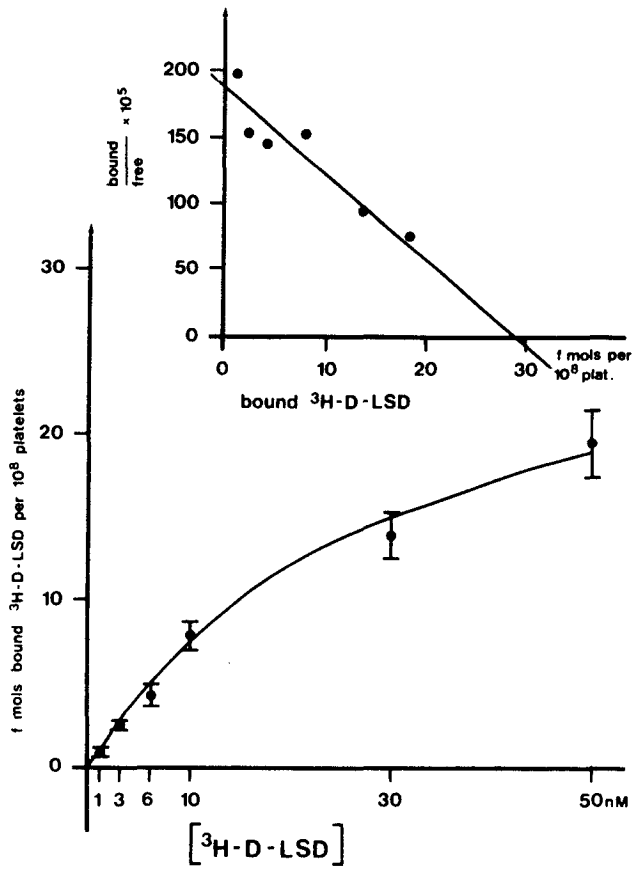

Fig. 3. Bottom, specific binding of D-[^{3}H]LSD (= total binding of D-[^{3}H]LSD minus binding of D-[^{3}H]LSD in the presence of a 1000-fold excess of unlabeled D-LSD) to rabbit platelets previously treated with 3.5% butanol. Ordinate: femtomoles bound D-[^{3}H]LSD per 10^8 platelets. Each point is an average with S.E.M. of four experiments. Top, Scatchard analysis of the values from below. Ordinate:

$$\frac{\text{bound D-[}^3\text{H]LSD}}{\text{free D-[}^3\text{H]LSD}} \times 10^5.$$

TABLE 2

K_d, B_{max} and nonspecific binding (in percentage of total binding) of D-[^{3}H]-LSD in rabbit platelets isolated by 3 different procedures

In each experiment the three different preparations were obtained from the same pool of PRP. Each value is an average with S.E.M. of four experiments. Significance, nonspecific binding: butanol *vs.* tris and dextran ($P < .01$.)

Isolation	K_d	B_{max}	Nonspecific Binding
	nM	*fmol/10^8 platelets*	%
Dextran gradient	48.2 ± 12.8	35.4 ± 11.0	49.0 ± 4.7
Washing, tris	37.9 ± 10.1	51.9 ± 12.7	38.6 ± 2.8
Washing, butanol	41.4 ± 11.0	49.4 ± 12.9	22.3 ± 3.5

considerable potency in counteracting the specific binding of D-[^{3}H]LSD to platelets, whereas nonradioactive L-LSD was two orders of magnitude less potent. Among the other drugs tested, methergoline was the most potent inhibitor (3.5 × 10^{-8} M) followed by hallucinogenic drugs (bufotenin, psilocybin, and N'N'-dimethyltryptamine, table 2). Neuroleptics, especially spiroperidol and butaclamol, were very weak antagonists and there was no stereospecificity of butaclamol. Strong inhibitors of 5 HT uptake (chlorimipramine and imipramine) as well as 5 HT itself had very little if any antagonistic effect. In general, the drugs were more potent in counteracting the D-LSD-induced shape change than D-[^{3}H]LSD-binding.

Discussion

The present results confirm earlier findings that D-LSD induced a shape change reaction in rabbit platelets (Graf and Pletscher, 1979). They also showed that the shape changes caused by D-LSD and 5 HT differed from each other in time course and magnitude. However, various 5 HT antagonists of the ergoline and neuroleptic type which markedly inhibited the 5 HT-induced shape change (Graf and Pletscher, 1979) also strongly counteracted that due to D-LSD and there was stereoselectivity of butaclamol in both cases. Furthermore, the potencies of the compounds in antagonizing the two types of shape change were of a similar order (Graf and Pletscher, 1979).

These findings indicate that the shape change caused by D-LSD is probably mediated by the 5 HT-receptor of the platelets.

It has been previously shown that the receptor mediating the 5 HT-induced shape change is not identical with the site responsible for 5 HT uptake into the platelets (5 HT-transport sites) (Drummond, 1976). The present results indicate that the D-LSD-induced shape change was also not mediated by the site responsible for 5 HT uptake. On the one hand, strong inhibitors of the 5 HT uptake, especially imipramine, had only a weak antagonistic effect on the D-LSD-induced shape change reaction (table 1). On the other hand, as we have shown in human platelets for spiroperidol (Laubscher and Pletscher, 1979) and in rabbit platelets for spiroperidol and methergoline, these 5 HT antagonists were virtually inactive in inhibiting the uptake of 5 HT (EC_{50} values $> 10^{-6}$ M).

The binding experiments show that D-LSD bound with high affinity and reversibly to stereospecific sites on the platelets. In preliminary experiments, platelet membranes isolated as indicated by Brunette and Till (1971) also exhibited specific, saturable binding of D-[^{3}H]LSD, the K_m value being similar to that in intact platelets. The binding sites of D-[^{3}H]LSD are therefore probably localized mainly on the plasma membranes of the platelets. The kinetics of the D-[^{3}H]LSD-binding were different from those of the D-LSD-induced shape change reaction. Thus, the K_d in the binding studies was about 30 times higher than the EC_{50} in the shape change experiments. The shape change reaction had already reached its maximum at a D-LSD-concentration at which only about 10% of the D-[^{3}H]LSD-binding sites were occupied (compare figs. 2 and 3). This discrepancy could be explained by assuming that the sites labeled in the binding studies are those which transport 5 HT, *i.e.*, membrane sites different from the receptors mediating shape change. However, this possibility is unlikely since we have shown that D-LSD is a very weak inhibitor of 5 HT uptake ($IC_{50} > 10^{-6}$ M), probably due to its low affinity for the 5 HT uptake sites. In addition, imipramine, which is known to bind to the 5 HT-transporter of the plasma membrane (Talvenheimo *et al.*, 1979), and chlorimipramine, another uptake inhibitor of 5 HT, were virtually inactive in displacing D-[^{3}H]LSD from its binding sites. Therefore, the site responsible for the D-LSD-induced shape change (*i.e.*, the 5 HT receptor) is probably different from the 5 HT uptake site.

Neuroleptic drugs, *i.e.*, spiroperidol, D-butaclamol and methiothepine, were rather weak in displacing D-[^{3}H]LSD from its binding sites, especially when compared with their considerably more potent effect as inhibitors of the D-LSD-induced shape change. Also, there was no stereoselectivity of butaclamol. This indicates that neuroleptics do not specifically interact with the D-[^{3}H]LSD-binding site, although these drugs markedly interfered with the D-LSD-induced shape change reaction.

In addition, 5 HT had virtually no effect on D-[^{3}H]LSD-binding, although our results showed that the shape changes induced by D-LSD and by 5 HT were probably mediated by the same receptor. Therefore, the 5 HT receptor responsible for the D-LSD-induced shape change reaction does not seem to be identical with the site responsible for D-[^{3}H]LSD-binding. It has not yet been established that these two sites are functionally linked. However, this possibility should be considered since D-[^{3}H]LSD showed only one high affinity, stereospecific binding site, which was different from the 5 HT-receptor. The finding that the 5 HT-receptor and the D-[^{3}H]LSD binding site behaved differently toward certain drugs does not necessarily mean that there is no functional link. Thus, drugs causing or antagonizing a shape change mediated by the 5 HT-receptor may have a binding site which is different from that for D-[^{3}H]LSD, *e.g.* at the 5 HT-receptor itself. On the other hand, it cannot be excluded that D-[^{3}H]LSD also has a specific binding site at the 5 HT-receptor, which could not be detected with the methods that were used.

Whether or not the neuroleptics bind to the same sites as 5 HT has not yet been clarified. With [^{3}H]spiroperidol, as with haloperidol (Boullin *et al.*, 1978) no displaceable, specific binding could be found, possibly because it was hidden by nonspecific binding. High affinity binding of [^{3}H]-5 HT has been detected in rat platelets (Drummond, 1976; von Hahn *et al.*, 1980). However, we were not able to demonstrate any such binding in rabbit platelets, possibly because it was too small compared with the binding of [^{14}C]-5 HT to the uptake sites and the diffusion of [^{14}C]-5 HT into the platelets.

In contrast to the neuroleptics and 5 HT, methergoline was as potent as unlabeled D-LSD in displacing D-[^{3}H]LSD). Therefore, the primary site of action of methergoline might be the D-[^{3}H]LSD-binding sites. The hallucinogenic 5 HT-agonists, psilocin, bufotenin and N'N'-dimethyltryptamine also displaced D-[^{3}H]LSD. Their potencies were about equal or only slightly less than their potencies as shape change agonists (Graf and Pletscher, 1979), suggesting that these drugs are able to interact with the D-LSD-binding sites as well.

The binding sites for D-LSD in membranes of brain neurons seem to differ in certain respects from those in platelets. Thus, not only ergoline derivatives (methergoline and D-LSD) but also neuroleptics were strong and stereospecific antagonists of the D-LSD-binding and 5 HT was much more potent than in platelets in displacing D-[^{3}H]LSD (Bennett and Snyder, 1976; Leysen *et al.*, 1978; Peroutka and Snyder, 1979). In the brain, two types of 5 HT-receptors have been proposed to exist, *i.e.*, receptors for 5 HT (5 HT_1) and those for neuroleptics (5 HT_2). D-LSD is thought to bind to both types of receptors (Peroutka and Snyder, 1979). The results of D-[^{3}H]LSD-binding to platelets reported here are hardly consistent with this hypothesis and hence, with respect to D-[^{3}H]LSD-binding, platelets cannot be regarded as exact models for neurons (fig. 4).

D-LSD also affects dopaminergic neurons in the brain (Pieri

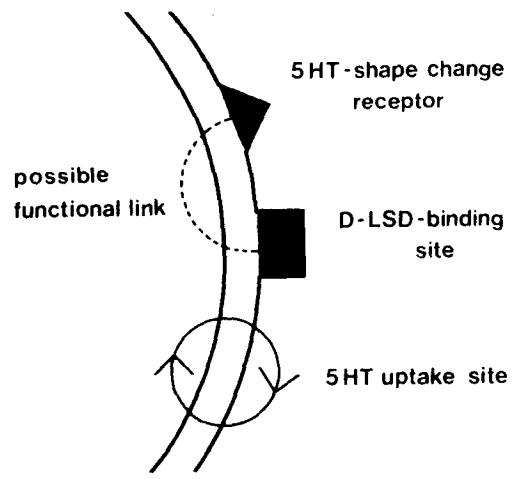

Fig. 4. Hypothetical model of various sites at the platelet membrane.

et al., 1974; von Hungen *et al.*, 1974; Da Prada *et al.*, 1975). Whether platelets contain dopamine (DA)-receptors to which D-[^{3}H]LSD might bind cannot be excluded. This seems, however, unlikely as DA did not induce either a shape change reaction or aggregation of rabbit platelets (Graf and Pletscher, 1979, experiments in this laboratory) and specific DA-receptors could not be demonstrated in binding experiments with human platelets (Boullin *et al.*, 1978).

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