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Human Platelet Imipramine Recognition Sites: Biochemical and Pharmacological Characterization

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WENNOGLE, L. P., B. BEER AND L. R. MEYERSON. *Human platelet imipramine recognition sites: Biochemical and pharmacological characterization.* PHARMAC. BIOCHEM. BEHAV. 15(6)975–982, 1981.—The influence of the membrane environment on the integrity of the human platelet [^{3}H]-imipramine recognition site was examined. When platelet membranes were isolated in a buffer containing enzyme inhibitors (EDTA, EGTA and antiproteases) a significantly greater number of high affinity [^{3}H]-imipramine binding sites was observed. A calcium-stimulated degradation of imipramine sites was also demonstrated. This degradation occurred *in vitro* over physiologically relevant time periods. Furthermore, inactivation of imipramine binding was achieved by very low concentrations ($1C_{50}$=5 μg/ml) of phospholipase A_2. Specific serotonin reuptake inhibitors were potent displacers of [^{3}H]-imipramine binding; histamine (H_1), alpha-adrenergic (α_1), and muscarinic agents were much less active. The receptor was shown to be proteinaceous in nature due to its sensitivity to proteases, heat denaturation and chemical modification with N-ethylmaleimide. From these results it is proposed that membrane lipid perturbations, catalyzed by calcium, may control expression of platelet [^{3}H]-imipramine sites. The relation of this recognition site to aminergic systems and the possible relevancy to the action of antidepressants are addressed.

Antidepressants Imipramine Platelets Tricyclics Receptor characterization Calcium Phospholipase A_2

DEPRESSION is a complex disease state whose underlying etiology remains unknown. Nevertheless, depressed patients can be effectively treated with a variety of drugs, such as the tricyclic antidepressive agents [23, 25, 29]. Tricyclic antidepressives like imipramine are potent inhibitors of biogenic amine reuptake; other antidepressive agents such as pargyline are monoamine oxidase inhibitors. Treatment with either class of drugs results in the increased availability of neurotransmitter substances, either by increasing the lifetime of these neurotransmitters at synaptic junctions or by curtailment of catabolism. However, these biochemical effects occur shortly after drug administration while clinical reversal of depression usually requires several weeks of drug therapy [30].

To explain the slow onset of antidepressive agents, proposals have focused on transsynaptic receptor adaptations. Chronic treatment with tricyclic agents decreases the density (down regulates) beta-adrenergic [3, 28, 33], alpha-adrenergic [11, 14, 43] and serotonin receptors [33, 38, 40]. However, these effects are likely to be secondary to drug treatment since imipramine has little effect on beta or serotonin receptors *in vitro* [33].

In order to probe the primary site of action of antidepressive agents, a high affinity binding site for [^{3}H]-tricyclic antidepressives has recently been identified [6, 17, 22, 31, 34–36]. The pharmacology of the [^{3}H]-imipramine recognition site has been most extensively studied and its characteristics appear similar in both brain and platelets [21,31]. This high affinity [^{3}H]-imipramine binding was correlated to serotonin reuptake systems [22,32]. Based on specific neuronal tract lesions, the [^{3}H]-imipramine receptor is located on presynaptic serotonergic nerve terminals [32,41].

Interestingly, chronic treatment with tricyclic antidepressive agents also down regulates high affinity [^{3}H]-imipramine binding sites [19,21]. Furthermore, clinical studies have demonstrated that drug-free severely depressed patients possess a lower density of platelet [^{3}H]-imipramine binding sites compared to matched controls [2,8]. The possible involvement of endogenous modulators has been advanced to explain changes in [^{3}H]-imipramine binding site densities [19,21].

Thus, it was of interest to biochemically characterize the imipramine receptor and to assess its participation in aminergic function. The present communication focuses upon *in vitro* manipulation of the chemical environment of human platelet membranes in order to evaluate the processes which influence this biologically relevant target site.

METHOD

Drugs and Reagents

Drugs were obtained from the following sources: fluoxetine (Lilly, Indianapolis, IN); mianserin (Organon, West Orange, NJ); loxapine and amoxapine (Lederle, Pearl

River, NY); cocaine and amitriptyline (Merck, Rahway, NJ); imipramine and desmethylimipramine·HCl (Ciba-Geigy, Summit, NJ); pargyline (Abbott, Chicago, IL); mescaline sulfate (S. B. Penick & Co., Lyndhurst, NJ); chlorpromazine (Smith, Kline & French, Philadelphia, PA); trazodone (Mead-Johnson, Evansville, IN); citalopram, Lu 10-171-B and talsupram, Lu 5-003-C (Lundbeck, Copenhagen); a quaternary amine derivative of amitriptyline: *N,N,N*-trimethyl-3- (10,11-dihydro-5H-dibenzo[a,d]- cyclohepten-5-ylidene)-propanaminium iodide; a quaternary amine derivative of 10,11 Δ-amitriptyline: 3-(5*H*-dibenzo[*a,d*]- cyclohepten-5- ylidene-*N,N,N*,-trimethyl-propanaminium iodide; and a quaternary amine derivative of N-methyl protriptyline: *N,N,N*,-trimethyl- 5H-dibenzo[*a,d*] cycloheptene- 5-propanaminium iodide, as well as the tertiary amine compound 10,11 Δ-amitriptyline: 3-(5*H*-dibenzo [*a,d*] cyclohepten- 5-ylidene)- N,N,-dimethyl- propanamine were synthesized by Dr. T. McKenzie of American Cyanamid Company. Platelet-enriched plasma was obtained from Sera-Tec and the New York Blood Center; [^{3}H]-imipramine was obtained from New England Nuclear. Pepstatin, aprotinin, A23187, pronase, phospholipase A_2 (porcine) and pyrilamine were from Sigma; three times crystallized trypsin (TPCK) was from Worthington; reagents for SDS-PAGE electrophoresis were from Biorad. All other reagents were of the highest available quality.

Membrane Isolation

Buffer I was 0.05M Tris (pH 7.5), 0.12M NaCl, 0.005M KCl. Buffer II was the same buffer, supplemented with the following antiproteases: 3mM EDTA, 1 mM EGTA, 0.1 mM PMSF (solubilized in DMSO and diluted to 0.01%), 5 U/ml aprotinin and 0.5 μg/ml pepstatin (solubilized in ethanol and diluted to 0.01%).

Blood was drawn from healthy human donors, collected in 3% sodium citrate and processed within 24 hr. Plastic containers were used throughout all isolation procedures. Whole blood was centrifuged at 192 ×G for 10 min and the plasma (supernatant) was further centrifuged at 2500 ×G for 10 min to sediment intact platelets. The platelet pellet was gently resuspended in 50 volumes of Buffer II (for the isolation of platelets and their membranes this buffer was used at pH 6.8 to prevent platelet aggregation) and the sedimentation step was repeated. Platelets were disrupted by homogenization 3 times for 15 s in a Tekmar tissue homogenizer at setting 100. In certain experiments, as noted, membranes were also sonicated 3 times for 10 sec at 0°C at setting 6 in a Branson sonifier. Crude platelet membranes were isolated by pelleting twice at 18,000×G for 20 min and membranes were finally resuspended in Buffer II (pH 7.5) at a protein concentration of 3 mg/ml and stored at 4°C. Membranes prepared in the absence of antiproteases were derived from the same pool of platelet-enriched plasma.

Binding Analysis

[^{3}H]-Imipramine binding was performed by a modification of the procedure described by Paul *et al.* [31]. Reaction mixtures for [^{3}H]-imipramine binding contained the following components: membrane preparation (300 μg protein) in Buffer I, [^{3}H]-imipramine (0.1–10 nM) and varying concentrations of test substance in a total incubation volume of 250 μl. Samples were incubated in triplicate for 90 min at 0°C. Reactions were terminated by addition of 5 ml of Buffer I and filtration was performed through prewetted Whatman GF/B glass fiber filters. Filters were washed two times with 5 ml of chilled Buffer I to remove unbound radioactive ligand. Corrections were made for nonspecific binding by assaying parallel incubations which contained 10 μM desipramine. Filters were placed in scintillation vials containing 10 ml Beckman Ready Solv-HP scintillation fluid. Radioactivity was determined with a Beckman LS-7500 liquid scintillation spectrometer. Drug IC_{50} values are reported as the concentration that displaces the specific binding of 3 nM [^{3}H]-imipramine by 50%. Data from saturation isotherms were analyzed by the method of Scatchard [39] and best fitting lines were determined by least squares linear regression analysis. Statistical renderings are expressed as standard error of the mean (S.E.M.).

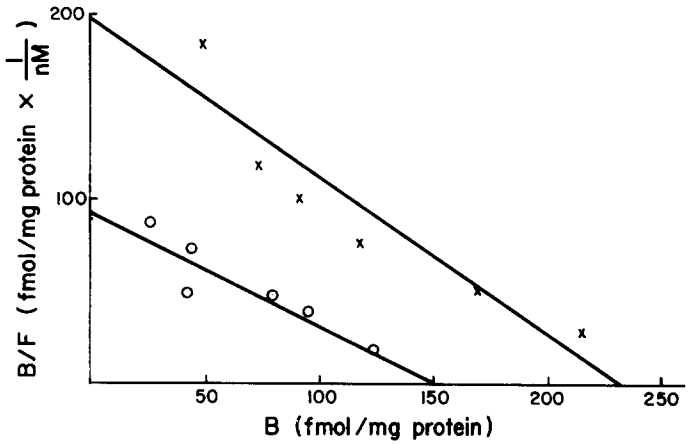

FIG. 1. The effect of antiproteases on the isolation of platelet membranes. Scatchard analysis of [^{3}H]-imipramine binding was performed with platelet membranes isolated either in the presence (×-×) or absence (○-○) of an antiprotease containing buffer as described in Method. Straight lines represent linear regression analysis of the data.

Miscellaneous Techniques

Enzymes and chemical modification reagents were dissolved and used in Buffer I. SDS-polyacrylamide gel electrophoresis was performed using a modification of the Laemmli system with 10% acrylamide gels and 0.013% bis acrylamide as described by Anderson and Gesteland [1]. Tissue samples were dissolved in sample buffer (4% SDS, 20% sucrose, 0.125 M Tris pH 6.8, 20% β-mercaptoethanol) and heated 2 min at 100°C. Gels were fixed, stained with Coomassee brilliant blue, destained and photographed using standard procedures. Protein concentrations were determined by the method of Bradford [7] with gamma globulin as standard.

Abbreviations

The following abbreviations were employed: IMI—imipramine; EDTA—ethylenediamine tetraacetic acid; EGTA—ethylene glycol-bis-(β-aminoethylether) N,N,-tetraacetic acid; PLA_2—phospholipase A_2; NEM—N-ethylmaleimide; Tris—tris(hydroxymethyl) aminomethane; PMSF—phenylmethyl sulfonyl fluoride; DMSO—dimethyl sulfoxide; SDS—sodium dodecyl sulfate; PAGE—polyacrylamide gel electrophoresis; DTT—dithiothreitol; LSD—lysergic acid diethylamide.

TABLE 1

THE EFFECT OF ENZYMES ON [^{3}H]-IMIPRAMINE BINDING TO HUMAN PLATELET MEMBRANES

Enzyme	Temperature	IC_{50}
Trypsin	37°C	0.42 mg/ml
Pronase	25°C	0.50 mg/ml
Phospholipase A_2	25°C	0.005 mg/ml

Platelet membranes were washed and suspended in Buffer I (Method) and incubated with varying concentrations of enzyme at the appropriate temperature for 30 minutes. The concentration of enzyme yielding 50% inhibition of binding of 3 nM [^{3}H]-imipramine (Method) is indicated as the IC_{50} value.

RESULTS

Isolation of Platelet Membranes

The density of specific [^{3}H]-imipramine recognition sites was consistently higher when platelet membranes were isolated in a buffer containing an antiprotease cocktail. Membranes isolated in the presence of antiproteases contained 1.48±0.12 times the number of sites per milligram protein (B_{max}) compared to matched control membranes isolated without antiproteases (four individual experiments). In a typical experiment (Fig. 1) B_{max} values of 235 and 150 fmol/mg protein were found, with and without antiproteases, respectively. As seen by Scatchard analysis (Fig. 1), the dissociation constant (K_d) for [^{3}H]-imipramine was not affected by membrane isolation in the presence of antiproteases (1.52±0.15 versus 1.39±0.16 nM with and without antiproteases, respectively). The antiproteases themselves had no effect on binding parameters when added to the assay mixture. Scatchard analysis of saturation isotherms with [^{3}H]-imipramine conducted with platelet membranes isolated in the absence of antiproteases showed no change in K_d or B_{max} after addition of antiproteases. Therefore, the effect of antiproteases was not due to an unmasking of cryptic sites.

Effect of Enzyme Treatment on Platelet Imipramine Binding

The imipramine binding site was sensitive to various enzyme treatments (Table I). Of the two proteases studied, both pronase and trypsin were capable of destroying the imipramine binding site at rather high concentrations, 0.5 and 0.42 mg/ml, respectively. The trypsin effect was totally inhibited by excess soybean trypsin inhibitor, indicating the effect was not due to a trypsin contaminant. An SDS-PAGE gel of platelet membranes treated with trypsin (Fig. 2) indicates that protease digestion was extensive at 0.42 mg/ml trypsin. Nonetheless, this concentration of enzyme was effective only in reducing 50%of the [^{3}H]-imipramine binding. In contrast, phospholipase A_2 was capable of destroying imipramine binding at considerably lower concentrations, with an IC_{50} value of 5 μg/ml.

Calcium-Mediated Lability of the Imipramine Recognition Site

After incubation of platelet membranes at 37°C, there is a loss of specific [^{3}H]-imipramine binding sites (Fig. 3). This loss is time dependent (Fig. 4), stimulated by calcium and

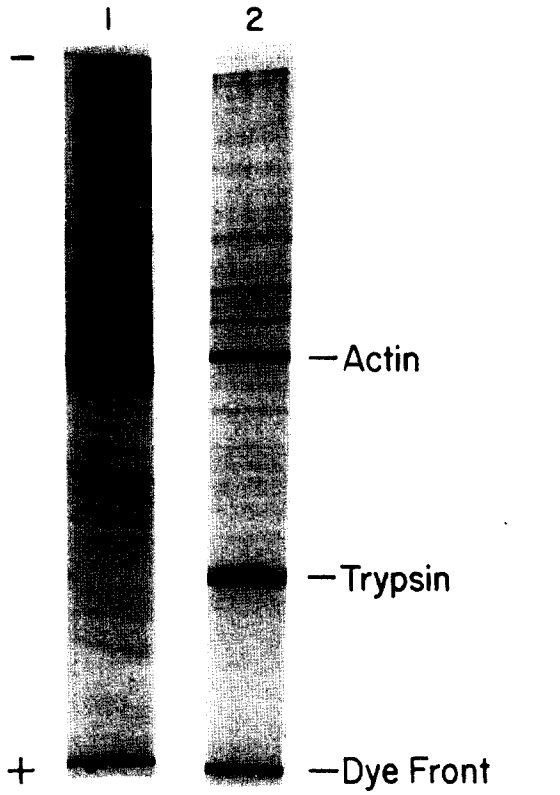

FIG. 2. The effect of trypsin on SDS polyacrylamide gel electrophoretic analysis of platelet membrane preparations. Crude platelet membranes were incubated either without trypsin (gel 1) or with 0.47 mg/ml trypsin (gel 2) for 30 min at 37°C and analyzed by SDS-PAGE as described in Method.

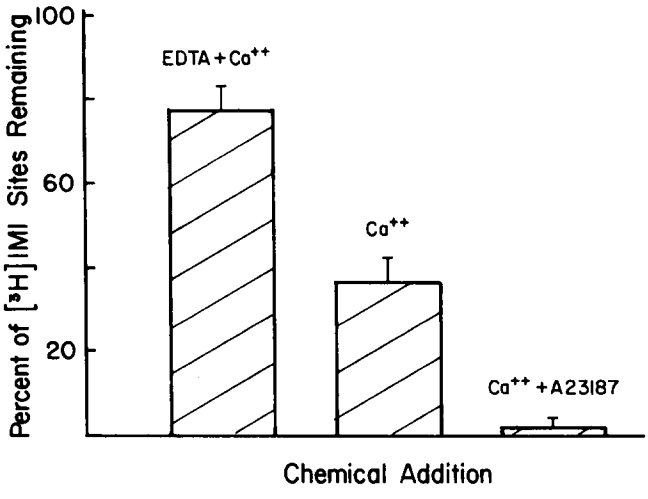

FIG. 3. The lability of the [^{3}H]-imipramine binding site. Platelets (1.2 mg/ml protein) were incubated in Buffer I at 37°C for 20 hr either with 7.5 mM EDTA plus 3 mM Ca^{++} with 3 mM Ca^{++} alone, or with 3mM Ca^{+} plus 10 μM A23187 as indicated. Reactions were terminated by cooling to 0°C and EDTA was added to 7.5 mM. [^{3}H]-imipramine binding was performed as described in Method. The results are an average of 6 independent determinations and standard error is indicated.

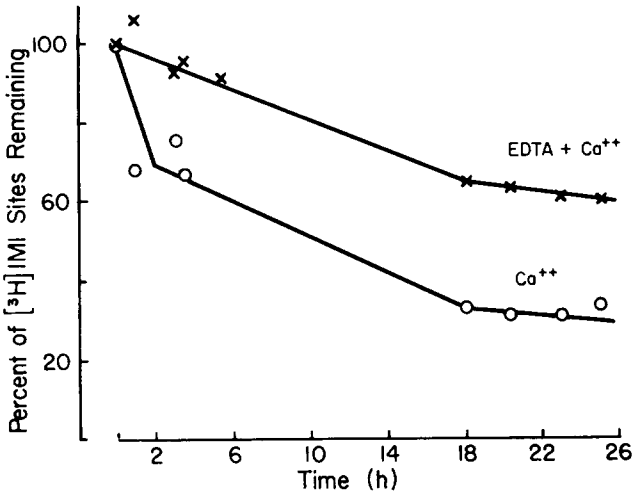

FIG. 4. Time course for the Ca^{++}-mediated loss of platelet imipramine binding sites. Platelets were incubated at 37°C either in the presence of 3 mM Ca^{++} (O-O) alone or 3 mM Ca^{++} plus 7.5 mM EDTA (×-×). At the indicated time intervals the reactions were terminated as described in Fig. 3 and assayed for [^{3}H]-imipramine binding (Method).

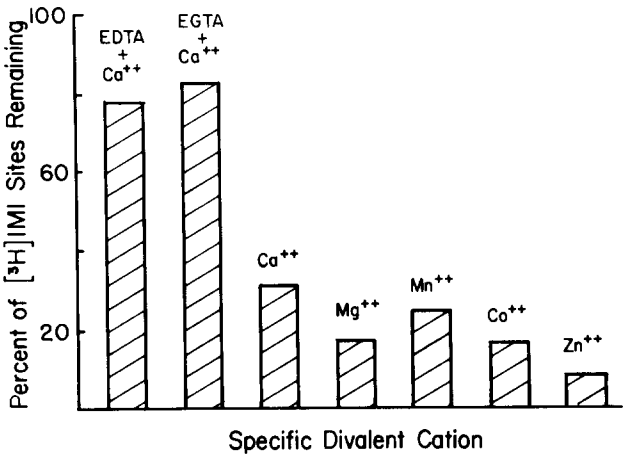

FIG. 5. The effect of specific divalent cations on the stability of the imipramine binding site. Reactions which contained 3 mM divalent cation and 0 or 7.5 mM EDTA or EGTA, as indicated, were incubated at 37°C for 20 hr. Incubations were stopped as described in Fig. 3 and [^{3}H]-imipramine binding analysis was performed.

other divalent cations (Fig. 5), and inhibited by the chelating agents EDTA and EGTA. The calcium ionophore A23187 further stimulated the calcium induced degradation of the receptor (Fig. 3). The initial velocity of receptor loss (in the presence of 3 mM Ca^{++} and 100 fmol/mg protein [^{3}H]-imipramine sites) was approximately 23.3 fmol/hr/mg protein. At the concentration tested, there appeared to be little difference in the ability of different divalent cations to catalyze the decay of the receptor. The addition of calcium alone, at the concentrations tested, had no effect on imipramine binding.

Chemical Modification

The effect of selected protein chemical modification reagents on the integrity of the imipramine binding site is shown in Table 2. Dithiothreitol (DTT), a compound known to specifically reduce disulfide bonds [27], had no effect on imipramine binding. Similarly, ethyl acetimidate, a reagent specific for primary amino groups [18], had little effect on imipramine binding. In contrast, N-ethylmaleimide (NEM) markedly reduced imipramine binding with an IC_{50} value of 41 μM. The ability of NEM to reduce imipramine binding indicates the probable participation of free sulfhydryl groups in this recognition site.

Miscellaneous Biochemical Data

The [^{3}H]-imipramine recognition site was destroyed by heat treatment of platelet membrane preparations. Heating membranes at 65°C for 1 min inactivated approximately one half the specific binding sites (Fig. 6).

Platelet membrane preparations can be stored frozen in liquid nitrogen for up to 6 months with negligible effect on [^{3}H]-imipramine binding parameters. In general, slightly higher levels of non-specific binding resulted from this storage procedure.

TABLE 2

THE EFFECT OF PROTEIN MODIFIERS ON THE NUMBER OF PLATELET IMIPRAMINE BINDING SITES

Agent	Concentration	Percent Sites Remaining
Dithiothreitol	20 mM	99.9
N-Ethylmaleimide	0.067 mM	36.1
Ethyl acetimidate	3.3 mg/ml	86.9

The effect of treatment of platelet membranes with various chemicals on [^{3}H]-imipramine binding was measured as described in Method. Membranes were treated in Buffer I at 25°C for 30 minutes and binding was performed directly.

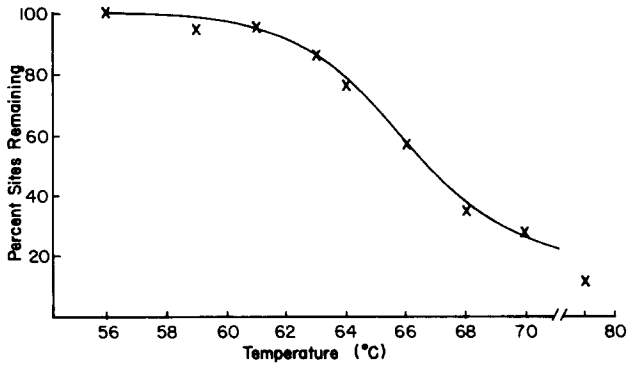

FIG. 6. Thermal denaturation of platelet [^{3}H]-imipramine recognitions sites. Membranes (1.5 mg/ml) in Buffer I were heated for 1 min at the temperature indicated and cooled. [^{3}H]-imipramine binding (3 nM imipramine) was performed as described in Method.

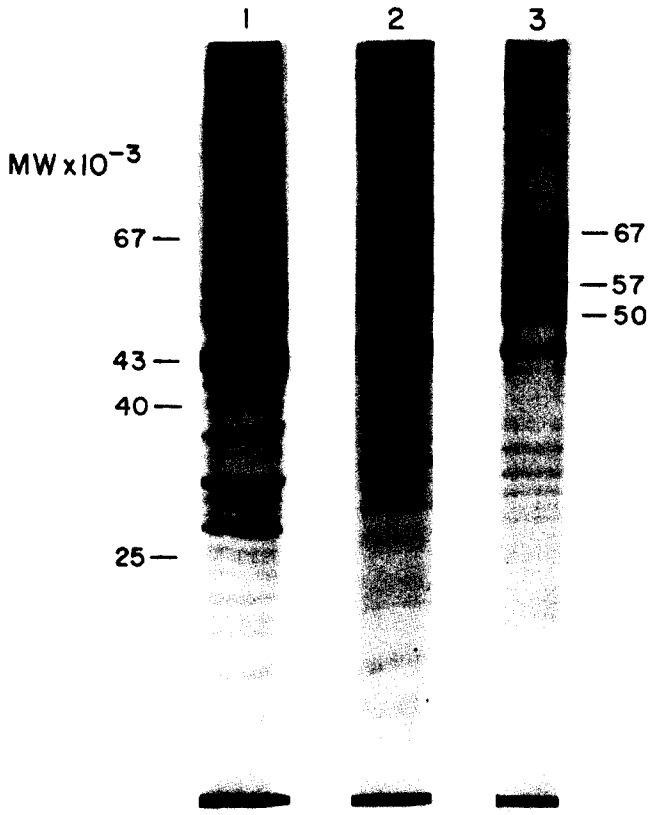

FIG. 7. Purification of platelet membranes. Whole platelets (a), crude platelet membranes (b), and platelet membranes (c) purified by discontinuous sucrose gradient centrifugation were analyzed by SDS-PAGE as described in Method.

Sub-Platelet Fractionation

It was possible to enrich specific imipramine binding by density gradient fractionation of platelets. Sonicated platelet membranes were subjected to discontinuous sucrose gradient sedimentation [46]. A fraction was obtained at the 0.6–1.2 M sucrose interface with a 2.8 fold higher B_{max} for imipramine binding (747 fmol/mg protein vs. 270 fmol/mg protein for crude membranes). This fractionation presumably represents an enrichment of the imipramine receptor in a particular platelet organelle or possibly in the platelet plasma membrane fraction. An SDS-PAGE gel of whole platelets, crude membranes and gradient-isolated membranes is presented in Fig. 7. This gel shows that purification of membranes by discontinuous sucrose gradient centrifugation results in an enrichment of proteins of approximate apparent molecular weight 50,000, 57,000 and 67,000 daltons. However, the specific activity of imipramine binding sites was only 747 fmol/mg. It can be estimated, based on a typical receptor protein of approximately 250,000 daltons, that the imipramine binding site would represent 0.02% of the total protein present in this sample.

It has been reported that there is a significant variation in the B_{max} for imipramine in platelet membranes from one plasma donor to another [8]. Such variations in samples prepared from different plasma sources were also noted in the present study. In the presence of antiproteases, values as low as 230 and as high as 525 fmol/mg protein were found. Incomplete platelet disruption could account for this variation [4].

Differential Inhibitor Specificity

The inhibitory potential (IC_{50}) of various compounds for displacing [^{3}H]-imipramine from platelet membranes (Table 3) are essentially in agreement with results from other laboratories [23,31]. Selective serotonin reuptake inhibitors such as fluoxetine and citalopram were quite potent in displacing [^{3}H]-imipramine. In addition, quaternary-amide derivatives of amitriptyline: *N,N,N*-trimethyl-3-(10,11-dihydro-5H-dibenzo[*a,d*]cyclohepten-5-ylidene)-propanaminium iodide; protriptyline: *N,N,N*-trimethyl-5*H*-dibenzo-[*a,d*] cycloheptene-5-propanaminium iodide; and 10,11 Δ-amitriptyline: 3-(5*H*-dibenzo [*a,d*] cyclohepten-5-ylidene)-*N,N,N*,-trimethyl-propanaminium iodide were roughly equipotent with their tertiary-amine analogues. Other antidepressive compounds such as amitriptyline, quipazine, amoxapine and trazadone were effective at various concentrations, as indicated. Amoxapine and trazodone are new (second-generation) antidepressant drugs; they were effective in displacing [^{3}H]-imipramine only at moderately high concentrations. Compounds such as cocaine, pyrilamine and chlorpromazine, which are not regarded to be effective antidepressants, were found to have some activity. We tested the effect of a number of nucleotide derivatives of guanine and adenosine on binding activity and all were without effect.

DISCUSSION

The data presented support the premise that the human platelet imipramine recognition site is proteinaceous in nature. Firstly, the receptor was sensitive to heat denaturation and was inactivated in the temperature range typical for receptor proteins [37]. Secondly, highly purified trypsin destroyed imipramine binding sites. Finally, receptor-ligand interaction was inhibited by chemical modification of membranes with N-ethylmaleimide, a chemical selective for protein sulfhydryl groups [27]. Reaction of N-ethylmaleimide with amino groups is also possible, but the amino-specific labeling reagent ethyl acetimidate was ineffective in inhibiting platelet imipramine binding. The highest content of receptors was found in a membrane fraction which corresponds to the density of plasma membranes, although the existence of binding sites on alpha or serotonin granules cannot be excluded.

It is clear from the phospholipase A_2 sensitivity of imipramine binding that the membrane lipid environment is important for the integrity and maintenance of the receptor. This effect may represent non-specific changes in the lipid environment, such as viscosity alterations. Support for this contention derives from the fact that detergents have been shown to inactivate imipramine binding to platelets [44]. Alternatively, phospholipases could inactivate a specific boundary lipid, crucial to the structure and function of the receptor site. This data plus the calcium-stimulated lability of the receptor site suggests that endogenous, calcium-activated phophalipases may control imipramine receptor number.

Imipramine has been shown to interact with serotonergic systems. Recent studies suggest that the presynaptic serotonin reuptake system is the site specifically labeled by [^{3}H]-imipramine binding [22,32], although binding to a distinct locus proximal to the reuptake carrier system may al-

TABLE 3

EFFECT OF SELECTED PHARMACOLOGIC AGENTS ON PLATELET ^{3}H-IMIPRAMINE BINDING

	IC_{50} (nM)		IC_{50} (nM)
Histaminergic Agents		Monoamine Oxidase Inhibitors	
Pyrilamine (H_1)	1,005	Pargyline	>10,000
Cimetidine (H_2)	>10,000	Isocarboxazide	>10,000
Diphenhydramine	>10,000	Nialamide	>10,000
Cholinergic Agents		Adrenergic Agents	
Atropine	>10,000	Clonidine (α_2)	>10,000
Scopolamine	>10,000	Phentolamine	>10,000
Oxotremorine	>10,000	Norepinephrine	>10,000
Dopaminergic Agents		Tricyclic Antidepressants	
Haloperidol	>10,000	Imipramine	2.1
Chlorpromazine	231	Desipramine	193
Clozapine	9,000	Amitriptyline	7.0
Loxapine	22,800	N-Methylamitriptyline*	15.2
Apomorphine	>10,000	10,11 Δ-Amitriptyline*	138
		N-Methyl, 10,11 Δ-Amitriptyline*	62
Serontonergic Agents		N,N-Dimethylprotriptyline*	738
LSD	>10,000	Amoxapine	912
Serotonin	10,400	Doxepin	554
Quipazine	201		
		Uptake Inhibitors and Atypical Agents	
		Fluoxetine	16.4
		Cocaine	1,700
		Citalopram	17.7
		Talsupram	5,130
		Mianserin	>10,000
		Trazodone	1,360

IC_{50} value is equivalent to the concentration of drug necessary to inhibit the specific binding of ^{3}H-imipramine (3 nM) to human platelet membranes by 50%.

*See Method for IUPAC nomenclature.

losterically alter the activity of the serotonin reuptake process. This aminergic carrier system certainly represents a major fraction of the specific binding to human platelet membranes which are rich in such activity [12,13]. However, imipramine has been shown to affect other receptor ssytems, such as histaminergic, α-adrenergic and muscarinic [33]. Based on the available data, it is impossible to assess the contribution of these other receptors to the total number of sites labeled by [^{3}H]-imipramine. Human platelet membranes have very low densities of alpha adrenergic receptors [42] and these appear to be of the α_2 subtype [24]. Moreover, the muscarinic cholinergic sites of platelets exist in negligible concentrations [10]. Additionally, cholinergic-characterizing agents (atropine and scopolamine) were very weak in displacing [^{3}H]-imipramine in platelet membranes. The specific serotonin reuptake inhibitors fluoxetine and citalopram were potent displacers of [^{3}H]-imipramine. Pyrilamine, an H_1 antagonist, was also effective at moderate concentrations. Curiously, chlorpromazine (a dopamine anatagonist and calmodulin inhibitor) inhibited imipramine binding at intermediate concentrations.

It is clear that substitution of the side chain nitrogen profoundly influences binding properties of compounds related to imipramine. Recently, specific binding sites for [^{3}H]-desipramine, the N-demethylated derivative of imipramine, have been detected in rat cerebral cortex [3,44]. These sites appear pharmacologically distinct from high affinity [^{3}H]-imipramine sites. However, in contrast to imipramine binding, the potencies of antidepressants in displacing [^{3}H]-desipramine correlates well with their inhibition of presynaptic uptake of norepinephrine [44]. These observations suggest that [^{3}H]-imipramine preferentially labels serotonin reuptake sites while [^{3}H]-desipramine binding preferably recognizes norepinephrine uptakes sites.

Quaternary ammonium derivatives of the tricyclic parent structures were roughly equipotent to their tertiary amine analoques in displacing [^{3}H]-imipramine binding. This has also been shown to be true for the inhibition of serotonin reuptake [16,45]. The specificity of these derivatives have not yet been explored.

Biochemical characterization of the imipramine recognition site has yielded two main observations. First, the imipramine receptor site is influenced by its lipid environment. Perturbations of this environment resulted in alterations of the apparent receptor number. Second, *in vitro* data suggest that the receptor is labile and that degradation is stimulated by calcium.

Since platelets are anucleated, transcriptional and translational processes leading to receptor synthesis are unlikely to occur. Thus, examination of stem cells such as

megakaryocytes or hemocytoblasts for their ability to regulate [^{3}H]-imipramine binding would warrant investigation. It could very well be that these loci or even bone marrow components are indeed ultimately responsible for the observed decreases in human platelet [^{3}H]-imipramine binding in clinically depressed patients [2,8]. Nonetheless, the biochemical and pharmacologic observations presented herein, may provide a more basic foundation for examination of platelet [^{3}H]-imipramine recognition sites and their interrelation with depressive illness.

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