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Rapid report

Evidence for separate serotonin and catecholamine compartments in human platelets

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Abstract

Serotonin (5-HT) and the catecholamines (CA), noradrenaline (NA) and adrenaline (Ad), are platelet dense-granule constituents which influence platelet activity and vessel tone. Platelets accumulate 5-HT via an active process whilst CA uptake occurs mainly by passive diffusion. The platelet contents and collagen-stimulated efflux of 5-HT, NA and Ad were examined in normal individuals to establish whether relationships exist between these monoamines. Regression analysis revealed that platelet 5-HT was not related to platelet NA or Ad levels. Platelet NA, however, correlated positively with Ad ($r = 0.61$, $P < 0.01$). Collagen-induced release of all three monoamines occurred in a dose-dependent manner. The collagen EC_{50} values for 5-HT and CA release, however, differed and were greater for 5-HT release: 9.6 ± 0.8 vs. 3.8 ± 0.2 μg/ml collagen, 5-HT vs. NA, $P < 0.001$; 9.6 ± 0.8 vs. 3.9 ± 0.5 μg/ml, 5-HT vs Ad, $P < 0.001$. These data may reflect differences regarding the triggering mechanisms for 5-HT and CA release and provide evidence for separate compartments of intra-platelet 5-HT and CA and possibly distinct populations of 5-HT and CA containing dense granules and/or platelets.

Keywords: Serotonin; Noradrenaline; Adrenaline; Platelet; Dense granule; Subpopulation

The biogenic monoamines serotonin (5-hydroxytryptamine, 5-HT), which has its origins in the enterochromaffin cells of the gut, and noradrenaline (NA) and adrenaline (Ad), which are sympathoadrenal in origin, are transported in the circulation by platelets packaged in the dense granules [1,2]. Platelets absorb these monoamines from the plasma as they lack the enzymes necessary for their synthesis [3–5]. 5-HT is accumulated by platelets via an active Na^+, energy and temperature dependent process which can be blocked by Na^+,K^+ ATPase inhibitors, e.g., ouabain [6]. By contrast, platelet NA and Ad uptake occurs principally by passive diffusion [2,7].

It has been proposed that intra-platelet monoamine content may determine platelet sensitivity and influence vessel tone through their release at discrete sites so increasing local plasma monoamine concentrations [8]. The monoamines have been implicated in various pathological conditions associated with abnormal arterial function and increased platelet activity including diabetes mellitus [9–12], essential hypertension [13–16], familial hypercholesterolaemia [17–19] and atherosclerosis [20,21].

Differential release of platelet granular contents is a recognized phenomenon [22–25]. Thus on platelet activation, while maximal efflux of dense and α granule contents is rapid and almost total (80–100%), and is seen with all platelet agonists, lysosomal secretion is slow, incomplete and requires high concentrations of strong agonists, e.g., thrombin and collagen [26]. Blood platelet heterogeneity — i.e., evidence for two classes of platelets in both man and rat — has been reported, based on the presence or absence of an acid phosphatase which acts on *para*-nitrophenyl phosphate [27]. To date, however, no evidence for the existence of distinct monoamine compartments or sub-populations of monoaminergic platelet dense granules or platelets has been recorded. This study was therefore undertaken to investigate these possibilities.

For the estimation of platelet 5-HT, NA and Ad antecubital venous blood was collected from 19 normal subjects (11 male, 8 female), aged between 22 and 53 years, into

Abbreviations: Ad, Adrenaline; CA, catecholamine; 5-HT, 5-hydroxytryptamine (serotonin); NA, noradrenaline.

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EDTA (2.7 mmol l^{-1}) and centrifuged (300 × g, 10 min) at room temperature to yield platelet-rich plasma (PRP) [28]. PRP samples were separated into platelet and platelet-poor plasma (PPP) fractions by high-speed centrifugation (2500 × g, 20 min) [29] and frozen at −85°C until time of monoamine analysis. PPP and platelet CA were extracted using alumina and determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD)[29]. 5-HT in PPP and platelet fractions was also measured by HPLC-ECD[28]. The protein content of platelet pellets was determined using the method of Lowry et al. [30].

For release experiments blood was collected into 106 mmol l^{-1} trisodium citrate (TSC) (9:1, blood/TSC, v/v) from 11 subjects (6 male, 5 female, 22–53 years) and PRP prepared as described above. Platelet monoamine release was studied using 1 ml aliquots of PRP preincubated in a water-bath at 37°C for 5 min followed by 10 min incubation in the presence of increasing concentrations of collagen (0, 5, 10, 20, 40, 80 and 160 μg ml^{-1}; Immuno, Dunton Green, Kent, UK). After incubation platelets were sedimented by high-speed centrifugation and the resulting supernatants (PPP) frozen (−85°C) before going for monoamine analysis as above. Release data were calculated relative to PRP platelet count (range (226–527) × 10^9 l^{-1}), counts having been performed on samples prior to incubation using a Model STKS Coulter Counter (Coulter Electronics, Hialeah, FL, USA).

Data are expressed as means ± S.E.M. and were analyzed statistically using the unpaired Student's t-test and regression analysis.

The results of platelet 5-HT, NA and Ad measurements are shown in Table 1. The platelet content of 5-HT exceeded the contents of NA and Ad by approx. 1397-fold and 20950-fold respectively, values of similar orders of magnitude to those reported by Da Prada and Picotti [2]. Regression analysis indicated that platelet 5-HT levels did not correlate with platelet NA or Ad contents. Platelet NA and Ad were, however, related positively ($r = 0.61$, $P < 0.01$, Fig. 1).

Collagen-induced release of 5-HT, NA and Ad all occurred in a dose-dependent manner, maximal release occurring at concentrations of collagen between 40 and 80 μg ml^{-1} (Table 2). Under resting conditions (0 μg ml^{-1} collagen), supernatant (PPP) concentrations of NA and Ad represented far greater proportions of maximal release than those determined for 5-HT. Thus, resting concentrations of 5-HT, NA and Ad were approx. 2%, 46% and 47% of the concentrations obtained with 80 μg ml^{-1} collagen, respectively. The collagen EC_{50} value for 5-HT release (9.6 ± 0.8 μg ml^{-1} collagen) exceeded the EC_{50} values for NA (3.8 ± 0.2 μg ml^{-1}) and Ad (3.9 ± 0.5 μg ml^{-1}) by 153% and 146%, respectively.

Table 1
Platelet 5-HT, NA and Ad levels in normal human subjects

	Platelet monoamine (pmol mg^{-1} platelet protein)
5-HT	1466.5 ± 134.3
NA	1.05 ± 0.07
Ad	0.07 ± 0.01

Data are presented as means ± S.E.M., $n = 19$.

In this study it has been shown that in normal human platelets the levels of the dense granule component 5-HT are not related to those of NA or Ad. By contrast, platelet NA and Ad were related positively. Although the platelet release of all three monoamines induced by collagen occurred in a dose-dependent fashion, the EC_{50} values for 5-HT and CA release differed significantly, being greater for 5-HT. The EC_{50} values for NA and Ad release were remarkably similar.

Taken together these data would indicate that platelet 5-HT and CA reside in separate compartments, NA and Ad probably occurring in the same one. It is therefore possible that 5-HT and CA, in fact, occur associated with different dense granules or even platelets. To establish this definitively will require more detailed studies involving the subfractionation of platelet organelles and whole platelets. Alternatively, specific markers for 5-HT and CA could be employed.

5-HT is stored in the dense granules in a high-density lattice structure in association with adenine nucleotides (e.g., ATP, ADP) and bivalent cations (Ca^{2+} in the case of human platelets) [31]. These complexes are thought to involve the anchorage of 5-HT moieties between adjacent adenosine moieties with the 5-hydroxyindole rings parallel to the flat purine rings [32]. It was concluded from NMR studies that this molecular arrangement restricts free motion of the 5-HT moieties, consistent with a strong interaction between the indole and adenine rings [32]. A similar mechanism probably operates with respect to the solid Ca^{2+}-nucleotide complexes, and coupled with the 5-HT nucleotide interactions, probably underlies the osmotic stability and high specific weight of the dense granules.

Platelets absorb 5-HT in preference to NA and Ad via an energy-dependent, high-affinity uptake mechanism [6]. The boundary membrane of the dense granule also possesses a transport system selective for 5-HT whilst, as for the platelet membrane, NA and Ad are transported with relatively low affinity [6,24]. It is possible therefore that much of the intra-platelet CA occurs in the cytosol, so making it more readily available for release on platelet stimulation. Such a situation would explain the finding in this study that the collagen EC_{50} values for NA and Ad release were considerably lower than that for 5-HT release. It should be noted that subcellular fractionation experiments indicated that both CA and 5-HT were most concentrated in the fraction consisting of pure 5-HT organelles [2]. This observation would therefore appear to be inconsistent with the theory expounded above. These studies, however, were conducted using rabbit platelets which are

Table 2
Platelet 5-HT, NA and Ad release in normal human PRP (pmol per 10^8 platelets)

	Collagen ($\mu g\ ml^{-1}$)							
	0	5	10	20	40	80	160	EC_{50}
5-HT	8.3 ± 1.9	81.8 ± 11.7	186.2 ± 21.5	267.6 ± 32.2	331.3 ± 40.6	379.2 ± 49	407.1 ± 50.2	9.6 ± 0.8
NA	0.37 ± 0.07	0.66 ± 0.08	0.74 ± 0.1	0.79 ± 0.1	0.84 ± 0.11	0.83 ± 0.11	0.85 ± 0.11	3.8 ± 0.2 *
Ad	0.08 ± 0.02	0.1 ± 0.02	0.11 ± 0.02	0.12 ± 0.02	0.14 ± 0.03	0.17 ± 0.04	0.14 ± 0.03	3.9 ± 0.5 *

Data are expressed as means ± S.E.M., $n = 11$.
* Significantly different from EC_{50} value obtained for 5-HT release with $P < 0.001$.

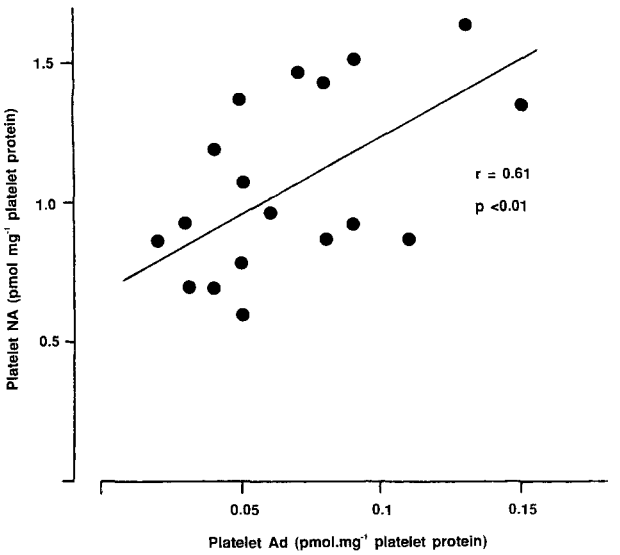

Fig. 1. Correlation between platelet NA and platelet Ad.

substantially different from human platelets in their composition [6].

Another explanation for my findings may relate to the strength of binding of the various monoamines in the dense granules. Thus, if 5-HT is more tightly bound in the dense granule complexes than NA or Ad, as is likely, a similar consequence with regard to platelet monoamine release as described above, might be expected.

The observation that platelet 5-HT did not correlate with either NA or Ad, whilst NA and Ad were correlated positively, is consistent with the hypotheses presented. If correlations had been observed between 5-HT and CA this would have indicated that these monoamines reside in the same intracellular compartment.

Finally, one should consider the possibility that 5-HT and CA secretion occurs through different mechanisms. This would require considerable further investigation, as indeed is the case to confirm or reject the theories already outlined.

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