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## Platelets as a model for neurones?

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*Summary.* The multiple biochemical and pharmacological similarities existing between blood platelets and 5-hydroxytryptamine (5-HT)-containing neurones of the CNS point to the platelets as a reliable model for the biochemical characterization of 5-HT releasers and uptake blockers which interfere with the storage and the active carrier mechanism of 5-HT in the neurones, respectively. In addition, the affinity displayed by dopamine and by dopaminergic neurotoxin $MPP^+$ for the platelet 5-HT transport and storage indicates also some similarities between platelets and the dopaminergic system of the CNS. Since human platelets contain almost exclusively monoamine oxidase type B (MAO-B), they can be used as a source for the purification and characterization of this human enzyme. Human platelets thus offer an excellent peripheral model to indirectly assess the degree and duration of MAO-B inhibition occurring in the CNS. To date, knowledge of the many biochemical mechanisms underlying platelet physiology is still fragmentary. In fact, the functional role of binding sites located on the platelet cytoplasmic membrane, i.e. their coupling to a specific transmembrane signalling mechanism, is still in need of a precise biochemical and physiological characterization.
*Key words.* 5-HT releasers; monoamine oxidase type B; 5-hydroxytryptamine; 5-HT blockers; platelet receptors; MPTP; $MPP^+$; 5-HT storage; Ro 19-6327.

### *Introduction*

Progress in the neurosciences has often been the result of studying simple biological models. Blood platelets and chromaffin cells of the adrenal medulla are useful and relatively simple models for the study and the understanding of some complex mechanisms operating in the amine-containing neurones of the central nervous system (CNS).

This symposium, dedicated to Prof. Alfred Pletscher, will confirm that blood platelets fulfill the prerequisites for being considered a valid and relatively simple model for pharmacological studies on central serotoninergic neurones. Human platelets possess at least three organelles which are closely related in their function to those of 5-hydroxytryptamine (5-HT) neurones:

– the cytoplasmic membrane with an active transport system for 5-HT and with binding sites for many drugs and neurotransmitters;
– the subcellular organelles (also called dense bodies) which store 5-HT and other monoamines using a $H^+$ (proton)-translocating ATPase;
– mitochondria with monoamine oxidase (MAO), the enzyme which catabolizes, by oxidative deamination, 5-HT and other monoamines.

The use of platelets to study drugs interfering with the active transport of monoamines (e.g. tricyclic antidepressants), their storage sites (e.g. reserpine) or their metabolism (e.g. MAO-B inhibitors) is facilitated by the fact that blood platelets are easily obtained by venipuncture. For this reason platelets became an attractive model also for clinical studies. In clinical research, platelets are studied with the hope that changes in their aminergic mechanisms would reliably reflect alterations in the central aminergic neuronal system e.g. in psychiatric diseases and in essential hypertension. To date, however, the utility of platelets as a biological marker for well-established physiopathological states is still controversial.

In many respects, blood platelets deriving from the mesoderm differ from neurones which derive from the ectoderm. For instance, platelets are anucleated cells that, in contrast to neurones, do not have the enzymatic machinery needed for the biosynthesis of the monoamines. An exhaustive and pre-

cise biochemical and pharmacological comparison between platelets and 5-HT neurones is almost impossible because too many mechanisms characteristic of the 5-HT neurones are still unknown. Nevertheless, as we will see in this short review, evidence is available which supports the concept that platelets are a useful model for the study of some aspects of neurobiological research.

In this article we will discuss the use of platelets for studying the effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and that of its metabolite 1-methyl-4-phenyl-pyridinium ($MPP^+$), a compound highly toxic for central dopaminergic neurones. In this context, we would like to refer to numerous reviews which appeared on platelets as a model for monoaminergic neurons[86, 90, 92, 103], on the biochemistry and pharmacology of the platelets[25, 30, 47, 104] as well as on the utilization of platelets as a marker in psychiatric diseases[62, 71, 104, 109, 115].

*Platelets and 5-HT neurones: major similarities*

The similarities and differences between platelets and 5-HT neurones, listed in the table, suggest that additional comparative investigations are needed. For instance, it should be clarified whether α-granules[31, 81], the largest platelet secretory granule (≥ 500 nm in diameter) storing several peptides (e.g. the platelet-derived growth factor, β-thromboglobulin, platelet factor-4)[25] share any similarity with the large dense-core vesicles (≥ 80 nm in diameter) which possibly store peptide neurotransmitters or trophic factors in the neurones. Moreover, it is not sufficiently clear whether the platelet organelles which store 5′-phosphonucleotides (mainly adenosine-5′-triphosphate, ATP and guanosine-5′-triphosphate, GTP) are closely related to the small synaptic vesicles (40–60 nm in diameter) which secrete classical neurotransmitters and are localized in typical clusters within the nerve terminals.

A protein which avidly binds 5-HT and which is thought to be involved in the storage of 5-HT in the CNS has been isolated from the rat brain[106]. Since such a serotonin binding protein is absent in rat platelets, it was inferred that platelets are not a useful model to study the 5-HT storage in the CNS[106]. The concept that platelets belong to the APUD (amine-precursor-uptake and decarboxylation) system[84] needs also further investigation. Since both the megakaryocyte, a giant nucleated cell, as well as its progeny, the anucleated platelets[105], contain a neuron-specific enolase (NSE), it was proposed that platelets could originate from the diffuse neuroendocrine system[11]. This crucial point should be investigated with antibodies directed against the protein p38 (38,000 dalton protein), an intrinsic membrane protein specific of the small synaptic vesicles observed in the nerve terminals of the neurones[59]. Antibodies against the protein p38 have recently revealed a new population of vesicles, distinct from the typical secretory granules in various neuroendocrine cells outside the CNS. This new class of small vesicles has been observed e.g. in chromaffin cells of the adrenal medulla, in the endocrine cells of the pancreas and in all cell types of the anterior pituitary[34, 80].

Recently, exocytotic release of neuronal secretory vesicles was clearly observed in synapses but also in nonsynaptic sites of the CNS of various animal species including rats[9]. This nonsynaptic release leading to a diffuse 'hormonal-like' liberation of (peptidic?) messengers and observed in the CNS, resembles in some respects the exocytotic release occurring in platelets.

Comparison between platelets and 5-HT neurones

| | Platelets | Neurones |
|---|---|---|
| Active transport for 5-HT | + | + |
| $5\text{-HT}_2$ receptors | + | + |
| $^3$H-Imipramine binding sites | + | + |
| Subcellular storage of 5-HT in vesicles | + | + |
| MAO type B | + | + |
| Biosynthesis of 5-HT | − | + |
| 5-HT transporter at the plasma membrane (imipramine-sensitive) | + | + |
| 5-HT transporter at the vesicular membrane (reserpine-sensitive) | + | + |
| Neuron-specific enolase | + | + |
| Serotonin binding protein | − | + |
| Protein p38 | ? | + |

*Two sites for the active transport of 5-HT into the platelets: an imipramine-sensitive transporter at the plasma membrane and a reserpine-sensitive $H^+$-translocating ATPase at the granular membrane*

In 1954, Humphrey and Toh observed that dog platelets accumulated 5-HT in vitro[58] and in 1956 it was demonstrated that reserpine depleted 5-HT from brain[6] and platelets in vivo[100]. Thereafter, in 1957, Carlsson et al.[13] showed that reserpine releases 5-HT from the platelets in vitro whereas in 1961 Stacey[102] demonstrated that imipramine blocks the uptake of 5-HT in platelets in vitro. In 1966 Carlsson[12] postulated that noradrenaline is taken up by the catecholaminergic neurones by two mechanisms, one imipramine-sensitive, operating in the cell membrane and another reserpine-sensitive located at subcellular storage organelles[12]. In 1966, Tranzer et al.[110], using electron microscopy and biochemical methods, showed that in platelets 5-HT is stored in typical subcellular highly osmiophilic organelles which disappeared upon injection of reserpine (fig. 1 a, c, e, g). Based on this observation, in 1967 Pletscher et al.[89] postulated that the two transport mechanisms observed in catecholaminergic neurones, were also operating in platelets, at the plasma membrane (imipramine-sensitive) and at the granular membrane (reserpine-sensitive).

In 1967 Da Prada et al.[28] obtained pure fractions of 5-HT organelles by density gradient centrifugation of rabbit platelet homogenates (fig. 1 c). The ultrastructural localization of 5-HT in typical 5-HT organelles was confirmed in platelets from several species including man. Since isolated 5-HT organelles contained high concentrations of 5-HT, ATP and bivalent cations it was postulated that these molecules were stored inside the 5-HT organelles in the form of high molecular weight aggregates[5, 6, 25, 31].

Using the uranaffin reaction (fig. 1 b, d, f, h), a technique which reveals the adenine nucleotides in amine-storing organelles, even 5-HT organelles with a very low content of 5-HT could be detected by electron microscopy[95]. By this method it was possible to demonstrate the occurrence, in the megakaryocytes, of vesicles probably containing high amounts of adenine nucleotides (fig. 1 b). In physiological conditions, these precursors of the 5-HT organelles seem to be devoid of 5-HT and therefore, are not detectable by the osmiophilic reaction (fig. 1 a).

The fact that these organelles accumulate exogenous 5-HT and become osmiophilic[111] strongly supports the view that the megakaryocytes are unable to synthesize 5-HT and that, in physiological conditions, the transporter at the plasma membrane can take up 5-HT from the plasma only when the platelets have entered the circulation.

Recently it was observed, by electron-microscope autoradiography, that mature megakaryocytes of the mouse spleen can also accumulate exogenous dopamine in their vesicles which represent the precursors of the platelet 5-HT or-

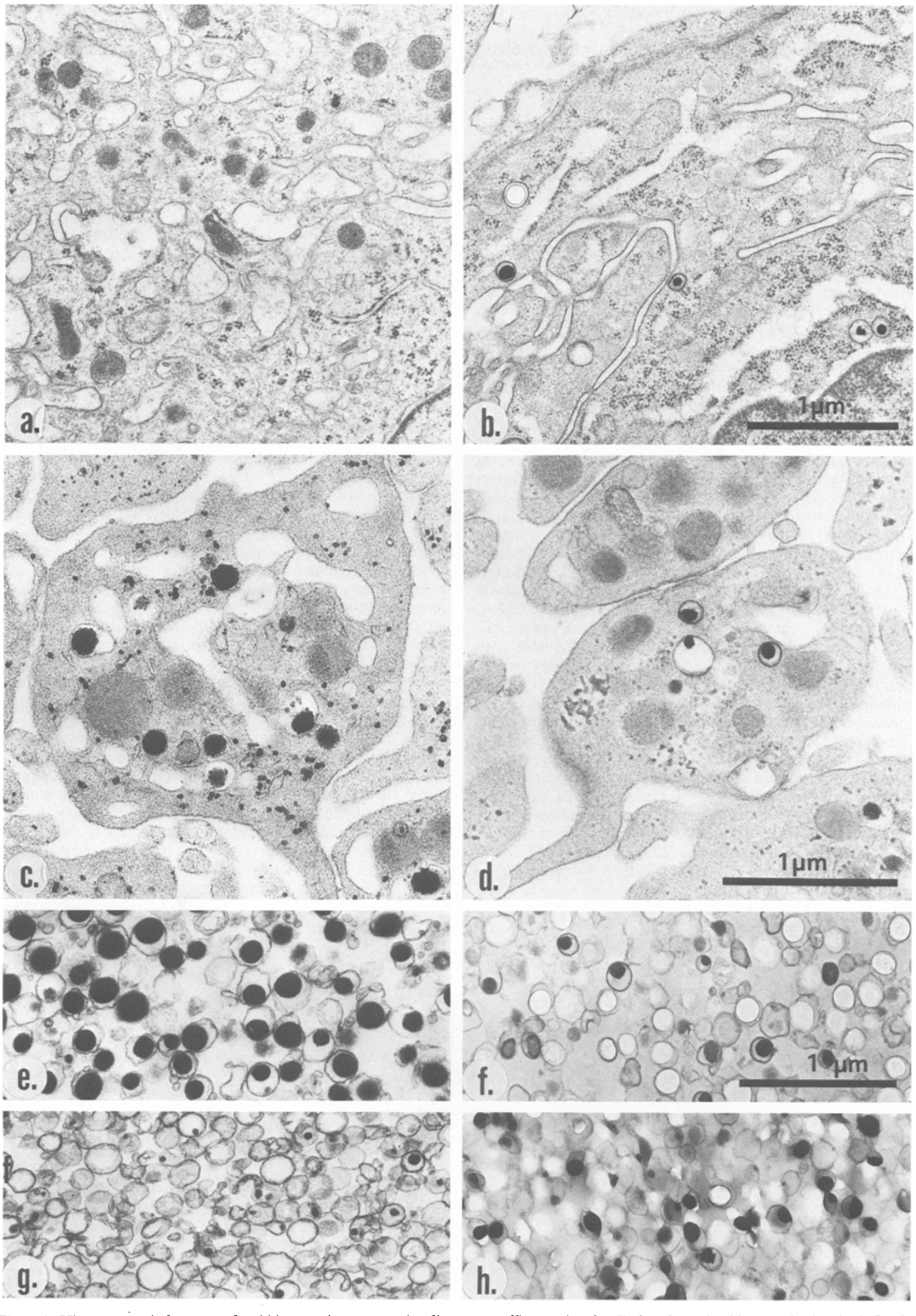


Figure 1. Ultrastructural features of rabbit megakaryocytes (*a, b*), platelets (*c, d*) and isolated 5-HT organelles (*e–h*). Tissues were either fixed conventionally (glutaraldehyde-osmium) or treated with the uranaffin reaction (*a, c, e, g* and *b, d, f, h* respectively). In megakaryocytes amine-storing organelles (lacking 5-HT) can only be detected with the uranaffin reaction for 5′-phosphonucleotides. In platelets both fixation methods reveal 5-HT storing organelles. The isolated organelles are similarly reactive but are only stained with the uranaffin reaction once depleted (by reserpine) of their 5-HT.

ganelles and which are already preformed in the megakaryocytes[19].

By the multidisciplinary approaches mentioned above, involving electron microscopy as well as biochemical and physico-chemical methods, it was possible to gain much insight into the *mechanism of action of reserpine*. For instance, it was shown that after reserpinization, it is still possible, by density gradient centrifugation, to isolate 5-HT organelles depleted of 5-HT but still containing 5'-phosphonucleotides[29]. In addition, it was observed that labeled reserpine, is virtually exclusively localized in the membrane of the platelet 5-HT organelles[27].

Autoradiographical investigations revealed that after injection of labeled reserpine to rats, the radioactivity (consisting of intact reserpine) accumulated in distinct areas of the brain and peripheral sympathetically innervated organs[96]. Thus, reserpine was labeling specifically serotoninergic, dopaminergic and noradrenergic neurones whose amino-storing organelles represented the main target site for the alkaloid[61].

In addition to reserpine, it was shown that also the antimalaria compound mepacrine, accumulated avidly into the 5-HT organelles, inducing the release of 5-HT from the platelets[25]. In contrast to reserpine, mepacrine accumulated in the interior of the 5-HT organelles due to the acidic intravesicular milieu[31].

Ionophores causing a collapse of $H^+$ gradient[41, 60] induced nonexocytotic release of 5-HT and noradrenaline from the intracellular storage organelles of the platelets[3]. The energetics underlying the transport of the amines at the level of the membrane of the chromaffin and of the 5-HT granules is almost identical. Indeed, a membrane bound $H^+$-translocating ATPase in conjunction with the extremely low permeability of the granular membrane to $H^+$ is responsible in both cases, for generation and maintenance of a $\Delta pH$ (inside acidic) and a transmembrane potential ($\Delta \Psi$, inside positive). Apparently, the electrochemical gradient is utilized by the transporter which can translocate a biogenic amine molecule in exchange for one or more protons[31, 41, 60]. Noteworthy, reserpine, but not imipramine, inhibits almost completely the transporter of the 5-HT organelles and of the chromaffin granules[31]. Recently, the amine transporter of the chromaffin granules was purified by affinity chromatography[44]. Interestingly enough, it has been shown that reserpine inhibits the photoaffinity binding of [$^3$H] ANA-5-HT (4-azido-3-nitro-phenylazo-5-HT) to the purified transporter (a 45-kd polypeptide)[44, 99].

*The transport of 5-HT into the platelets* is a well-defined process already discussed in several reviews[86, 103]. Many pieces of evidence indicate that the mechanism at the cell membrane by which 5-HT is actively transported into the platelets and into the serotoninergic neurones is very similar. This transport mechanism is energy-dependent, imipramine-sensitive and requires $Na^+$ and $Cl^-$ ions. The findings presented in figure 2 stress the fact that by using a low substrate concentration ($< 10^{-7}$ M) and short incubation times (1 min or less) many typical and atypical 5-HT blockers inhibit, in a similar rank order of potencies, the uptake of 5-HT in platelets and in synaptosomes. Thus, for the series of inhibitors tested, a good correlation between the $IC_{50}$ values for the inhibition of saturable 5-HT uptake into platelets and synaptosomes of rats was obtained. These results, in which several chemical classes of potential antidepressants are compared, support the notion that platelets are a simple and reliable model of 5-HT neurones in uptake studies. Therefore, platelets seem to be predictive of the effectiveness of 5-HT re-uptake blocker antidepressants at the synaptosomal plasma membrane.

The high-affinity saturable uptake mechanism by which platelets accumulate 5-HT can also transport dopamine and noradrenaline. However, the mechanism of the uptake of dopamine and of noradrenaline in platelets does not share the characteristics of the high affinity uptake that these monoamines display in synaptosomal preparations[72–74]. Therefore, for both dopamine and noradrenaline the platelets seem to be a poor investigative model. In any case, the above results indicate that the 5-HT transporter at the platelet plasma membrane is somewhat less selective for 5-HT than the transporter of 5-HT in neurones. This lack of selectivity is clearly indicated by the fact that the 5-HT transporter of human and animal platelets has a discrete affinity for $MPP^+$, a compound that in synaptosomal preparations, is almost exclusively taken up by the dopamine transport system (see later).

The purification and the characterization of the transporter at the platelet plasma membrane is under intensive investigation[15]. The aim of these studies is to isolate and characterize, by reconstitution experiments, the supramolecular complex consisting of the 5-HT transporter and of the imipramine binding protein. Recent investigations indicate that a pre-labeling with the pseudoirreversible ligand $^3$H-3-cyanoimipramine (Ro 11-2465), a potent 5-HT uptake blocker[22], will be useful for the purification of the membrane-bound intrin-

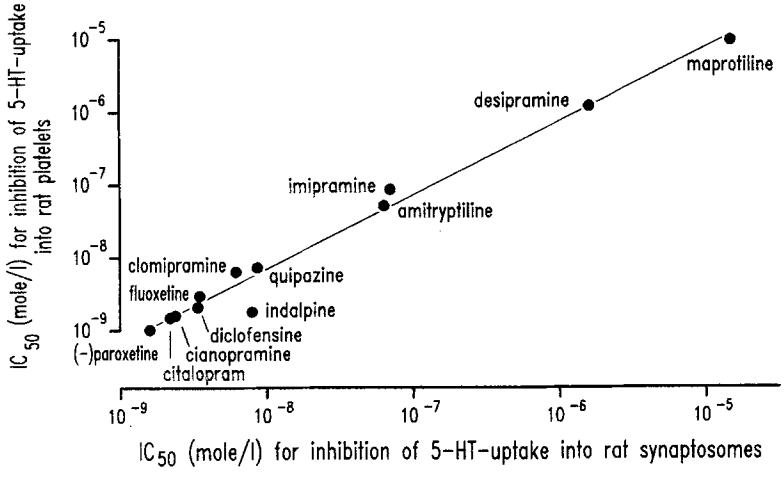


Figure 2. Comparison of the $IC_{50}$ values of a series of uptake inhibitor antidepressants on saturable [$^3$H]5-HT uptake. Six to 10 different drug concentrations were studied in the presence of 5 nmol/l [$^3$H]5-HT. The incubations (1 min at 37 °C) were performed in modified Tyrode. Rat platelets and whole brain synaptosomes were rapidly isolated by filtration on Millipore filters (0.2 μm pore size)[22]. The $IC_{50}$ values for 5-HT uptake in rat platelets correlate with the $IC_{50}$ values for rat synaptosomes ($r = 0.998$, $p < 0.001$).

sic molecule of the transporter-imipramine binding site complex[32, 33]. Several recent lines of evidence indicate that the imipramine-binding site and the 5-HT transporter are closely related or even one and the same. An allosteric coupling between the $^3$H-imipramine labeled sites and the 5-HT transporter seems to exist[76]. Furthermore, some evidence suggest that the high-affinity imipramine binding site, present both in platelets and brain[64, 83] and which is closely associated with the 5-HT transporter, might be considered as a receptor for a putative not yet identified endogenous ligand with a regulatory function in the uptake of 5-HT[4, 6, 63]. A 43,000-dalton glycoprotein purified to homogeneity from human plasma binds to human platelet membranes and stimulates the uptake of 5-HT[1].

The potent 5-HT uptake inhibitor 2-nitroimipramine was developed as a high-affinity probe of the imipramine binding site in platelet membranes[94]. Recently, photoaffinity labeling of [$^3$H]2-nitroimipramine to the 5-HT uptake/tricyclic binding site complex was reported[114].

*MPTP and MPP$^+$ as probes for the study of the enzyme MAO-B, the 5-HT transporter at the plasma membrane and at the organelles storing 5-HT*

In the CNS, MPTP, following conversion into MPP$^+$ by the monoamine oxidase type B (MAO-B), selectively destroys the dopaminergic neurones of the substantia nigra in humans and other primates, producing the symptoms of Parkinson's disease[75]. In the brain, the neurotoxic quaternary compound MPP$^+$ was shown to be actively transported into the dopaminergic neurons by a carrier-mediated, mazindol-sensitive mechanism. It was therefore postulated that neurotoxicity required both the conversion of MPTP to MPP$^+$ by MAO-B located in the dopaminergic and 5-HT neurones and in the glia, as well as the uptake of MPP$^+$ by dopamine neurones. In fitting with this view, the pretreatment of animals with MAO-B inhibitors and with dopamine uptake blockers prevents MPTP neurotoxicity.

We could show that human platelets, which are rich in MAO-B activity[31], accumulate [$^3$H]MPTP which was rapidly converted to [$^3$H]MPP$^+$. Accumulation of the radioactivity was markedly impaired when MAO-B inhibitors were added to the incubation medium, whereas 5-HT uptake blockers or MAO-A inhibitors were inaffective[20]. As for neurones, the MAO-B activity present in the platelet plays an essential role in the accumulation of [$^3$H]MPTP because only low amounts of radioactivity accumulated in platelets with low levels of MAO-B activity (e.g. guinea pig and rabbit platelets)[20]. In the study presented in figure 3, human blood was collected from healthy volunteers and EDTA (1 % w/v in saline) was used as anticoagulant. In some experiments the platelets were directly incubated (1 h at 37 °C) as platelet-rich plasma (PRP) in the presence of 5 nM [$^3$H]MPTP with or without MAO-B inhibitors or 5-HT uptake blockers. In other parallel experiments, the platelets were isolated from the plasma by centrifugation, washed twice in EDTA-containing Tyrode, and finally resuspended and incubated (1 h, 37 °C) in Tyrode containing EDTA (0.8 % w/v) with 5 nM [$^3$H]MPTP and the drugs. As shown in figure 3 both the irreversible (pargyline and selegiline) and the reversible (Ro 16-6491 and Ro 19-6327) MAO-B inhibitors[14] markedly reduced the accumulation of [$^3$H]MPTP-derived radioactivity. On the other hand, the specific 5-HT uptake blockers markedly inhibited the accumulation of the radioactivity only in the PRP experiments. These results and additional

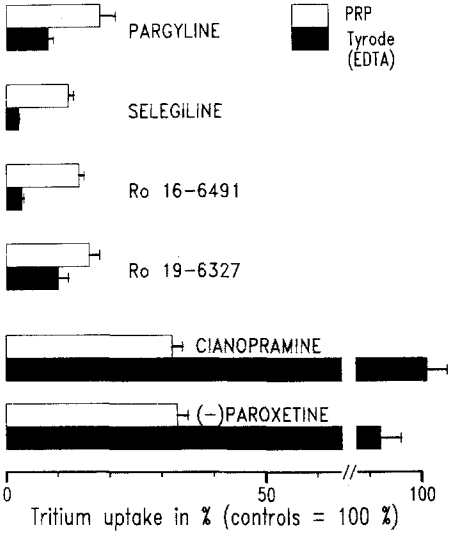


Figure 3. Effect of MAO-B inhibitors (1 μM) and 5-HT uptake blockers (1 μM) on the accumulation of 5 nM [$^3$H]MPTP-derived radioactivity in human platelets incubated (1 h, 37 °C) in plasma (PRP) or in modified Tyrode containing EDTA (0.8 % w/v saline). Means ± SEM, N = 3 (in quintuplicate). Control absolute values (DPM/mg platelet protein): PRP = 99,600; Tyrode (EDTA) = 198,700.

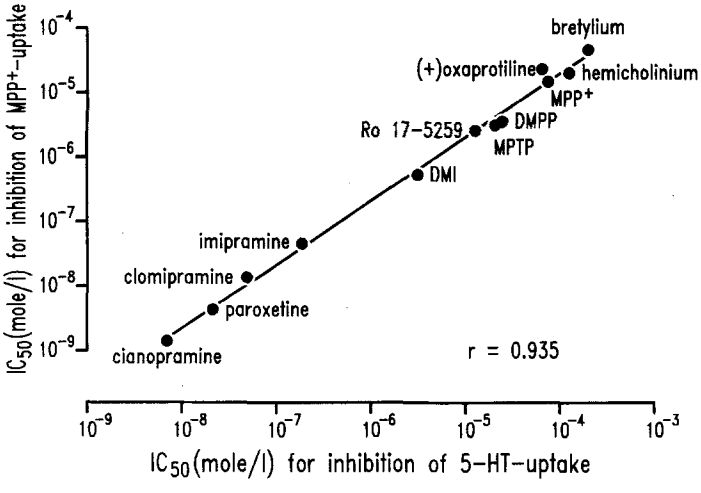


Figure 4. Comparison of the $IC_{50}$ values for a series of amine uptake inhibitors and quaternary compounds on saturable [$^3$H]MPP$^+$ and [$^3$H]5-HT uptake in human platelets incubated in plasma (15', 37 °C). $IC_{50}$ values were obtained from inhibition curves based on 5 different drug concentrations and are means of 3 experiments performed in triplicate[16].

experiments (not shown) strongly support the view that EDTA (1% w/v) can be used as an anti-coagulant when the uptake experiments are performed in PRP. In contrast, when the platelets are washed and incubated in buffers containing EDTA, some modifications of the plasma membrane are likely to occur (e.g. light permeabilization of the membrane and/or allosteric modification of the carrier). This result and the observation that EDTA reduces by nearly 50% the $V_{max}$ for saturable 5-HT transport[73] suggest that EDTA, should be used with caution, as anticoagulant, in uptake experiments.

Recent experiments provide evidence that the quaternary compound $MPP^+$ is actively accumulated into human and animal platelets by the same energy-dependent carrier mechanism that is utilized for the transport of 5-HT and dopamine[16, 21]. The kinetic analysis of the uptake of [$^3$H]$MPP^+$ by human platelets showed that the affinity of the drug for the 5-HT carrier (apparent $K_m$ = 22.6 μM) is slightly lower than that of dopamine ($K_m$ = about 100 μM) but about 50 times higher than that of 5-HT (apparent $K_m$ = 0.46 μM)[16].

In human PRP, the uptake of [$^3$H]$MPP^+$ was markedly blocked by metabolic inhibitors (ouabain and KCN) and by selective 5-HT uptake blockers (e.g. cianopramine, paroxetine, clomipramine) (fig. 4). These findings and the fact that the high-affinity uptake of 5-HT in platelets was inhibited competitively by $MPP^+$ leads to the conclusion that the compound is actively transported into the platelets by the 5-HT transporter[16]. As measured in parallel experiments, the $IC_{50}$ values for several uptake blocking antidepressants and quaternary compounds correlate with their respective potency as inhibitors of the uptake of [$^3$H]5-HT (fig. 4). Additional experiments with rabbit platelets indicate that [$^3$H]$MPP^+$ transported into the platelet is, at least in part, accumulated in the 5-HT storage organelles. Subcellular fractionation experiments performed on homogenates of rabbit platelets loaded with [$^3$H]$MPP^+$ and submitted to density gradient ultracentrifugation showed that the distribution of radioactivity and the content of the endogenous 5-HT throughout the density gradient was virtually the same. The fact that the peak concentrations for [$^3$H]$MPP^+$ and 5-HT correspond to the fraction of the pure fraction of the 5-HT organelles clearly proves that [$^3$H]$MPP^+$, once transported into the platelets, can be stored in the 5-HT organelles[16]. Several indirect experiments support the notion that the [$^3$H]$MPP^+$ taken up by platelets is accumulated in the 5-HT organelles. For instance, in vitro, typical platelet 5-HT releasers (thrombin, reserpine, mepacrine etc.) as well as the ionophore monensin and nigericin, markedly released the radioactivity from human platelets previously loaded with $MPP^+$[16]. Preliminary in vivo experiments showed that [$^3$H]$MPP^+$ injected intravenously to rats accumulated in trace amounts almost exclusively in platelets. In these animals, the concentration-ratio between platelets and plasma [$^3$H]$MPP^+$ attains a value of about 4000. Noteworthy, the concentration gradient for [$^3$H]$MPP^+$ between rat platelets and plasma is about 5 times higher than that for catecholamines measured in man and rabbits[26].

Unlabeled $MPP^+$, injected repeatedly i.p. to rats (10 mg/kg, twice daily for 4 days) resulted, 16 h after the last injection, in a marked decrease of the endogenous platelet 5-HT. This 5-HT decrement was long-lasting since the amine was still significantly low (about 40% decrease) 96 h after the last $MPP^+$ injection.

As shown in electron micrographs (fig. 5a, b) the osmiophilic cores typical of the 5-HT organelles virtually disappeared 16 h after the last $MPP^+$ injection (fig. 5b). A reappearance of the osmiophilic bodies was found at a time at which the 5-HT content of the platelets was partially restored (96 h after the last $MPP^+$ injection) (fig. 5c). Under our experimental conditions no appreciable ultrastructural damage was detected in the platelets, indicating that the toxicity of $MPP^+$ is essentially directed only towards the dopaminergic cells of the substantia nigra.

A summary of the interactions between MPTP and $MPP^+$ and the platelets or the neuronal and glial elements of the CNS is given in figure 6. The two possible mechanisms which might ultimately lead to the degeneration of dopaminergic neurones, i.e. inhibition of mitochondrial NADH dehydrogenase or formation of free radicals by the interaction with neuromelanin[75] are also illustrated (fig. 6). Since $MPP^+$ is taken up by the 5-HT organelles of the platelets it is proposed that $MPP^+$ might be accumulated in the dopaminergic vesicles (fig. 6). Altogether our results show that blood platelets are a very useful cell model for studying in vitro and in vivo the mechanisms leading to cell-specific accumulation of the neurotoxin $MPP^+$. MPTP and $MPP^+$ represent, therefore, new tools for a more precise characterization of the similarities and dissimilarities existing between platelets and neurones.

*Human and Rhesus monkey platelets as peripheral models to study MAO-B activity*

In this symposium the role of the MAO on the metabolism of the monoamines is dealt with by Dr Youdim. However, I would like to stress a few achievements in our own laboratories concerning MAO-B. Only human and Rhesus monkey platelets show a relatively high MAO activity which is of the B type, for which 5-HT is a poor substrate. Human platelets have been used as an enzyme source to raise monoclonal antibodies against the MAO-B which indicate that human 5-HT neurones of the raphe nuclei are particularly rich in MAO-B activity[37].

It is interesting to note that this antibody is able to recognize MAO-B molecules in all human tissues investigated, including brain[37]. Using this monoclonal antibody a radioimmunoassay was developed which allows measurements of the total concentration of the enzyme (active plus inactive) as well as, in combination with catalytic activity assays, the calculation of the molecular activity of the enzyme in platelets[43]. The measurement of these two parameters should give accurate information on the genetic expression and control of the enzyme in platelets[43].

In recent studies, using labeled Ro 16-6491 (a very specific and reversible, mechanism-based MAO-B inhibitor), it was possible to demonstrate selective binding of this new ligand to human brain mitochondrial and platelet membranes[14]. The high-affinity specific binding of [$^3$H]Ro 16-6491 observed in the saturation curves of figure 7, indicate that [$^3$H]Ro 16-6491 has a slightly higher affinity for frontal cortex than for platelets MAO-B ($K_D$ = 47 and 108 nM, respectively)[14]. Both in human frontal cortex and platelets, Scatchard analysis of saturation curves for [$^3$H]Ro 16-6491 showed a single population of binding sites. The nonspecific binding increased linearly by increasing the [$^3$H]Ro 16-6491 concentration and at 20 nM of the ligand it was about 10% and 20% of total binding for brain and platelets, respectively[14].

In the experiments shown in figure 7, Ro 19-6327, a highly potent and selective reversible MAO-B inhibitor, chemically related to Ro 16-6491 and being proposed for clinical trials[24], was administered to Rhesus monkeys and the time course of the MAO-B activity in intact platelets measured using a newly developed assay which utilizes [$^3$H]MPTP as the substrate[23]. The results in figure 8 show that the MAO-B activity of the monkey platelets, following Ro 19-6327 (10 mg/kg p.o.), was rapidly inhibited for more than 48 h.

Human and Rhesus monkey platelets represent a peripheral

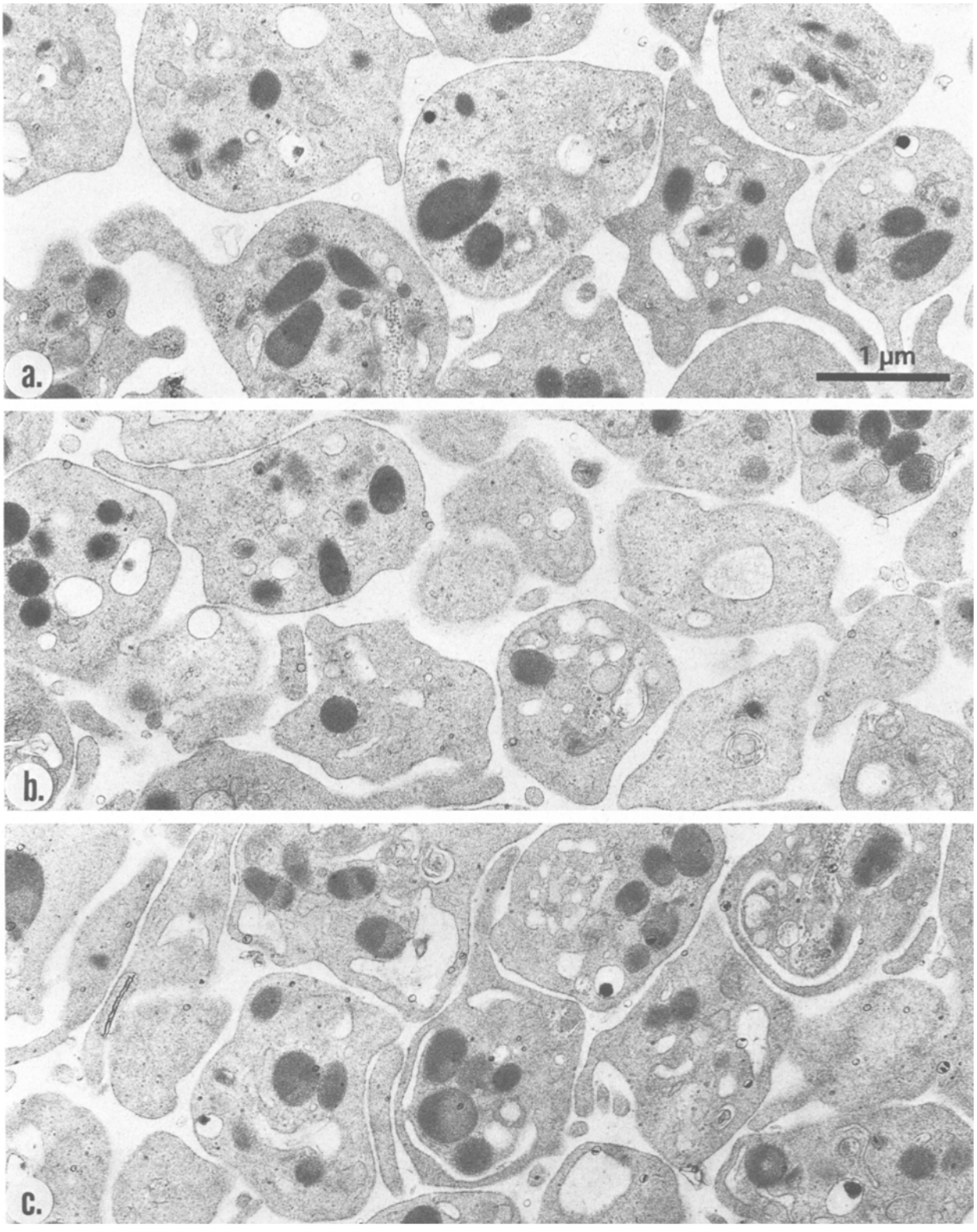


Figure 5. Ultrastructural features of rat platelets fixed by a conventional method (glutaraldehyde–osmium). *a* control; *b, c* $MPP^+$ (10 mg/kg i.p. twice daily for 4 days), 16 h and 4 days after the last injection, respectively.

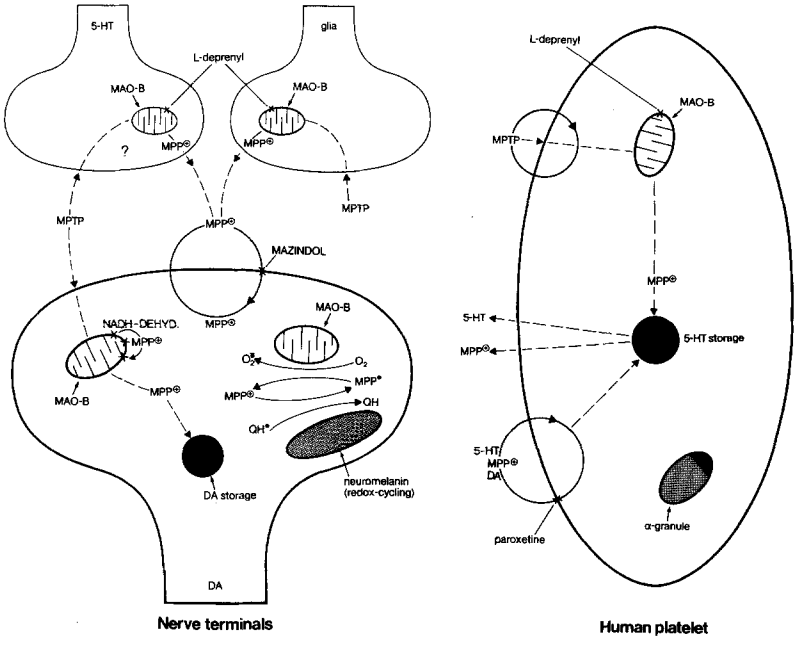


Figure 6. Schematic illustration of the multiple interaction occurring in the platelets, in the neurones and in the glia between MPTP and its metabolite $MPP^+$ (for details see the text).

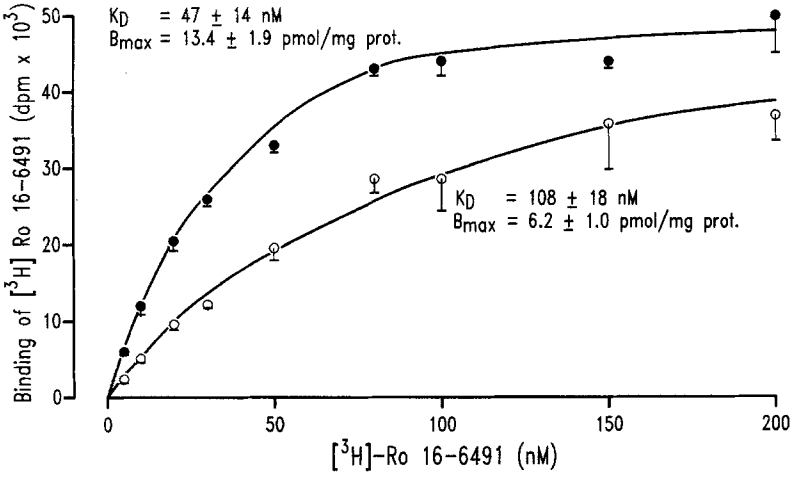


Figure 7. Specific high affinity binding of [$^3$H]Ro 16-6491 to human frontal cortex crude mitochondrial preparations (●) and platelet membranes (○) at various ligand concentrations. Means ± SEM of 3 experiments performed in duplicate.

source of cells containing virtually only the B type of MAO activity. Therefore, platelets represent an excellent model for pharmacological studies of MAO-B and may be used to assess indirectly the degree of MAO-B inhibition in the CNS.

*Receptor sites at the platelet plasma membrane and their signal transducing system*

Blood platelets possess several binding sites on their plasma membranes only few of them have been identified as true receptor sites with pharmacological characteristics similar to the receptors present in membranes of neurones[77]. The study of a single receptor system of the CNS is often very difficult due to the extreme complexity of the nervous tissue and to the presence of multiple receptors and receptor subtypes. Therefore, the use of the blood platelet model may provide useful information about the structure and the function of some receptor systems which are also present in the CNS. Thus, in intact isolated platelets it has been possible to investigate 5-HT receptors and the entire sequence of events that follows the receptor-ligand interaction (see below).
Among the platelet receptors that are present also in neurones, the most extensively characterized are the 5-HT and the α-adrenoceptors. Various peptides, like vasopressin, substance P, and nerve growth factor, appear also to have specific receptors on membranes of platelets[50].

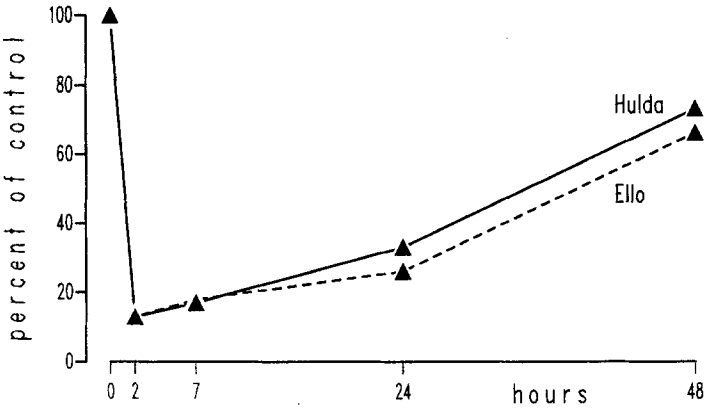


Figure 8. Time course of MAO-B inhibition measured in intact platelets from 2 Rhesus monkeys (*macaca arctoides*) administered Ro 19-6327 (10 mg/kg p.o.). MAO-B activity was measured using 2.5 nM [$^3$H]MPTP as substrate[60]. Absolute values (pmol/h/$10^9$ platelets): Ello = 2.9; Hulda = 3.

*5-HT receptor*

The 5-HT receptor of platelets, clearly distinct from the 5-HT carrier system, has been characterized both by a functional parameter, the 'shape change reaction', and by radioligand binding experiments. According to its pharmacological profile, this receptor seems to be of the 5-$HT_2$ subtype[36, 85]. The stimulation of platelet 5-HT receptors with 5-$HT_2$ agonists, causes a typical functional alteration, in which platelets change from their normal discoid shape into a spherical form with extrusion of pseudopods[7, 48, 66].

This reaction is specifically antagonized by drugs (ketanserin, spiroperidol, D-butaclamol, LSD) that show high affinity for the 5-$HT_2$ receptor in brain[67, 87]. Specific, high-affinity binding of [$^3$H]5-HT to platelets has been found[38] and [$^3$H]ketanserin, a specific 5-$HT_2$ antagonist, binds with similar affinities (about 1 nM) to rat brain membranes and cat platelets[70].

Stimulation of the 5-$HT_2$ receptor in platelets does not appear to activate the adenylate-cyclase system[88]. The same is observed in brain where only the 5-$HT_1$ effects are mediated by the adenylate cyclase system[8]. In contrast, evidence exists that the effects of 5-$HT_2$ receptor stimulation are mediated in platelets, and probably also in brain, through the activation of the polyphosphoinositide turnover, which yields inositol triphosphate ($IP_3$) causing a rise of platelet cytoplasmic free $Ca^{++}$[2, 35, 39]. The phosphoinositide-dependent and -independent mechanisms involved in platelet activation have been recently reviewed[65].

*α-Adrenoceptor*

Stimulation of platelets with an α-adrenergic agonist, such as adrenaline or noradrenaline, causes inhibition of adenylate cyclase activity, with a decrease of cyclic AMP production and a tendency of platelets to aggregate[54–56]. It is now generally acknowledged that the adrenoceptors of human platelets are of the $\alpha_2$ subtype[18, 45, 49, 53]. The affinities of various ligands, with a very similar pharmacological profile in respect to the action of different agonists and antagonists, are very similar for brain and platelet receptors. However, caution should be exercised in the interpretation and extrapolation of results from platelets to other α-adrenoceptors[52]. The platelet α-adrenoceptors have been purified by affinity chromatography[93] and will be better characterized in the near future.

*Vasopressin receptor*

The vasopressin receptor present on human platelet membranes appears to belong to the class of $V_1$-receptors[98, 112, 113]. The binding of [$^3$H]arginine-vasopressin ([$^3$H]AVP) and the morphological effects of AVP (shape-change reaction and aggregation) on platelets are strongly dependent on the presence of divalent cations such as $Mg^{++}$[40, 91, 97]. A similar enhancement by $Mg^{++}$ of [$^3$H]AVP binding has also been reported in brain neurones[17]. The functional effect of AVP on platelets appear to be mediated, similar to cells of the anterior hypophysis, by an activation of the polyphosphoinositide turnover[101, 112]. Recently, a solubilization method for human platelet vasopressin receptors has been reported[107]. Therefore, the vasopressin receptor purification will probably be purified very soon.

*Concluding remarks and trends*

The available findings, summarized in this review, show that blood platelets are a reliable and predictive model for the pharmacological characterization of drugs supposed to interfere with the mechanisms of storage and uptake of 5-HT in the CNS. Platelets are an excellent investigational tool for MAO-B inhibitors in preclinical studies. Moreover, in clinical investigations platelets offer a reliable and noninvasive means of indirectly monitoring the degree of 5-HT uptake and/or MAO-B inhibition.

These measurements are currently carried out in clinical pharmacological studies with the aim of assessing the therapeutic dose of newly developed 5-HT blockers[69] or MAO-B inhibitors[42].

A more accurate assessment of the dissimilarities of storage and uptake between platelets and 5-HT neurones will be achieved only when the supramolecular complex 5-HT uptake/imipramine-binding site and the transporter at the storage sites will be isolated in pure form. In any case, the affinity that dopamine, the neurotoxin $MPP^+$ and its precursor MPTP share for the platelet 5-HT transport and storage systems, point to some similarities between platelets and dopaminergic neurones of the CNS. This is also supported by the fact that human platelets, similarly to brain tissue, can sulphoconjugate dopamine by the enzyme phenolsulphotransferase[108].

The coexistence in the platelet 5-HT organelles of different monoamines and 5-phosphonucleotides[30] suggests that platelets, similarly to neurones[57], release multiple chemical messenger candidates. For instance, the huge amounts of 5'-phosphonucleotides released by an exocytotic mechanism from the 5-HT organelles might indicate that platelets have purinergic functions and might be used as a model for purinergic nerves[10]. Interestingly, platelets contain melatonin as well as the enzymatic equipment for its biosynthesis, i.e. serotonin-N-acetyltransferase and hydroxyindol-O-methyltransferase[68]. To date, it is not clear whether the 5-HT neurones of the CNS are also able to synthesize melatonin.

Many findings support the view that platelets are a valid model for several aspects of the serotoninergic neurones. However, the use of platelets as a model for dopaminergic, glutaminergic and GABAergic neurones is only partially justified[82]. Platelets can be considered as multitransmitter storage sites similarly to neurones. To date it is not clear whether this redundance has a functional relevance or merely represents a residual vestige of the evolution.

Platelets are extensively used to investigate the second messenger signalling mechanisms involved in platelet activation. A better comprehension of the second messenger systems underlying platelet activation[65] will certainly be of help in elucidating physiological and pharmacological responses related to the neuronal system.

Finally, due to the multifunctional role of the platelets, it is probable that some binding sites already detected at the platelet plasma membrane, will be characterized as receptors in the near future. The most interesting are the $\beta_2$ adrenoceptor[51], the peripheral benzodiazepine receptor[82], the $H_2$ histamine receptor[46], the angiotensin II receptor[79] and the interferon $\gamma$ receptor[78].

The physiology and the pharmacology of the platelets is studied with increasing interest by several investigators. The platelet model, which is already a classical one for the study of the central serotoninergic system, may in the near future be used for the elucidation of other transmitter-mediated mechanisms operating in the CNS.


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