STRONTIUM IONS STIMULATE PHOSPHOINOSITIDE METABOLISM IN HUMAN BLOOD PLATELETS

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Received 17 August 1981; revision received 14 September 1981

1. Introduction

Phospholipid metabolism is thought to play a key role in the activation of a wide variety of cell types [1,2]. In platelets, arachidonate is released from membrane phospholipids [3] and subsequently converted to thromboxane A_2, a potent inducer of platelet aggregation and secretion [4]. In addition, one or possibly more thromboxane-independent mechanisms of platelet activation exist which also appear to involve phospholipid metabolism [5,6]. In response to thrombin [7,8] and to collagen [7], platelet phosphatidylinositol is degraded by phopholipase C with the formation of diglyceride [9] and subsequently phosphatidic acid [10]. The mechanism by which these events are coupled to secretion and aggregation is not clear, although it has been postulated that the phosphatidylinositol effect may be associated in some way with a rise in cytosol calcium concentration [1]. We have shown that secretion of 5-hydroxytryptamine in platelets can be triggered by the addition of strontium ions [11], possibly by mimicking a rise in intracellular calcium. We report here that strontium ions cause a specific turnover of the platelet phosphatidylinositol pool accompanied by an apparent stimulation of polyphosphoinositide metabolism. The latter class of phospholipids is also thought to be involved in the regulation of cell activation during stimulation and may thus play a role in the control of granule secretion and other processes which occur during aggregation.

2. Materials and methods

Platelet-rich plasma was prepared as in [11] and incubated at 37°C for 2 h with [1(3)-^{3}H]glycerol (Amersham; 100 μCi/10^9 platelets) or with [5,6,7,8,9,11,12,14,15-^{3}H]arachidonic acid (Amersham 5 μCi/10^9 platelets). Platelets were then isolated by centrifugation and resuspended in 15 mM Tris–HCl, 140 mM NaCl buffer (pH 7.4) [11] at a 5×10^8/ml. Aliquots (1 ml) of platelet suspension were incubated at 37°C and lipids subsequently extracted with 3 ml $CHCl_3$:MeOH:13 N HCl (200:100:1). The upper phase was re-extracted with 2 ml $CHCl_3$ and the organic phases pooled and dried in a vacuum oven. Phospholipids were separated by 2-dimensional TLC as in [12] and neutral lipids by a unidirectional TLC method using ether:hexane:acetic acid (70:30:1) as solvent. Lipid spots were stained with iodine vapour, the spots scraped and counted for radioactivity following the addition of 2 ml MeOH and 10 ml Bray's scintillant.

3. Results and discussion

When platelets labelled with [^{3}H]glycerol were incubated in the presence of 8 mM $SrCl_2$, radioactivity was lost from phosphatidylinositol with a maximum effect at ~10 min (fig.1a). Platelets labelled with [^{3}H]arachidonic acid, which is incorporated more effectively than glycerol into phosphatidyl serine [13], showed a similar though less marked loss in radioactivity from this phospholipid in the presence of 8 mM $SrCl_2$ (fig.2a). No comparable changes in radioactivity were observed in phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid or lysophosphatidylcholine spots (table 1). Similarly, no changes were found in radioactivity associated with diglyceride or monoglyceride (not shown) suggesting that $SrCl_2$ was not acting via activation of phospho-

Published by Elsevier/North-Holland Biomedical Press
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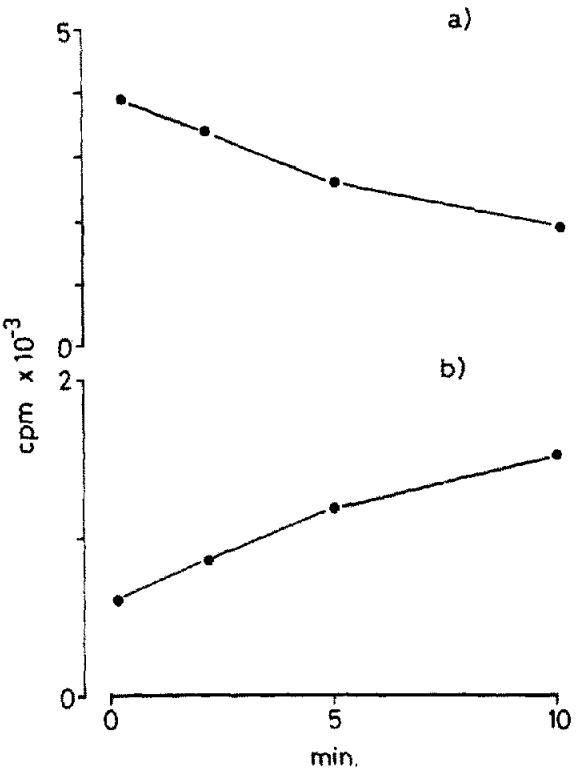

Fig.1. Effect of 8 mM $SrCl_2$ on radioactivity associated with phosphatidylinositol (a) and polyphosphoinositides/lysophosphatidylinositol (b). Platelets were prelabelled with [^{3}H]-glycerol. Each value represents the mean of duplicate determinations; all experiments were performed at least 3 times with essentially similar results.

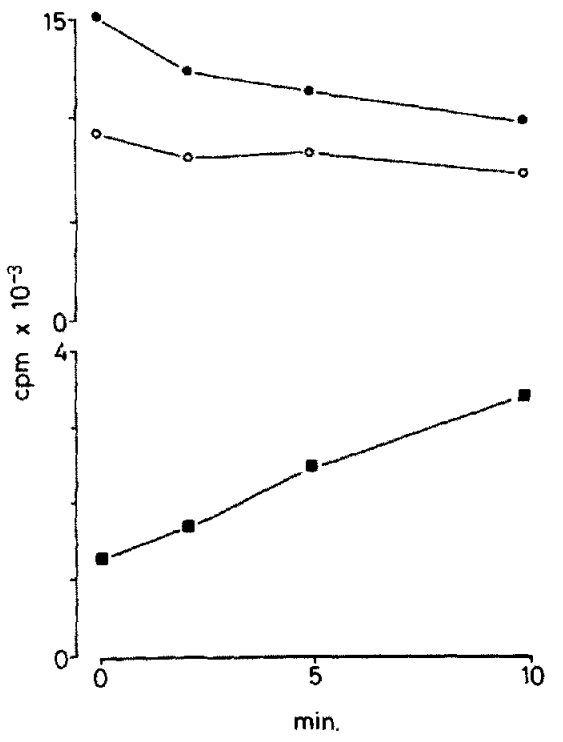

Fig.2. Effect of 8 mM $SrCl_2$ on radioactivity associated with phosphatidylinositol (•) phosphatidyl serine (○) and polyphosphoinositides/lysophosphatidylinositol (▪). Platelets were prelabelled with [^{3}H]arachidonic acid. Each value represents the mean of duplicate determinations; all experiments were performed at least 3 times, with essentially similar results.

Table 1
Effect of $SrCl_2$ on radioactivity associated with the principal phospholipid spots in platelets pre-labelled with [^{3}H]glycerol

Incubation 10 min	cpm/5 × 10^4 platelets					
	PI	PPI/LPI	PC	PE	PA	LPC
Saline	4146	580	5113	1309	59	363
$SrCl_2$, 2 mM	3589	738	5111	1188	77	280
$SrCl_2$, 4 mM	2871	1748	4625	1136	75	295
$SrCl_2$, 8 mM	2621	2280	4713	1140	80	271

Abbreviations: PI, phosphatidylinositol; PPI, polyphosphoinositides; LPI, lysophosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; LPC, lysophosphatidylcholine

Each value represents the mean of duplicate determinations; each experiment was performed at least 3 times

lipase C. This indirectly implies that arachidonate for production of thromboxane B_2 is released in response to $SrCl_2$ via the activation of a phospholipase A_2, possibly the enzyme corresponding to the activity reported to release arachidonate from phosphatidylinositol and phosphatidylserine in response to thrombin [3]. Here, we were unable to determine which of these phospholipids is the major source of released arachidonate.

The loss of radioactivity from phosphatidylinositol produced by $SrCl_2$ was associated with an increase in radioactivity in a spot which co-chromatographed with diphosphatidylinositol, triphosphatidylinositol and lysophosphatidylinositol (fig.1). A similar accumulation of radioactivity was observed in this spot whether using platelets labelled with [^{3}H]glycerol or [^{3}H]arachidonic acid (fig.2). Since arachidonate is esterified almost exclusively in the 2 position of the

Table 2
Effects of various drugs and inhibitors on changes in PI and PPI labelling in response to 8 mM $SrCl_2$

Incubation 10 min	cpm/5 × 10^6 platelets	
	PI	PPI
Saline	6336	456
$SrCl_2$, 8 mM	4387	2216
+ $CaCl_2$, 8 mM	6468	892
+ 10 μg PGF_1/ml	5598	864
+ Indomethacin, 20 μM	5708	904
+ Aspirin, 100 μM	6207	844
+ Imidazole, 5 mM	4480	2392

Each value represents the mean of duplicate determinations; each experiment was performed at least three times

phospholipid [14], the labelled material appears to be one or both of the polyphosphoinositides rather than lysophosphatidylinositol. Thus the metabolism of phosphatidylinositol induced by Sr^{2+} appears to be associated with accelerated polyphosphoinositide turnover. Both the effective concentration range of $SrCl_2$ (table 1) and the time course characteristics of phosphatidylinositol and polyphosphoinositide labelling corresponded closely to the effects of $SrCl_2$ on 5-hydroxytryptamine secretion in [11]. Furthermore, the effects of Sr^{2+} on phosphoinositide turnover were inhibited by Ca^{2+}, prostaglandin E_1, indomethacin and by aspirin (table 2) which were also found to inhibit the secretion response [11]. In contrast, imidazole, a thromboxane synthetase inhibitor [15] did not inhibit either phosphoinositide metabolism or 5-HT secretion. These findings suggest that metabolism of the inositol phospholipids induced by Sr^{2+} may be closely associated with platelet secretion responses. The exact role played by polyphosphoinositides in cells receiving stimulation is not known. In [16] diphosphatidylinositol labelling in platelets increased during shape change and aggregation in response to ADP and in [7] to collagen. In rat brain, polyphosphoinositide metabolism appears to be influenced by the ionophore A23187 [17], suggesting that Ca^{2+} may be important in regulating the metabolism of these phospholipids. Thus the possibility exists that Sr^{2+} may be able to mimic intracellular Ca^{2+} and trigger either the formation or breakdown of polyphosphoinositides. Whether these processes play a role in modulating secretion in platelets remains to be ascertained. If so, it seems likely that compounds such as aspirin and indomethacin may be able to affect platelet reactivity by inhibiting phospholipid metabolism in addition to the actions of these drugs upon prostaglandin production [18].

The use of Sr^{2+} as a potential calcium probe may provide useful information concerning the role of Ca^{2+} in modulating secretory responses in platelets and other cell types during stimulation. Our results suggest that one likely role of Ca^{2+} in platelet function may be in regulating the turnover and interconversion of inositol phospholipids.

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