Increased expression of procoagulant activity on the surface of human platelets exposed to heavy-metal compounds

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One of the essential roles for platelets in haemostasis is in the potentiation of blood clotting due to the contribution of anionic phospholipid from the surface of the cells, as an essential cofactor to the proteolytic reactions of coagulation (platelet procoagulant activity). Only a limited number of agonists are known to initiate platelet procoagulant activity. In this study the rate of thrombin formation on the platelet surface was observed to increase in a dose-dependent manner upon treatment of washed platelets with heavy-metal compounds. Unlike the immediate increase observed upon treatment of platelets with calcium ionophore, A23187, the change due to these agents was progressive, approaching a maximum after 10 min. The maximum-fold acceleration of the rate of thrombin formation compared with control platelets was calculated for $HgCl_2$ (56-fold), $AgNO_3$ (42-fold) phenylmercuri-acetate (24-fold) and thimerosal (14-fold), compared with 70-fold observed for calcium ionophore. The increase in procoagulant activity due to $HgCl_2$ coincided with a large increase in intracellular calcium and phosphorylation of 22 and 45 kDa proteins. It is considered that the mechanism responsible for the increase in procoagulant activity is exposure of anionic phospholipids. This was detected by a 2-fold increase in the binding of ^{125}I-annexin V upon addition of $HgCl_2$, compared with resting platelets (3-fold on treatment of platelets with calcium ionophore). In contrast to the generation of activity by A23187 and other known agonists of this reaction, heavy-metal compounds appeared to cause little or no release of microparticles from the platelet surface. Since $HgCl_2$ did not cause aggregation of platelets or significant release of serotinin, these findings may give further support to the need for exposure and ligation of glycoprotein IIb:IIIa for vesiculization to occur. Treatment of platelets with heavy metals may constitute a new approach to investigating the early changes in the cell membrane which lead to increased expression of anionic phospholipid.

INTRODUCTION

Platelets play two important roles in normal haemostasis. First, by aggregating, they constitute the initial haemostatic plug which immediately curtails bleeding from broken blood vessels. Secondly, the platelet surface can become activated and potentiate blood clotting, a property referred to as platelet procoagulant activity. This is usually observed as an increase in the rate of activation of prothrombin by factor Xa in the presence of factor Va and Ca^{2+}, referred to as the prothrombinase reaction. The change to the surface of the platelet responsible for procoagulant activity is due, principally, to a reversal of the polarity of the phospholipid membrane: the anionic phospholipids, which are normally maintained by a translocase system at a higher concentration on the inner leaflet, become exposed on the outer membrane surface (reviewed in [1,2,3,4]). The physiological importance of this property of platelets is demonstrated by the moderately severe bleeding disorder, Scott syndrome, in which stimulated platelets reveal abnormally low levels of anionic phospholipid exposure and a correspondingly lower procoagulant activity [5,6].

The generation of platelet procoagulant activity does not occur with all agonists. 'Weak' agonists such as ADP, adrenalin and platelet-activating factor hardly affect the procoagulant properties of the platelet surface even though irreversible thromboxane-dependent aggregation can proceed to near completion. In contrast, thrombin, collagen, complement attack complex C5b-9 and calcium ionophore have been demonstrated to enhance the generation of platelet procoagulant activity in the order of potency: ionophore > collagen/thrombin > C5b-9 > collagen > thrombin [4]. Treatment of platelets with local anaesthetics (dibucaine and tetracaine) or with sulphydryl oxidizing agents (diamide or pyridyldithiolethanolamine) also cause an increase in procoagulant activity which is dependent upon extracellular calcium [4].

We recently observed an increase in the procoagulant activity of U937 monocyte-like cells upon treatment with mercuric and other heavy-metal compounds [7]. Both the tissue factor activity of the cell and the ability of the surface to support the prothrombinase reaction were rapidly increased, concomitant with a large increase in intracellular calcium concentration ($[Ca^{2+}]_i$). In the present study we have identified these heavy metals as potent agonists of platelet procoagulant activity. The characteristics of induction of the procoagulant surface are distinct from that promoted by other platelet agonists in that the degree of microvesiculization is low.

EXPERIMENTAL

Materials

Human α-thrombin was obtained as a gift from Dr. J.-M. Freysinnet (Strasbourg, France), human factor X from Enzyme Research Laboratories (Swansea, U.K.) and bovine factor V

Abbreviations used: PRP, platelet-rich plasma; PKC, protein kinase C; $[Ca^{2+}]_i$, intracellular calcium concentration; [^{14}C]5-HT, [^{14}C]5-hydroxytryptamine; MLC, myosin light chain; $[Ca^{2+}]_{cyt.}$, cytosolic calcium; Tg, thapsigargin.

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