Storage of 5-Hydroxytryptamine in Human Blood Platelets

It has been shown by combined biochemical and electron microscopic techniques that the 5-hydroxytryptamine (5HT) of blood platelets of rabbits and guinea-pigs is stored together with 5'-phosphonucleotides (especially adenosine-5'-triphosphate (ATP)) in specific subcellular organelles[1,2]. The mode of storage of 5HT in human blood platelets is not clear. Some authors claim that the amine is not particle-bound[3,4], whereas others postulate a localization of 5HT in 'Bull's eye' α-granules[5] or in specific organelles which are said to be very rare[1,6] or quite numerous[7].

In the present work, an attempt was made to isolate and characterize specific subcellular organelles storing 5HT and nucleotides in human blood platelets.

Isolated human platelets have been homogenized by ultrasonication and submitted to ultracentrifugation in a continuous Urografin® gradient[8]. The various fractions were analyzed for their content of 5HT[9], ATP[10] and proteins[11] or submitted to electron microscopy using glutaraldehyde and osmium tetroxide as fixatives[1]. The same procedures were applied to isolated human platelets preincubated in plasma containing 500 μg 5HT/ml for 1 h at 37 °C.

The highest concentration of 5HT and ATP (per μg protein) was present in a fine layer attached to the bottom of the centrifugation tube. The concentration of 5HT in the bottom layer was more than 100 times that in the whole platelets (Table), and a similar difference existed for ATP ($19.3 \pm 3.7 \times 10^{-4}$ and $27.5 \pm 1.4 \times 10^{-6}$ μmoles ATP/μg protein in organelles and whole platelets, respectively). The other fractions (e.g. those consisting of pure α-granules and mitochondria) did not exhibit relevant concentrations of the amine (Figure 1). On electron microscopy the bottom layer contained vesicle-like organelles of different shape with minor amounts of contaminants, e.g. mitochondria, α-granules and glycogen. The vesicles were either empty or filled to varying degrees with electron dense material. Part of these exhibited an extremely high electron density (Figure 3A).

The following findings indicate that the highly electron-dense organelles found in the bottom layer represent specific storage sites for endogenous and exogenous 5HT:

1. The bottom layer exhibited by far the highest content of endogenous 5HT when compared to the other subcellular fractions, e.g. the α-granules (Figure 1).

2. The highly osmiophilic organelles were found exclusively in the bottom layer. The strong osmiophily of these organelles is probably due to an accumulation of 5HT, since other constituents of the organelles, e.g. nucleotides, histamine and protein, did not show an intense osmiophilic reaction under the present experimental conditions[1].

3. The highly osmiophilic organelles seen in the intact platelets as well as in the bottom layer differed distinctly in their ultrastructure from other subcellular organelles such as the α-granules (Figures 2A and 3A).

4. The bottom layer obtained from human platelets was similar to that isolated from platelets of other species (rabbits and guinea-pigs) with regard to 5HT content and presence of highly osmiophilic organelles. In the animals it has been demonstrated that these organelles represent 5HT storage sites[1,2,12].

5. After incubation of the platelet-rich plasma with 5HT the content of this amine in the platelets as well as in the bottom layer increased by more than 10 times (Table). Simultaneously, the number of the highly osmiophilic organelles in the platelets as well as in the bottom layer rose considerably (Figures 2 and 3). The electron density of the other subcellular organelles of the platelets, e.g. the classical α-granules as well as the α-granules with a dense pole ('Bull's eye' α-granules) was not enhanced.

It can therefore be concluded that in platelets of man as in those of other species at least part of the 5HT is stored together with ATP in specific organelles. Furthermore, the present results as well as earlier findings with animal platelets and megakaryocytes[13] indicate that the 5HT-storing organelles are probably not derived from α-granules as proposed earlier[7].

Whether the majority of the platelet 5HT is localized in the highly osmiophilic organelles remains to be proven. Up to now, only about 15% of the total platelet 5HT could be recovered in these organelles. It may, however, be assumed that with further improvement of the isolation technique this recovery can be substantially increased.

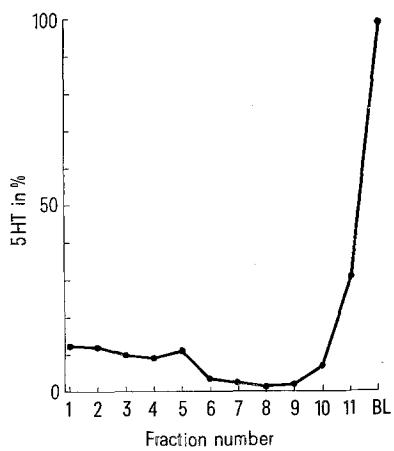

Fig. 1. Distribution of endogenous 5HT in various subcellular fractions of human platelets. The values have been calculated in μmoles 5HT/μg protein and are indicated in percent of the value found in the bottom layer (BL = 100%) containing the 5HT organelles. Fractions 6 and 8 contain the α-granules and mitochondria, respectively. Typical experiment.

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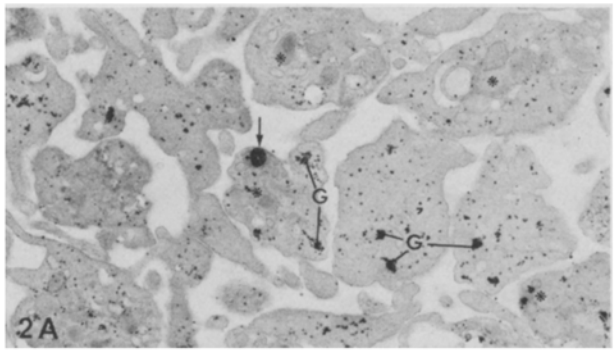


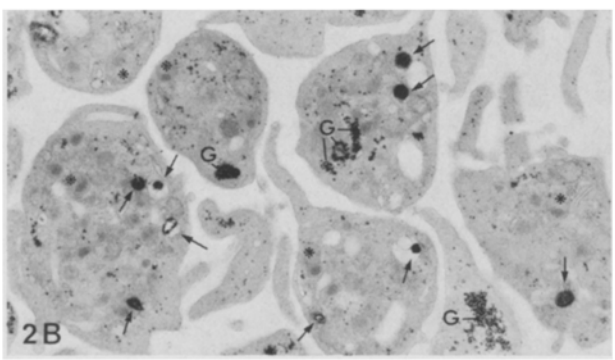
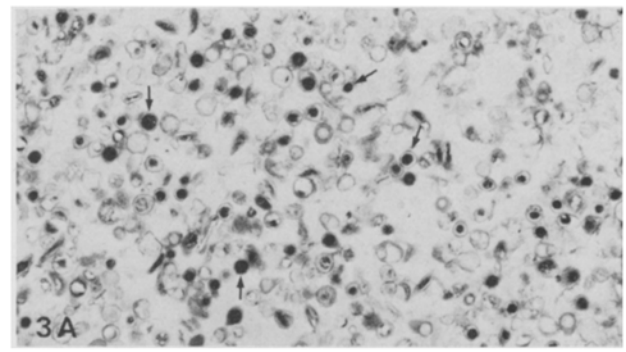


Fig. 2. Isolated human platelets. A) normal; B) after incubation in 5HT-rich human plasma (500 μg/ml for 60 min). The arrows point to the very electron-dense 5HT organelles. Some classical α-granules and α-granules with a dense pole ('Bull's eye' α-granules) are labeled with*. G, glycogen particules. Glutaraldehyde, OsO_4 fixation; lead citrate stain. × 11,500.



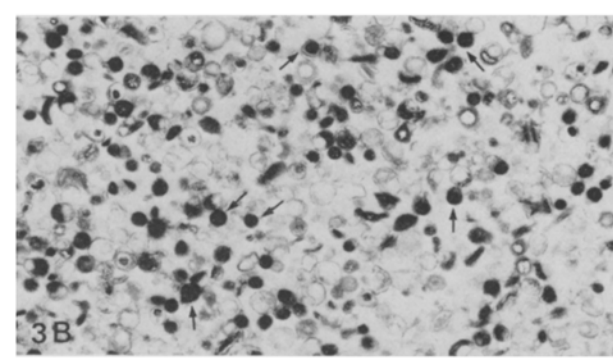


Fig. 3. Electron micrographs of the bottom layer rich in 5HT of the human platelets represented in Figure 2. A) from normal platelets; B) from platelets incubated with 5HT. Arrows point to some typical very dense 5HT organelles. Glutaraldehyde, OsO_4 fixation; lead citrate stain. × 11,500.

In rabbit and guinea-pig platelets, for instance, the percentage amount of the total platelet 5HT found in the highly osmiophilic organelles rose from 5 to 50% with the improvement of the isolation technique.

The bottom layer obtained after density gradient centrifugation of homogenates of human platelets shows a relatively low absolute 5HT content ($2.45 \pm 0.45 \times 10^{-4}$ μmoles/μg proteins), but a considerably higher ATP concentration ($19.3 \pm 3.7 \times 10^{-4}$ μmoles/μg proteins ($p < 0.01$)). In agreement with the low 5HT content, the proportion of highly osmiophilic versus empty organelles is also small, can, however, be markedly increased by incubation of the platelets with exogenous 5HT (Figure 3). One might therefore speculate that, as in guinea-pigs, the non-osmiophilic vesicles represent ATP-containing 'precursors' which may be transformed into typical 5HT organelles when exposed to sufficient amounts of 5HT. In the intact platelets these precursor vesicles cannot be distinguished on electron microscopy from vacuole-like structures of other nature, since the electron density of the 5HT organelles is due to the presence of 5HT and not of ATP. This hypothesis would explain why intact human platelets show only very few 5HT organelles which become considerably more numerous upon exposure of the platelets to 5HT, e.g. in the carcinoid syndrome[14,15] or after incubation with exogenous 5HT (Figure 2).

Concentration of 5HT in μmoles/μg protein of whole human blood platelets and isolated 5HT storage organelles of human platelets

	Platelets	Organelles
Normal	$2.43 \pm 0.21 \times 10^{-6}$ (11)	$2.45 \pm 0.54 \times 10^{-4}$ (7)
5HT incubation	$2.50 \pm 0.38 \times 10^{-5}$ (6)	$2.95 \pm 0.24 \times 10^{-3}$ (2)

Means with standard error. Number of experiments in parentheses.

Zusammenfassung. Menschliche Blutplättchen zeigten die höchste 5-Hydroxytryptamin (5HT)- und Adenosin-5'-Triphosphat (ATP)-Konzentration in einer subzellulären Fraktion, die elektronenoptisch aus Vesikeln von unterschiedlicher Dichte bestand. Diese waren deutlich verschieden von anderen subzellulären Organellen, z.B. den α-Granula. Inkubation der Plättchen mit 5HT erhöhte die Elektronendichte und den 5HT-Gehalt der Vesikel, veränderte aber die α-Granula nicht. Es wird geschlossen, dass in menschlichen Blutplättchen ein Teil des 5HT zusammen mit ATP in spezifischen subzellulären Organellen gespeichert ist.

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