Platelets and neurobiological research

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The beginning of my friendship with Alfred Pletscher can be dated exactly because in 1967 he accepted my invitation to give the first Michael Cross Memorial Lecture at the Royal College of Surgeons in London. It was a splendid lecture [15]; over all these years I remember the excitement transmitted by Alfred to his large audience.

We met again in Bristol in 1970 at a symposium of the Society for Endocrinology on 'Subcellular organisation and function in endocrine tissues'. I see from the still interesting and admirably produced book of that symposium that Alfred's contribution immediately preceded mine; his on storage of monoamine-nucleotide aggregates, and mine on 5-hydroxytryptamine in blood platelets; mechanisms of uptake and release. Apparently I was the first in the discussion of his paper when I said: 'I was delighted by the whole story, but particularly by your last remarks, because, towards the end of this morning, I wrote in my notes: Does ATP *stack*, as nucleotides do in nucleic acids? If they do stack, then there are, of course, analogies with other nucleotide-stacking situations in which drugs intercalate or interpose, at least partially. The molecular weights can then increase ad infinitum; the question is, what limits the molecular weight?' and Professor Pletscher gave a very pertinent and thoughtful reply. He in turn was the first to comment on my paper, saying: 'Did you say that the thrombin-induced release of 5-HT is due to expulsion of the whole granule?' and I gave what I hope was a similarly thoughtful reply.

This anecdote at the beginning is merely to try and establish some credentials for my being allowed to introduce this session, for which I wish to thank the organisers very warmly. The world-wide community of platelet researchers is aware by now that these peculiar bits of cytoplasm have turned out to be just as interesting to neurobiologists, and more specifically to psychopharmacologists, as to cardiovascular physiologists and pathologists. But the non-vascular interest is much the more recent. There was a period of several years during which these two legs supporting platelet research were very different, with the circulatory leg characterized by elephantiasis and the neurobiological leg by infantile paralysis. Fortunately, unlike the other disability, this leg rapidly gained strength until it is now the equal of the other; and much of this progress is certainly attributable to Alfred Pletscher. As we all know, it was the ability of platelets, alone among the blood cells, to take up and concentrate 5-hydroxytryptamine (serotonin), that caught the imagination of so many of us, including Alfred Pletscher's, and first led to the idea that platelets, apparently so different and certainly in such a different physiological situation from the cells of the central nervous system, might have important properties in common with them. Much effort was devoted by Pletscher's group and by mine, as well as by some others, to the elucidation of the mechanisms responsible for the uptake, storage and release of serotonin in platelets. With the discovery of tryptaminergic neurones in the brain, mechanistic parallels began to be established with platelets. This soon led to the use of platelets in determining the modes of action of centrally-active drugs, with very considerable success.

My own interest in platelets began with the idea that their capacity for accumulating serotonin might be similar to that of adrenal medullary cells for accumulating adrenalin, on which I was then working with Professor Hugh Blaschko in Oxford. Thus, my own work on platelets began with the idea that the serotonin in them might be bound to ATP, just as adrenalin is in adrenal medulla; that turned out to be the case. I then discovered that the ATP breaks down to ADP during blood clotting. These observations, together with the discovery by Hellem and colleagues of the aggregating effect of ADP on platelets, initiated the elucidation of the haemostatic function and the thrombotic malfunction of platelets.

At that time also I devised the optical platelet aggregometer [3]; it is almost exactly 25 years old today and a symposium soon to be held at Brown University in Providence will review progress. I suppose it is fair to say that this simple in vitro quantification of the fundamental function of platelets has been at the basis of much of the knowledge we now have about them: not only in haemostasis and thrombosis but also as neuro- and psycho-pharmacological models.

This, then, brings up the topic of this part of our symposium in honour of Alfred Pletscher. The contributions which he and his excellent coworkers have made to neurobiology by the clever use of platelets are outstanding. Those made up to 1980 were summarised by Professor Pletscher in a thoughtful review entitled 'Use and limitations of platelets as models for neurones: amine release and shape change reactions' [16]. His critical analysis of these platelet mechanisms have been fundamental for advances in understanding and in designing psychotherapeutic drugs; their significance for modern clinical medicine needs no emphasis.

Because I myself was intermittently active in this field and invariably stimulated by reading Alfred's publications and by discussions with him, perhaps I may be permitted to refer briefly to some relevant work of ours which in its neurobiological implications still awaits complete clarification. The work was done towards the end of my tenure of the Pharmacology Professorship in Cambridge, where we were fortunate to have comparatively liberal access to in- and out-patients of the local mental hospital. One research concerned the long-term effect of lithium on the uptake of 5-hydroxytryptamine by human platelets [6]. The mechanism by which lithium (Li) diminishes the clinical manifestations of manic-depressive patients is still uncertain.

There is considerable evidence for the involvement of brain amines in human affective disorders [17]. The long-term administration of Li to experimental animals increases the uptake of noradrenaline, 5-hydroxytryptamine (5-HT) and tryptophan by synaptosomes and diminishes the release of neurotransmitters from stimulated brain-slices.

In man, administration of Li increases the uptake of 5-HT by blood platelets which possess mechanisms for 5-HT uptake and storage similar to those of tryptaminergic neurones. It is not known, however, whether the effect of Li is to increase the transport of 5-HT across the outer membrane of the platelets or the capacity of the intracellular granules which store 5-HT. Our experiments suggested that both systems are affected by Li.

The content, uptake and storage of 5-hydroxytryptamine (5-HT) in platelets were determined in eight manic-depressive patients not on lithium (Li); in ten manic-depressive patients on Li; and in ten apparently normal persons as controls. Platelets from the patients, whether or not on Li, contained less 5-HT than platelets from normal people. 5-HT uptake and storage by platelets from untreated patients were significantly lower than those of control subjects. When patients had been on Li for 3 months or longer the dimin-

ished uptake and storage of 5-HT by their platelets were more than fully reversed.

The mechanism of this effect of Li remains obscure; evidently it does not depend on the presence of Li itself in the environment of the platelets.

The observations indicate that different functions of platelets are affected differently by Li, and that its long-term effects on the uptake and storage of 5-HT in the patients' platelets depend on Li-induced biosynthetic changes in components of amine transport and storage systems [2, 12].

There is evidence suggesting that mood depends inter alia on the concentrations of free amines in the brain. If platelets are a good model for central tryptaminergic neurones, the antimanic effect of Li could be explained on the basis of increased uptake and storage of 5-HT by synaptosomes, thereby reducing the concentrations of this amine available for acting on synapses.

Particularly intriguing to me was our demonstration of increases in aggregation by and uptake of 5-hydroxytryptamine with platelets from rabbits treated with chlorpromazine [1].

There was evidence that after patients have been treated with chlorpromazine for more than about a week, the effectiveness of 5-hydroxytryptamine (5-HT) in causing aggregation of their platelets is greatly increased [9]. This effect is specific in as much as there is no concomitant change in the response of the platelets towards the aggregating action of adenosine disphosphate (ADP). Furthermore, the effect is reversible, in that about a week after administration of chlorpromazine is stopped, the sensitivity of the patients' platelets to 5-HT decreases to what it was before chlorpromazine was administered. Finally, the effect is repeatable in the same patients. In order to analyse the mechanism of this drug-induced change in the response of platelets to 5-HT, it seemed useful to find out whether a similar effect could be demonstrated in another species.

Our results confirmed for rabbit platelets the discovery made with human platelets that their normally slight aggregability by 5-HT is greatly increased after chlorpromazine has been administered for more than a few days. The aggregability of rabbit platelets by ADP, like that of human platelets, was not affected, so that the chlorpromazine effect was to that extent specific for 5-HT. The increased aggregability of rabbit platelets by 5-HT during the administration of chlorpromazine occurred in two successive phases with one maximum after 7 to 10 and another after 18 to 24 days. This particularly curious effect has, apparently, not been described for human platelets. Secondly, it was now established, at least for rabbits, that their treatment with chlorpromazine also affects the high affinity uptake system for 5-HT in platelet membranes (Baumgartner and Born, 1969). Both the V_{max} and the K_m were increased, suggesting that the number of uptake sites is increased but that the average affinity of the sites for 5-HT is decreased.

Both effects, on aggregation and uptake, would be most simply explained by assuming that the administration of chlorpromazine for more than a few days induces increases in the number of platelet receptors that are specific for 5-HT [7, 8]. As the effects took about 3–4 days to manifest themselves, and as this is also the mean survival time of circulating platelets in rabbits, it would seem likely that such an increase in 5-HT receptors occurred in the megakaryocytes from which platelets are derived. This would be consistent with an increase in the biosynthesis of receptor proteins of which platelets themselves are incapable.

By analogy with other drug-induced receptor modulations, such an increased biosynthesis could be a consequence of the blocking in vivo of 5-HT receptors by chlorpromazine for which it has a high affinity [13]. A diminution in the ability of cells which normally do so to react to 5-HT, would induce a mechanism for increasing their production of 5-HT receptors. This would show itself in the case of platelets as increases in aggregation by an uptake of 5-HT.

However, this explanation does not take into account the fact that added 5-HT induces a characteristic change in shape in normal rabbit platelets [13, 14], indicating that they possess receptors for 5-HT with which it reacts very effectively. Furthermore the magnitude of the shape change, unlike that of aggregation, is not increased by chlorpromazine treatment. There is evidence that platelets can aggregate without having undergone demonstrable changes in shape. It may be, therefore, that the effect of chlorpromazine treatment is to increase the number or the availability of only those platelet receptors which, together with fibrinogen and calcium [5, 10] are essential for aggregation.

Both shape change and aggregation are inhibited by methysergide in low concentrations [7]. However, injection of methysergide into rabbits did not affect aggregation in the same way as did chlorpromazine. This may have been because the doses of methysergide were too small, or because the explanation of the chlorpromazine effect as a consequence of 5-HT receptor blockade is too simple.

Towards the end now I should like to say a little about the fascinating way by which platelets release their stored serotonin and associated substances into the surrounding medium. This has to begin with a story against myself because this crucially important reaction was definitely not discovered by the late Michael Cross and myself in the early days of aggregometry. We obtained optical records of the release reaction but failed to notice them as something having to be explained. A little later, similar tracings were correctly interpreted by MacMillan and Oliver. Ever since then I exhort my research students to look out for the anomalous and the contradictory in their experimental results if they want to discover something really new! The release reaction quickly gave rise to the chain-reaction or positive feedback hypothesis to account for the growth of platelet aggregates in vitro and in vivo [4] in loose analogy with the cascade hypothesis of coagulation. The clinical importance of the reaction arises out of its inhibition by aspirin because it is this fact which underlies the success of aspirin in diminishing the incidence in certain patient populations of myocardial infarction or of cerebral thromboembolic events.

The release reaction, as an example of exocytosis, is still incompletely understood. So I should like to end with something that has intrigued me since optical aggregometry first provided consecutive tracings of primary and secondary aggregation, the latter being the optical manifestation of the release reaction. The tracings, mutatis mutandis particularly of the time base, look very much like the pre- and action-potentials of nerve, there being in both a sharp threshold beyond which the first phase enters the second. The situation in nerve is described [11] as a gradual transition from the subliminal to the threshold response, leading to a propagated action potential; it is only when the local potential reaches the certain critical size that it develops into a true propagated disturbance; if it fails to achieve this it dies out as a localised monophasic wave – it is said to decrement to extinction. The aggregometer tracings could be described in very similar terms but, to the best of my knowledge, the mechanism of the threshold effect is much less well understood in platelets than in nerves. That is one task of many which should keep us all, and particularly our admired friend Alfred Pletscher, happily employed for a long time to come.

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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-