CALCIUM-CONTAINING PLATELET GRANULES

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INTRODUCTION

Calcium has long been recognized as an essential factor for platelet adhesion and subsequent blood coagulation, and anticoagulation can be achieved with the use of calcium chelators such as EDTA or citrate. The normal level of calcium in plasma is approximately 0.1 mg/ml; however, dry weight chemical analysis of platelets (5) has indicated that they contain as much as 3.0 or 4.5 mg calcium/g of platelets. We have examined normal platelets in an effort to determine where within the platelet this rather large amount of calcium is stored.

MATERIALS AND METHODS

Human blood was collected using either heparin or EDTA as an anticoagulant. Platelets were then separated from the blood (3) and fixed in paraformaldehyde (1), formaldehyde (2), or glutaraldehyde buffered with either phosphate or cacodylate and containing no calcium, or in the same fixatives containing 0.08 mg/ml calcium. The platelets were post-fixed in osmium tetroxide containing the appropriate buffer and embedded in Epon (4). Sections were picked up on silicone monoxide-coated grids and examined with a Philips EM 300 electron microscope. No lead or uranium stains were used on the sections. With the exception of the fixative to which calcium was added, the only solution containing a metal ion that the specimen came in contact with was osmium, and it has been demonstrated that osmium completely sublimes when the specimen is incinerated (7). We then utilized microincineration to localize the inorganic material in the platelet and X-ray spectroscopy[1] to identify it.

An initial control experiment with calcium- and phosphorous-containing specimens showed that the collection efficiency ratio for the characteristic X-ray of these elements were Ca to P = 1:1.72. To obtain equivalence, all calcium counts were multiplied by 1.72. In order to obtain adequate statistical accuracy, a count time of 100 s was found to be suitable. The experimental results were corrected for collection efficiencies and background counts were subtracted.

RESULTS

Electron micrographs of these preparations revealed well-preserved platelets with their usual granules, mitochondria, and other organelles. If the fixative contained no calcium, the dense 5-hydroxytryptamine (5-HT) (8) granules were obvious only in specimens collected in heparin and fixed in cacodylate-buffered glutaraldehyde. When the fixative contained calcium, the 5-HT granules were apparent in all specimens fixed in cacodylate-buffered solutions; but if phosphate was used as a buffering system, the 5-HT granules were observed only in the specimens collected in heparin and fixed with glutaraldehyde. It has been reported previously that 5-HT granules will be apparent only after certain fixatives (9).

When unstained sections of these preparations, with and without calcium in the fixative, were examined with the electron microscope, the only parts of the platelet with an inherent electron density were the 5-HT granules (Fig. 1). If the granules were present, microincineration subsequently indicated that they contained a significant amount of inorganic material when either a calcium-free or a calcium-containing fixative was used (Fig. 2). In an attempt to identify the inorganic material, we studied the sections with an analytical electron microscope.[1]

The initial scan was performed with a solid state detector, and then, for more accurate iden-

[1] AEI Scientific Apparatus Limited, Harlow, Essex, England (personal communication).

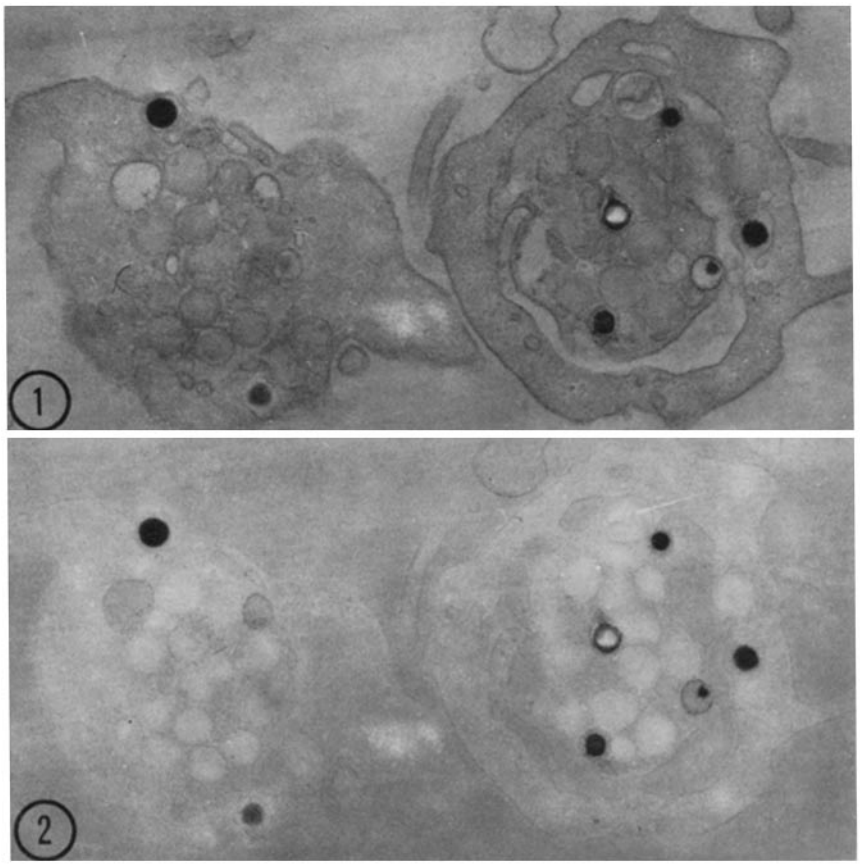

FIGURE 1 Unstained section of platelets demonstrating dense 5-HT granules. × 21,000.

FIGURE 2 Above section after microincineration at 450°C for 15 min. Dense granules remain unchanged, suggesting presence of inorganic material. × 21,000.

tification a focusing crystal detector was used. A 100-s counting time was found to be suitable for this analysis. The beam size of this instrument is just under 2,000 Å, and when the beam was centered over one of the 5-HT granules, an average count of 120 was obtained for calcium, and an average count of 1,800 was obtained for phosphorus. Areas of the platelet cytoplasm of comparable size and free of the 5-HT granules were used as controls. These control areas gave counts of zero for calcium and an average of 1,200 for phosphorus. Interpretation of these counts can be confusing. The zero count for calcium does not mean that there is no calcium in that part of the platelet cytoplasm; it simply means that there is much less calcium present there than in the 5-HT granule. The minimum amount of calcium detectable by X-ray spectroscopy is approximately 10^{-16} g; therefore, we can conclude from these counts that considerably more calcium is present in the 5-HT granules than in a comparable area of the platelet cytoplasm.

DISCUSSION

In our experience, if the 5-HT granule is apparent, then calcium can be proved to be present by the above described techniques. The functional significance of the calcium and how it is associated with the 5-HT in these granules is not known, but it may act to increase the storage of 5-HT by forming osmotically stable, high molecular weight aggregates of amines and nucleotides combined with bivalent cations (6). These investigators (6) reported that the concentration of calcium is at least 50 times higher in isolated dense granules than in intact platelets.

The above described techniques would be of interest in studying other amine-containing granules such as chromaffin granules of the adrenal medulla or mast cell granules to determine if they also contain high concentrations of bivalent metals.

SUMMARY

By using electron microscopy combined with microincineration and X-ray spectroscopy, calcium has been proved to be present in the 5-HT granules of platelets. The functional significance of this calcium is unknown, but it may act to increase the storage of 5-HT.

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