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Platelets as models: Use and limitations

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Key words. 5-Hydroxytryptamine; 5 HT uptake; neurotransmitters; platelet receptors; 5 HT-storage; LDL activation; phosphatidyl-inositol metabolites; $(Ca^{2+})_i$.

The idea of using platelets as an experimental system for neuropharmacology originated in 1955 when experiments with reserpine, an indole alkaloid from *Rauwolfia serpentina,* were carried out. This drug, which was used in the treatment of mental disorders (e.g. schizophrenia), was found to cause a marked depletion of 5-hydroxytryptamine (5 HT) in the brain of rabbits[6]. Since a few years before this discovery, platelets had been reported to contain 5 HT, the suggestion was made that they might be useful for elucidating the mode of action of *Rauwolfia* alkaloids. Indeed, reserpine was then shown to cause a decrease of the 5 HT content in platelets in vivo[7] and in vitro[4], and the in vitro experiments indicated that this decrease was due to a release of the amines from the cells (fig. 1). Later on, various synthetic drugs not related to *Rauwolfia* alkaloids were found to cause a decrease of cerebral 5 HT, and all these compounds also released the amine from platelets. Tetrabenazine, a derivative belonging to the group of 1,2,3,4,6,7-11 bH-benzo(a)quinolizines, was the first synthetic derivative shown to exhibit this effect (fig. 1)[4].

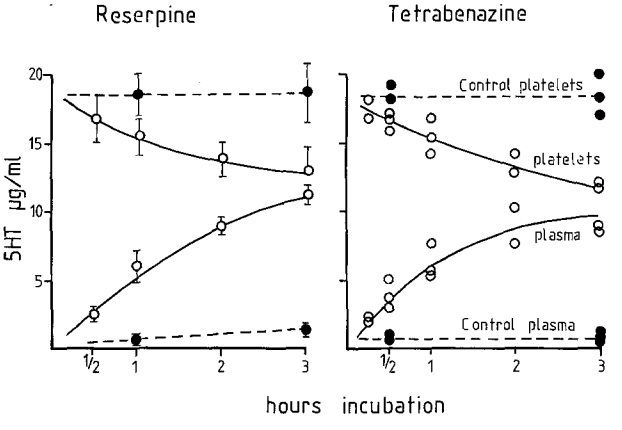

Figure 1. Effect of reserpine (1 µg/ml) and tetrabenazine (20 µg/ml) on the 5 HT content of blood platelets and plasma at 37 °C. The drugs were added to platelet-rich plasma (PRP) at zero time (taken from 4, 3).

Since platelets had also been shown to exhibit a specific uptake of 5 HT at the plasma membrane similar to that in cerebral neurons, the platelets were proposed to be a potential model for 5 HT neurons[3,5].

In the following years the use of platelets as models developed further and many of the speakers in this symposium have made essential contributions to this concept. On the basis of the results presented in this symposium and of other findings, I will try to give a short review and summary of the present knowledge about the neurotransmitter elements of platelets and their potential use as models for other tissues, especially the central nervous system.

Some neurotransmitter elements of human blood platelets are summarized in figure 2. They include an uptake system for 5 HT, intracellular storage organelles (dense bodies),

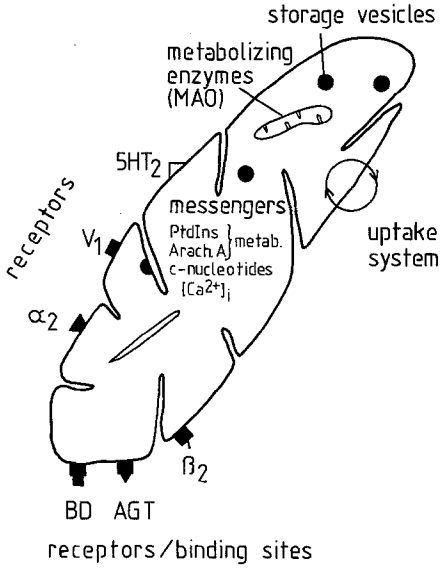

Figure 2. Neurotransmitter elements of blood platelets. V_1: vasopressin-1; BD: 'peripheral' benzodiazepines; AGT: angiotensin; α_2: alpha$_2$-adrenergic; β: beta-adrenergic.

monoamine oxidase (MAO), various membrane receptors and binding sites and intracellular messengers.

5 HT uptake

The 5 HT is taken up at the plasma membrane both by passive diffusion and by an active mechanism. The second type of uptake is much more effective than the former at low (physiological) concentrations of the amine. The active uptake depends on metabolic energy, Na^+ and Cl^-, and is saturable, i.e. mediated by a carrier. It shows close similarities to the 5 HT uptake of 5 HT neurons in the central nervous system. Thus the K_m of the 5 HT uptake is of the same order in platelets and neurons (between 10^{-8} and 10^{-7} M). Also drugs, especially antidepressants like imipramine, inhibit the specific 5 HT uptake in both platelets and neurons and their potencies are of similar order in both cells. The uptake system of platelets has a relatively high specificity for 5 HT since the K_m values for other amines e.g. dopamine and noradrenaline are several orders higher than that for 5 HT. However, experiments with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) indicate that the 5 HT transporter at the platelet membrane may be somewhat less selective for 5 HT than the transporter of 5 HT neurons (see Da Prada et al., this symposium).

The antidepressant imipramine shows high affinity binding to membranes of platelets and brain synaptosomes (in the nanomolar range). Furthermore, antidepressant drugs which inhibit 5 HT uptake also displace imipramine from its binding sites in platelets and neurons. The orders of potencies of the drugs are similar in both cell types. However, imipramine and 5 HT probably bind to different sites of the 5 HT carrier. The antidepressant seems to inhibit 5 HT uptake by causing an allosteric change in the carrier (for more details see Langer, this symposium).

The similarities between platelets and neurons regarding 5 HT uptake and imipramine binding has stimulated much clinical work, in which platelets were taken as models or markers for certain pathological conditions like mental depression, Down's syndrome, migraine etc. However, most of these investigations need further clarification before final decisions can be reached, although in certain conditions (e.g. subforms of mental depression), patterns with some consistency seem to emerge (see also Wirz and Langer, this symposium).

5 HT storage

Conclusive evidence of specific intracellular storage organelles for 5 HT and other basic compounds (dense bodies) was first provided in 1966 [8] (for more details see Da Prada et al., this symposium). Later on, the mechanism of this intragranular storage was investigated by several authors. Results indicate that an ATP-driven proton pump at the membrane of the storage organelles (granular membrane) probably establishes a proton gradient across this membrane (pH inside < outside). The 5 HT would then passively enter the storage organelles along this gradient, a reserpine-sensitive carrier being involved. Once within the organelles, 5 HT binds reversibly to intragranular nucleotides (e.g. ATP and ADP), a process which enhances the storage process (fig. 3).

The mechanism of amine storage in the intracellular organelles of neurons has not yet been fully elucidated. However, experiments with amine-releasing drugs provide some clues. Thus, drugs which act by different mechanisms, e.g. by causing a collapse of the transmembrane gradient of protons in storage organelles or by interfering with the carrier at the granular membrane, cause amine release from both platelets and neurons (see table 1). Therefore, similarities probably also exist between platelets and neurons regarding subcellular amine storage. It seems that the uptake of 5 HT into platelets and 5 HT neurons is governed by 2 processes, i.e. a specific uptake at the plasma membrane and a distinctly different uptake at the granular level, which is not highly specific for 5 HT since other basic substances (e.g. dopamine, noradrenaline, mepacrine) are also accumulated in the dense bodies.

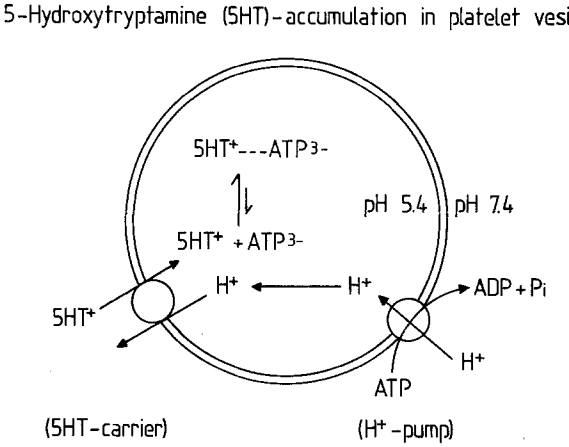

Figure 3. Mechanisms of 5 HT-storage in an intracellular storage organelle (dense bodies, vesicles) of platelets.

Table 1. Release of granular amines by drugs with different mechanisms of action. Similarities between human platelets and rat synaptosomes.

Mechanism Interference with	Releasing drugs	Response Platelets	Synaptosomes
Granular membrane carrier	Reserpine, benzoquinolizines etc.	+	+
Transmembrane proton gradient	Ionophores	+	+
Intragranular storage complex	Phenylalkylamines	+	?

Enzymatic inactivation

The main inactivating enzyme for monoamines in platelets is monoaminoxidase (MAO), which also occurs in neurons of the brain. The metabolic end-products in both tissues are 5-hydroxyindolacetic acid and to some extent 5-hydroxyindol-3-ethanol (5-hydroxytryptophol). Human platelets contain exclusively MAO-B, for which 5 HT is a relatively poor substrate. In the brain both MAO-A and MAO-B are present. However, the 5 HT neurons seem to contain MAO-A, for which 5 HT is a better substrate (see Youdim, this symposium).

As indicated by Da Prada et al. (this symposium) MAO-B metabolizes not only arylethylamines but also compounds like MPTP. This can take place in platelets as well as in brain leading to the neurotoxic metabolite methylphenylpyridinium (MPP^+). Other metabolizing enzymes, e.g. phenolsulfotransferase, and other 5 HT-metabolites, e.g. 5-O-methyl-N-acetyltryptamine[2] have also been found in platelets. The availability of an easily accessible source of MAO-B in human platelets has stimulated considerable clinical work, e.g. in mental depression and schizophrenia. However, although some interesting changes in enzyme activity have emerged in these pathological conditions, especially in some subtypes of depression, more work is needed before generally accepted conclusions are reached (see also Wirz and Youdim, this symposium).

Receptors

Neurotransmitter-receptors are present together with many other receptors at the platelet membrane. A new area of receptor research was opened up when labeled ligands with extremely high specific radioactivity became available (e.g. tritium-labeled compounds). With their help, a multiplicity of receptors and specific binding sites for neurotransmitters and neuromodulators could be identified on platelets. Some of them are indicated in figure 2.

The spectrum and potencies of neurotransmitter agonists and antagonists which interfere with these binding sites are similar to those in neuronal cells. This has, for instance, been demonstrated for the 5 HT ligand ketanserin by Leysen and De Chaffoy, for (^{3}H) D-LSD by Grahame-Smith (this symposium) and for (^{3}H) arginine-vasopressin by several authors including ourselves. In addition these ligands cause functional changes in the brain and in platelets (e.g. a shape change reaction), indicating that their sites of interaction on the cell are functional receptors.

From these and other findings it has been concluded that some neurotransmitter receptors of platelets can serve at least as partial models for those in brain.

A further dimension was added to research on neurotransmitters in platelets by the rapid development of knowledge about the cellular metabolic processes which follow receptor stimulation. The pace of progress in this area has been amazing in the last few years. Three pioneers of receptor-linked messenger-systems, Drs Feinstein, Rink and Marche, have dealt with the present state of the art in this symposium. There is evidence for at least four cellular pathways mediating neurotransmitter-induced platelet activation. These are summarized in a very simplified way in table 2.

These messenger systems show complex interactions which are far from being fully understood. The platelet seems to be an excellent model for studying these interactions and for elucidating the interrelation between the messengers and their target molecules, e.g. contractile proteins.

Platelets as models for neurons

The above and other findings led to the conclusion that platelets can be used as partial models for some neurotransmitter elements in neurons (e.g. 5 HT uptake, amine storage, receptors, metabolism). Of course, platelets are not neurons and even the above-mentioned neurotransmitter elements are not identical in the two cell types. Table 3 summarizes some of the similarities and dissimilarities between platelets and 5 HT neurons.

Although this incompleteness of the model calls for caution in its application, it might have the advantage of relative simplicity. The absence in platelets of a dense, complex network interconnecting the cells, such as that found in neuronal tissue, the missing biosynthesis of 5 HT, and the lack of a nucleus in mature platelets may be relevant in this respect.

Table 2. Platelets: intracellular messenger systems for neurotransmitters. PLC: phospholipase C; PKC: proteinkinase C; DAG: diacylglycerol; IP_3: Inositoltriphosphate.

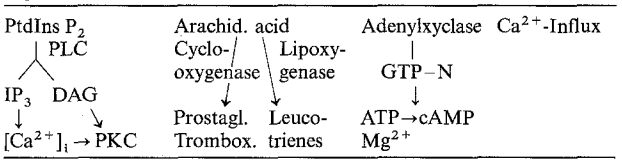

Table 3. Similarities and dissimilarities between platelets and neurons regarding their 5 HT system.

Element	Similarities	Dissimilarities
Biosynthesis		Tryptophan-hydroxylase 5 HT-turnover
Uptake	5 HT-Kinetics Imipramine binding Inhibition by drugs	Degree of specificity Ouabaine sensitivity
Storage	Dependence on proton gradient Release by exocytosis ionophores reserpine arylalkylam	Dependence on granular membrane potential
Metabolism	Oxidative deamination MPTP activation	MAO-subforms
Receptor	Ketanserin binding Pharmacological spectrum PtdIns-turnover $(Ca^{2+})_i$ (?)	Intrasynaptic regulatory influences

Models for other cell types

The use of platelets as models has not only been confined to neuronal cells but has also been extended to other cell types, especially vascular smooth muscle cells. The involvement of platelets in cardiovascular disorders has been known for a long time. In this symposium, Drs Vanhoutte and Baumgartner gave reviews on some of the latest developments. Several similarities between platelets and vascular muscle cells are known to exist. For instance, both cell types possess contractile proteins. Furthermore, they both exhibit neurotransmitter-receptors, i.e. alpha$_2$-adrenergic, 5 HT_2-, and V_1-receptors. Their stimulation leads to functional changes in both cases, e.g. shape change in platelets and contraction in vascular muscle, which are mediated by similar messengers (e.g. phosphatidylinositol-metabolites, $(Ca^{2+})_i$). Prof. Bühler reported some recent findings of his group indicating a possible use of platelets as models for vascular smooth muscle.

LDL and platelets

One of the most recent developments regarding similarities between platelets and other cells has been presented by

Table 4. LDL-induced increase (↑) and decrease (↓) of cellular metabolites in various tissues. PIP_2: phosphatidylinositol-diphosphate; DAG: diacylglycerol; IP_3: inositoltriphosphate; $(Ca^{2+})_i$: intracellular free calcium.* Preliminary results.

Metabolite	Platelets	Vascular muscles	Fibroblasts	Brain slices*
PIP_2	↓	↓	↓	
DAG	↑	↑	↑	
IP_3	↑	↑	↑	↑
$[Ca^{2+}]_i$	↑	↑	↑	

L. Block and A. Pletscher (not printed). In contrast to high density lipoprotein (HDL_3), human low density lipoprotein (LDL), which binds to specific sites of human platelets (K_D $3-6 \times 10^{-8}$ M), induced a shape change reaction, measured by densitometry and verified by electron microscopy. This effect was accompanied by an increased generation of diacylglyceride, phosphatidic acid and inositol 1,4,5-trisphosphate (IP_3), by a decrease in phosphatidylinositol 4,5-bisphosphate (PIP_2), and by an elevation of cytosolic free calcium concentration, $(Ca^{2+})_i$. The rise of $(Ca^{2+})_i$ was more pronounced in calcium-containing media, but was still significant in the absence of extracellular calcium, suggesting that LDL mobilizes calcium from intracellular stores. Both the functional and biochemical changes occurred at the same order of magnitude (1–40 µg/ml) of LDL and with similar time courses, and both were counteracted by HDL_3 (20–100 µg/ml), which is known to act as an inhibitor of LDL binding. The LDL-induced changes were paralleled by those of thrombin except that HDL_3 caused no inhibition in the case of thrombin. Inhibition of LDL-induced activation was also seen in the presence of albumin. The inhibitory action of plasma proteins may explain the lack of responsiveness of platelets to LDL when tested in platelet rich plasma.

These results indicate that LDL causes rapid platelet activation by stimulation of phospholipase C, resulting in an enhancement of phosphatidylinositol (PtdIns)-turnover with a rise in $(Ca^{2+})_i$. Further studies showed that low concentrations of LDL also activate the (PtdIns)-cycle in rat arterial smooth muscle cells, human skin fibroblasts, vascular endothelial cells, and, according to preliminary experiments, also in slices of rat brain (table 4) [1].

Therefore, low concentrations of LDL seem to bring about cell activation, possibly in a hormone-like way. This effect could play a role in pathophysiological processes (e.g. in arteriosclerosis) and here again, platelets might serve as a useful model.

'Praise' of platelets as models

The rationale for using platelets as models for cells can be summarized as follows:

1) Platelets are easily obtainable in native form from humans and, in contrast to animal cells, are undoubtedly representative for man.
2) It can be assumed that certain pathological disturbances of neurotransmitter dynamics and receptor function in mental and cardiovascular disorders are of a generalized nature. Platelets can therefore play a role in elucidating pathophysiological mechanisms and may serve as diagnostic tools or in some cases as monitors of therapy.
3) Platelets may be used for pharmacological screening (e.g. for 5 HT uptake inhibitors, amine releasers, receptor-active compounds), thus reducing the number of animal experiments required.

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Current activities in the Department of Research of the University Hospital, Basel

Human growth hormone in urine: development of an ultrasensitive radiometric assay

by T. Erb, G. Karolyi, A. Pampalone, A. N. Eberle, J. B. Baumann, E. Bürgisser* and J. Girard

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Summary. An immunoradiometric assay for human growth hormone (HGH) has been developed which has a detection limit of 1 ng/l and can measure HGH in unextracted urine from normal children and adults. The assay is based on a two-step procedure, using a solid-phase goat-anti-HGH immunosorbent for immunoextraction and [^{125}I]-labeled monoclonal HGH-antibody for detection and quantification. The assay is not affected by urea, NaCl or changes of pH from 5–8. The mean urine HGH concentration in normal children is 6.78 ± 7.6 (SD) pg/ml, in patients with HGH-deficiency 1.3 ± 0.9 pg/ml which increases to 11.7 ± 13.4 pg/ml on the day of growth hormone injection.
Key words. Human growth hormone (HGH); urine; IRMA; ultrasensitive assay.