Effect of Chlorpromazine and Reserpine on the Blood Platelets of Rabbit[1]

An Electron Microscope Study

In blood 5-hydroxytryptamine (5HT) is localized in platelets, from which it is released by reserpine and related substances[2]. Also chlorpromazine and allied drugs cause a decrease in the 5HT content of platelets, but the mode of action may be different from that of reserpine[3,4]. It is likely that 5HT in platelets is bound to subcellular particles[5] as it is in other tissues[6,7]. It is supposed that the action of reserpine occurs without morphological changes in platelets. However, in previous experiments we found that chlorpromazine decreased the packed platelets volume without decreasing the number of the platelets[8]. This is an indication of structural alteration which has been verified by the present investigation.

Methods. Arterial blood was collected by means of a polythene cannula from rabbits anaesthetized with ether. Blood was immediately mixed with 1/9 vol of 3.8% sodium citrate, and platelet-rich plasma was obtained by centrifugation by $200 \times g$. Employing gentle shaking at 37°C, samples of platelet-rich plasma were incubated for 1 h with or without $10^{-3} M/l$ of chlorpromazine hydrochloride[9]. After incubation, platelets were separated from plasma by using refrigerated centrifuge ($4000 \times g$, 15 min). Plastic tubes and pipettes were used. 5HT was measured from platelets by a spectrophotofluorometric method[10], and platelet counts were made in the phase contrast microscope. Because it is not possible within reasonable time to produce an almost total 5HT release *in vitro* with reserpine, this alkaloid (or solvent only) was injected intravenously at a dose of 0.5 mg/kg, 18 h before bleeding.

For electron microscopy, a strip of ordinary filter paper was dipped into the centrifugate containing packed platelets. The strip, to which platelets were attached, was then fixed in 1.2% potassium permanganate solution for 1 h. The specimens were dehydrated in rising series of ethyl alcohols and embedded in Araldite. Thin sections were cut on a Porter-Blum microtome and examined in a Siemens Elmiskop I electron microscope. A control specimen was brought with every treated sample so as to make the electron micrographs comparable. The platelets of three or four rabbits were investigated in each group.

Results. The number of platelets per mm^3 of platelet-rich plasma varied between 7.70×10^5 and 13.0×10^5. Incubation with chlorpromazine did not decrease the platelet counts as compared with the corresponding controls. Nor had reserpine pretreatment a clear influence on the number of platelets. More than 95% of 5HT was released from platelets by both of these treatments. In two experiments, incubation with chlorpromazine decreased the packed volume of platelets by 31 and 33%.

When studied in an electron microscope, the shape of the platelets was round or oval (Figure, A). The hyalomere was finely granular and seemed not to send out any re-

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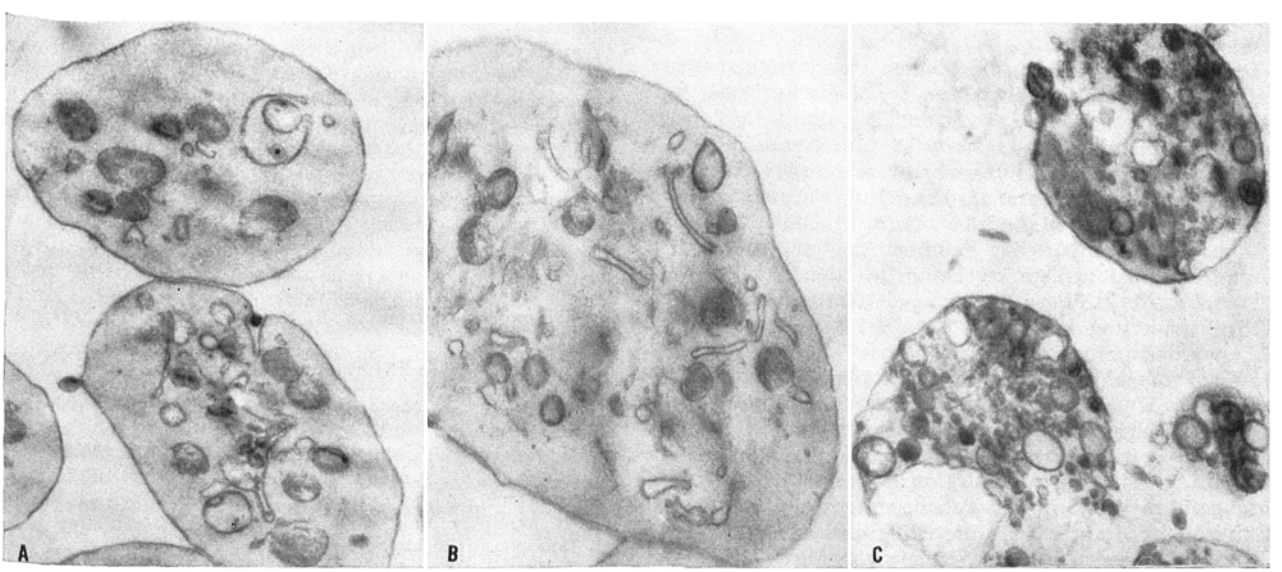

A. Normal rabbit platelets; B. Platelet treated with reserpine; C. Platelets treated with chlorpromazine. In the uppermost platelet an intact β-granulomere (mitochondria) is leaking through the broken cell membrane, the innermost platelet has lost a part of its cellular material which is probably seen on the right side of the Figure (×19000).

markable processes or pseudopods. This observation was made having examined several hundreds of platelets in each group. Some small infoldings of the cell membrane or limiting membrane were seen. The granulomere exhibited the usual organelles: α-granulomeres with homogenous ground substance, rather rare β-granulomeres or mitochondria, γ-granulomeres or microvesicles, and δ-granulomeres or vacuoles. Agranular membranes, representing either a part of Golgi complex or poorly developed endoplasmic reticulum, were observed.

In the present experiment, the administration of reserpine did not result in any alterations in the ultrastructure of the platelets, except that they seemed to be somewhat swollen (Figure, B). In contrast, the administration of chlorpromazine affected the platelets seriously (Figure, C). In most of the cases the limiting membrane was broken and the cytoplasmic material was leaking through this gap. At the same time, the easily vulnerable cytoplasmic organelles seemed to remain quite intact both inside and outside the platelet.

Discussion. Our observations on the ultrastructure of the normal rabbit platelet are well in accord with those reported in the rather extensive literature published since the pioneer works of RINEHART[11] and BERNHARD and LEPLUS[12]. For references the reader is referred to BRAUNSTEINER[13].

This work has shown that in blood platelets, at least *in vitro*, chlorpromazine produces structural changes, which can explain the 5HT releasing action of this agent. Chlorpromazine has been supposed to increase the permeability of the limiting membrane of platelets[3]. The ability of chlorpromazine to reduce the packed platelet volume[8] is conceivably connected with its deleterious effect on the cell membrane, clearly seen in electron micrographs, and thus providing direct evidence of the mode of action of this compound on platelets. Although the platelets had lost almost all their 5HT content, the electron micrographs showed that a considerable part of cytoplasm was still inside the platelet. This may indicate that chlorpromazine has an open way to affect also the membranes of cytoplasmic organelles and that it allows a rapid liberation of the stored amine. In fact, *in vivo* chlorpromazine seems primarily to act on the intracellular particles of rat brain. It decreased the ratio of particulate/supernate 5HT without decreasing the total amine[7].

Reserpine, on the other hand, produced no such morphological changes in the structure of platelets. This is in accord with the assumption that reserpine liberates 5HT from platelets by blocking the active amine transport, which maintains the high concentration of amine inside the cell[14].

Zusammenfassung. Chlorpromazin und Reserpin verursachen 5HT-Freisetzung von Thrombocyten. Chlorpromazin verursacht auch morphologische, elektronenoptisch sichtbare Veränderungen der Thrombocyten.

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Über die Bindung der Ca-Ionen an das Na^++K^+-aktivierbare Adenosintriphosphatase-System des Gehirns

Die Ca-Ionen spielen in der Zellmembrantätigkeit sehr wahrscheinlich eine wesentliche physiologische Rolle. Es ist bekannt, dass ein grosser Teil des Ca-Gehalts der Zellen in der Zellmembran lokalisiert ist[1]. Mehrere Angaben sprechen für eine Hemmung der Membranpermeabilität durch Ca-Ionen[1-3]. Besondere Beachtung verdient in diesem Zusammenhang die Na^+-K^+-ATPase[4]. Dieses Enzymsystem ist nämlich in der Zellmembran lokalisiert und kann durch Ca-Ionen stark gehemmt werden[5-9].

Die Na^+-K^+-ATPase wurde aus Rattenhirn durch Zellfraktionierung dargestellt[10]. Die ATP-spaltende Aktivität wurde in einem aus 50 m*M* Tris[11]-HCl-Puffer von pH 7,4, 5 m*M* $MgCl_2$, 5 m*M* Tris-ATP bzw. 100 m*M* NaCl und 20 m*M* KCl bestehenden Reaktionsgemisch bestimmt. Nach Ablauf der Inkubation (10 min, 37°C) wurde die Bestimmung des anorganischen Phosphats aus dem proteinfreien Filtrat nach LOHMANN und JENDRASSIK durchgeführt[12]. Die in der Tabelle dargestellte ATPase-Aktivität ist definiert als die Differenz aus (ATPase-Aktivität gemessen in Anwesenheit von Mg^{++} + Na^+ + K^+) und (ATPase-Aktivität in Anwesenheit von Mg^{++} allein).

Ca-Ionen beeinflussen die nur mit Mg^{++} oder mit Mg^{++} + Na^+ gemessene Aktivität gar nicht, sie hemmen jedoch eindeutig die in Gegenwart von Mg^{++} + Na^+ + K^+ bestimmte Enzymaktivität. Die halbmaximale Hemmung durch Ca-Ionen wurde in einer Konzentration von 0,5 m*M* festgestellt.

Um zu entscheiden, wie die Hemmung durch Ca-Ionen zustande kommt, prüften wir eine fragliche Bindung der Ca-Ionen durch das Enzympräparat. Es wurden Versuche in Anwesenheit von Citrat, Oxalat bzw. ÄDTA[13] mit einer 0,5 m*M* Ca-Konzentration durchgeführt. Die Ca-Ionen wurden entweder unmittelbar dem Reaktionsgemisch zugegeben, oder das Enzympräparat wurde mit

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