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STRONTIUM IONS INDUCE PRODUCTION OF THROMBOXANE B_2 AND SECRETION OF 5-HYDROXYTRYPTAMINE IN WASHED HUMAN PLATELETS

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Abstract—The addition of strontium chloride to suspensions of washed human platelets induced biosynthesis of thromboxane B_2 (TxB_2) and secretion of [^{14}C]-5-hydroxytryptamine (5HT) in a dose dependent manner. Both responses occurred maximally within four minutes and were inhibited competitively by the presence of calcium ions. Magnesium and lanthanum ions also inhibited both the production of thromboxane B_2 and secretion of 5HT in response to strontium ions. Inhibition of platelet thromboxane generation by aspirin resulted in an almost total inhibition of the secretion response after adding strontium ions. In addition, both the secretion of 5HT and thromboxane B_2 production in the presence of strontium ions were inhibited by dibutyryl cyclic AMP and by prostaglandin E_1. The results suggest that strontium ions induce platelet secretion largely via the liberation of arachidonate from membrane phospholipid with the subsequent biosynthesis of thromboxane A_2.

The platelet secretion reaction can be induced by ionophores for divalent cations such as A23187, which are thought to produce a rise in cytosolic calcium levels from either an extracellular or an intracellular compartment [1, 2]. In addition, platelet activation in response to several agents appears to be accompanied by an increase in cytosol calcium levels [3, 4]. However, little is known of the mechanism by which a rise in calcium levels in the platelet cytosol triggers platelet secretion. It has been suggested that calcium may modulate the release from platelet membrane phospholipid of arachidonate [5, 6]. Arachidonate is then converted via prostaglandin endoperoxides to thromboxane A_2, a potent inducer of platelet secretion and aggregation [7]. However, investigations of the role of calcium in platelet activation using intact cells are complicated by the apparent lack of permeability to calcium of the platelet plasma membrane. In particular, Robblee and Shepro [4] have shown evidence that the intracellular calcium compartment in platelets is only affected to a minor extent by the calcium concentration in the extracellular medium. For this reason, we have investigated the interrelationships of calcium, platelet thromboxane production and secretion in intact platelets using strontium, which by virtue of its small hydrated ionic radius compared to calcium, has potential value as a calcium probe by mimicking certain actions of calcium in biological systems [8, 9].

MATERIALS AND METHODS

Blood was obtained from healthy subjects who had taken no drugs for at least 10 days, and mixed with one tenth volume of 3.8% (w/v) trisodium citrate. Platelet-rich plasma was obtained by centrifuging whole blood at 200 *g* for 10 min at 20°. The platelet 5-hydroxytryptamine pool was labelled by incubating platelet-rich plasma with [^{14}C]-5-hydroxytryptamine (final concentration 2.5 μM) for 15 min at 37°. The platelet-rich plasma was then cooled to 2°, centrifuged at 1800 *g* for 10 min at 2°, and the platelets resuspended in 15 mM Tris–HCl 140 mM NaCl buffer (pH 7.4). Platelet suspensions were incubated at 37°.

Thromboxane B_2 and 5-hydroxytryptamine secretion measurements were performed as described previously [10].

[3′–^{14}C]-5-Hydroxytryptamine creatine sulphate (53 mCi/mmole) was purchased from the Radiochemical Centre, Amersham, U.K. Acetyl salicylic acid was purchased from the Sigma Chemical Co., London, and dibutyryl cyclic AMP from Boehringer Ingelheim, London. All other chemicals were of analytical grade. Synthetic prostaglandin E_1 and thromboxane B_2 were kindly donated by Dr. J. E. Pike, Upjohn Company, Kalamazoo, MI. Thromboxane B_2 antisera were a kind gift from Dr. J. B. Smith, Cardeza Foundation, Philadelphia, P.A.

RESULTS

The addition of strontium chloride (4 mM final concentration) to suspensions of washed human platelets resulted in a parallel rise in thromboxane B_2 production and secretion of [^{14}C]-5-hydroxytryptamine within 4 min (Fig. 1). No platelet aggregation was observed whether or not the platelets were stirred although platelets resuspended in the above manner were found to aggregate in response to thrombin (0.5 units/ml), collagen (4 μg/ml) or ADP (2 μM). The effects of strontium ions on thrombox-

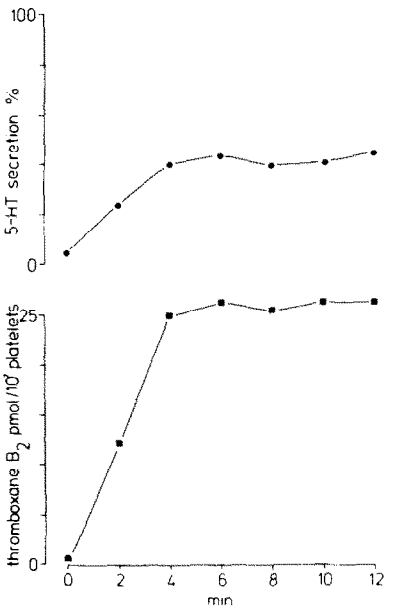

Fig. 1. Effect of strontium chloride on secretion of [^{14}C]-5-hydroxytryptamine and production of thromboxane B_2 by washed human platelets. Strontium chloride (final concentration 4 mM) was added to platelet suspension at time zero.

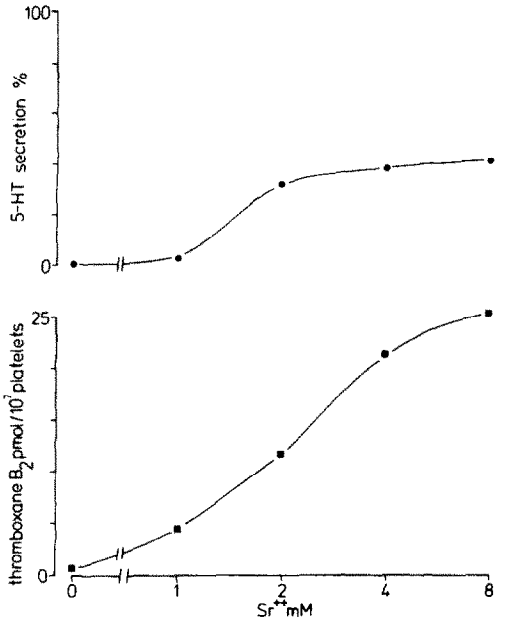

Fig. 2. Platelet [^{14}C]-5-hydroxytryptamine secretion and thromboxane B_2 production: Dose–response curves to strontium chloride.

ane B_2 production and on secretion were concentration dependent (Fig. 2). The presence of 2 mM calcium ions prevented both thromboxane B_2 generation and 5-HT secretion in response to 2 mM strontium ions (Table 1). This inhibition could be overcome by raising the concentration of strontium ions (Table 1), suggesting that calcium was competitively inhibiting the uptake of strontium or its action on the platelet. Magnesium ions produced a similar though less potent inhibition, whilst lanthanum ions, a transition metal thought to displace cell surface calcium and prevent calcium flux [11], completely inhibited thromboxane B_2 production and secretion (Table 1) induced by strontium ions. Preincubation of platelets for 5 min with 100 μm aspirin, a compound which inhibits the enzyme cyclooxygenase, and prevents the formation of prostaglandin endoperoxides and thromboxane A_2 [12], strongly inhibited 5-HT secretion in the presence of strontium ions and completely inhibited thromboxane B_2 production (Table 2).

Platelet secretion is known to be inhibited by compounds which increase intracellular cyclic AMP levels such as prostaglandin E_1 (PGE_1) [13]. 5-HT secretion induced by strontium ions was markedly inhibited in the presence of PGE_1 and also by dibutyryl cyclic AMP (Table 2). In addition, PGE_1 and dibutyryl cyclic AMP inhibited thromboxane B_2 production in response to strontium (Table 2).

DISCUSSION

Our results are consistent with the hypothesis that strontium ions are able to penetrate the cell plasma membrane and initiate cell activation, perhaps by mimicking a rise in cytosolic calcium concentrations [8, 9]. Strontium has been previously shown to induce histamine secretion from mast cells [8], although this effect was considerably slower than 5-HT secretion from blood platelets in response to strontium ions. Our results suggest that strontium ions induce the platelet secretion reaction by acti-

Table 1. [^{14}C]-5-Hydroxytryptamine secretion and thromboxane B_2 (TxB_2) production by washed human platelets: Interactions of strontium ions with other cations*

	Secretion (%)	TxB_2 (pmoles/10^7 platelets/8 min)
Basal	1.01 ± 0.47	ND
2 mM $SrCl_2$	47.7 ± 0.29	12.1 ± 0.86
2 mM $SrCl_2$ + 1 mM $CaCl_2$	0.76 ± 0.15	ND
8 mM $SrCl_2$ + 1 mM $CaCl_2$	6.16 ± 1.68	4.37 ± 0.12
16 mM $SrCl_2$ + 1 mM $CaCl_2$	27.0 ± 2.09	10.5 ± 0.86
2 mM $SrCl_2$ + 2 mM $MgCl_2$	22.2 ± 5.02	7.15 ± 0.91
2 mM $SrCl_2$ + 0.5 mM $LaCl_3$	0	ND

* Each value represents the mean ± S.E.M. of three separate determinations.
ND = Not detectable. Limit of detection was approximately 0.3 pmoles/10^7 platelets.

Table 2. [^{14}C]-5-Hydroxytryptamine secretion and thromboxane B_2 (TxB_2) production by washed human platelets in response to strontium ions: Effects of aspirin, prostaglandin E_1 and dibutyryl cyclic AMP (dbc AMP)*

	Secretion (%)	TxB_2 (pmoles/10^7 platelets/8 min)
Basal	5.93 ± 1.79	ND
2 mM Sr Cl_2	35.3 ± 3.77	10.2 ± 0.33
2 mM Sr Cl_2 + 100 μM aspirin	10.2 ± 4.31	ND
2 mM Sr Cl_2 + 10 μg/ml PGE_1	11.3 ± 4.77	ND
2 mM Sr Cl_2 + 1 mM dbc AMP	7.25 ± 3.72	1.22 ± 0.18

* Each value represents the mean ± S.E.M. of three separate determinations.
ND = Not detectable.

vating the release of arachidonate from membrane phospholipid with the subsequent synthesis of thromboxane A_2. When thromboxane A_2 formation was prevented with aspirin, only a small amount of secretion persisted, suggesting that the effects of strontium ions were mediated predominantly via production of thromboxane A_2. It is known that the ionophore A23187 can induce platelet secretion independently of thromboxane generation [10, 14], presumably by causing a rise in cytosol calcium levels. It is possible that the release of arachidonate from membrane phospholipid occurs as a result of the passage of strontium ions through the platelet plasma membrane and that the cellular calcium sequestering mechanisms prevent strontium ions reaching high enough concentrations in the cytosol to directly trigger secretion. Both thromboxane B_2 production and 5-HT secretion in response to strontium ions appeared to be competitively inhibited by calcium ions. These results are at variance with those of Murer, Day and Lieberman [15] who reported that calcium ions could actually induce secretion. However, these authors used platelet suspensions prepared in the presence of EDTA which may alter membrane permeability to cations. Our finding that secretion and thromboxane B_2 production induced by strontium were inhibited by lanthanum ions substantiates the hypothetical mode of action of lanthanum in preventing fluxes of calcium ions, and therefore presumably strontium ions, across external cell membranes [7].

The mechanism by which cyclic AMP inhibits platelet function is not fully understood. It has been shown that cyclic AMP inhibits the release of arachidonate from platelet membrane phospholipid [5, 16] possibly because it controls the compartmentalisation of calcium [5]. In addition, the uptake of calcium ions into platelet membrane vesicles is stimulated by cyclic AMP [17]. Our data strongly suggest that cyclic AMP can inhibit platelet secretion by preventing the liberation of endogenous arachidonate for subsequent conversion to thromboxane A_2. This could be the result of a direct action of cyclic AMP, by inhibiting the entry of strontium ions via the platelet plasma membrane, or possibly by sequestering strontium ions from the cytosol into an intracellular compartment. Further studies are required to investigate the interrelationships of cyclic AMP, divalent cations and thromboxane biosynthesis in the modulation of platelet secretory responses.

It is interesting to note that the addition of strontium ions to suspensions of washed platelets failed to elicit an aggregation response despite extensive secretion of 5-hydroxytryptamine and thromboxane biosynthesis. It is possible that uptake of strontium ions and subsequent formation of thromboxane occurred too slowly to induce platelet aggregation, since a maximal response occurred only after 4–5 min (Fig. 1). Alternatively, strontium ions may actually inhibit the aggregation process at a separate step, possibly by acting as a calcium antagonist.

In conclusion, our results using strontium as a probe for cellular calcium suggest that calcium is important in triggering platelet secretion responses, and may do so, at least in part, by regulating the biosynthesis of thromboxane A_2 from arachidonate released from membrane phospholipid.

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