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PROPERTIES OF PHOSPHATIDYLINOSITOL KINASE IN HUMAN PLATELETS.
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We have reported the specific ³²P-labelling in phosphatidyl-
 inositol-4-monophosphate (PIP) of intact platelets upon addition
 of the agents which elevate intracellular cAMP (Thrombos. Res. 44,
 155, 1986). This event may be catalyzed by the action of PI-kinase,
 the properties of which has not been elucidated yet. Thereby,
 attempts were made to assay and to characterize PI-kinase of
 human platelets. Fresh lysed platelets prelabelled with ³²P in
 cold Tris-HCl buffer containing 2mM EGTA were incubated at 37 C
 in the presence of MgCl₂ for designated times and the phospho-
 lipids were extracted and analyzed by thin layer chromatography.
³²P-labelling in PIP was gradually increased in consort with the
 decreased labelling in PI-4,5-bisphosphate. As the changes in the
 labelling was not affected by the presence of apyrase and as the
 radioactive inositol trisphosphate was not detected, it was
 suggested that the changes is due to the action of phosphomono-
 esterase rather than PI-kinase or phospholipase C. When ³²P-ATP
 was added to non-labelled lysed platelets upon incubation, ³²P was
 labelled only into PIP and the amount was markedly increased
 until 5min. after incubation. Since the labelling was strongly
 inhibited by apyrase, it likely reflects the activity of PI-kinase.
 The activity of PI-kinase thus measured required Mg²⁺ strictly
 for the activity and the maximal activity was obtained in the
 presence of 30mM Mg²⁺. In contrast, it was markedly inhibited in
 the presence of Ca²⁺ (as low as 2mM Ca²⁺ in the presence of 2mM
 EGTA), which was compatible with our previous findings with intact
 platelets. The activity of A-kinase was not inhibited by a low
 concentration of Ca²⁺. Furthermore, the activity was inhibited by
 cAMP or dbcAMP in a dose related manner and no enhancement of the
 activity was obtained by the addition of catalytic subunit of
 A-kinase, though a significant reduction in the activity was
 observed in the presence of inhibitor protein to A-kinase. From
 these observations, the following conclusions were obtained; 1) The
 activity of PI-kinase in lysed platelets may be determined by
 pulse labelling with ³²P-ATP. 2) It requires Mg²⁺ absolutely and
 is inhibited by a very low concentration of Ca²⁺. 3) PI-kinase is
 activated by A-kinase but the activated enzyme is inhibited by
 cAMP, suggesting the presence of feedback mechanism.

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PLATELET-FIBRIN CLOTS FORMED BY THROMBIN SELECTIVELY RETAIN
 PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP₂). John D. Vickers,
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It is established that stimulation of human platelets with
 thrombin for 60 s in the absence of fibrinogen increases the
 amount of PIP₂ compared with unstimulated controls (4.7 ± 0.24
 nmol/10⁹ plat. vs 3.83 ± 0.14 nmol/10⁹ plat., p<0.01, n=8). How-
 ever, stimulation with thrombin for 60 s in the presence of
 fibrinogen causes a large decrease in the amount of PIP₂ that
 can be extracted with acidified chloroform/methanol compared
 with unstimulated controls (1.62 ± 0.39 nmol/10⁹ plat. vs 3.84 ±
 0.44 nmol/10⁹ plat., p<0.001, n=6). Stimulation of rabbit plate-
 lets with thrombin in the presence of fibrinogen also decreases
 the amount of extractable PIP₂ (60% at 60 s, p<0.001, n=8).
 Similar decreases in amount can not be demonstrated for phospha-
 tidylinositol 4-phosphate, phosphatidylinositol, phosphatidic
 acid or phosphatidylcholine under the same conditions, indicat-
 ing that the decrease is specific for PIP₂. With rabbit plate-
 lets, polymerized fibrin formed by reptilase, which does not
 stimulate platelets or induce clot retraction, does not cause
 the decrease in extractable PIP₂ (3.06 ± 0.05 nmol/10⁹ plat.
 were extracted compared with 3.18 ± 0.07 nmol/10⁹ plat. without
 reptilase). However, stimulation of rabbit platelets with ADP in
 the presence of polymerizing fibrin formed by reptilase causes a
 larger decrease in extractable PIP₂ (to 2.54 ± 0.19 nmol/10⁹
 plat., p<0.05, n=4) than is caused by ADP and fibrinogen alone
 (to 2.87 ± 0.06 nmol/10⁹ plat., p<0.05, n=4). Inhibition by
 glycyl-L-prolyl-L-arginyl-L-proline of polymerization of fibrin
 formed by the action of thrombin prevents the large decrease in
 the amount of extractable PIP₂ (4.37 ± 0.30 nmol/10⁹ plat. were
 extracted) from human platelets. These results indicate that the
 interaction of polymerizing fibrin with stimulated platelets is
 required for the decrease in PIP₂. The decrease in extractable
 PIP₂ seen with polymerizing fibrin can not be explained by in-
 creased degradation of PIP₂ to IP₃ or PIP. Thus, when human or
 rabbit platelets are stimulated with thrombin in the presence of
 fibrinogen, an association of polymerizing fibrin with the stimu-
 lated platelets occurs that leads to decreased extractability
 of PIP₂. This may mean that PIP₂ forms a specific association
 with platelet proteins that are involved in clot retraction.

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TURNOVER OF THE PHOSPHOMONOESTER GROUPS OF POLYPHOSPHO-
 INOSITIDES IN UNSTIMULATED HUMAN PLATELETS.
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The metabolic activity of the polyphosphoinositides in
 unstimulated human platelets was studied by 1) short-term
 labelling with ³²P-P₁, 2) by replacement of ³²P-
 P₁ from pre-labelled platelets with unlabelled phosphate
 and 3) by depriving the cells of metabolic ATP. Under
 short-term labelling conditions, the 4- and 5-phosphates of
 phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol
 4,5-bisphosphate (PIP₂) had the same specific radioactivity
 as the γ-phosphate of metabolic ATP. The specific radioactivity
 of the 1-phosphates of phosphatidylinositol, PIP and PIP₂,
 was similar, but only 4-13 % as compared to the γ-phosphate of
 ATP. When ³²P-P₁ pre-labelled platelets were incubated
 with up to 25 mM of unlabelled phosphate, the displacement of
 the ³²P-label from PIP, PIP₂ and metabolic ATP followed
 similar kinetics. Inhibition of ATP regeneration in
³²P-P₁ pre-labelled platelets resulted in a rapid fall
 in ³²P-metabolic ATP with much slower fall in ³²P-PIP₂.
³²P-PIP increased initially and decreased thereafter in
 parallel with PIP₂. However, ATP turnover was not
 abolished, as indicated by the marked (25 % of the control)
 incorporation of extracellular ³²P-P₁ into PIP and
 PIP₂ in metabolically inhibited platelets. This low
 phosphate turnover may explain the relative resistance of PIP
 and PIP₂ to metabolic inhibition.
 We conclude that PIP and PIP₂ are present as a single
 metabolic pool in human platelets. Turnover of the 4- and
 5-phosphates of PIP and PIP₂ in unstimulated platelets is
 as rapid as that of the γ-phosphate of metabolic ATP, and
 accounts for about 7 % of basal ATP consumption.

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EVIDENCE THAT ABNORMAL PLATELET AGGREGATION IN SPONTANEOUSLY
 HYPERTENSIVE RATS IS LINKED WITH PHOSPHOINOSITIDES TURNOVER AND
 PHOSPHORYLATION OF 47,000 DALTON PROTEIN. Huzoor-Akbar and
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We have shown earlier that abnormal platelet aggregation in
 spontaneously hypertensive rats (SHR) is not caused by
 prostaglandins (Thromb. Res. 41, 555-566, 1986). In this study
 platelets from SHR and normotensive (Wistar Kyoto, WKY) rats
 were used to examine the role of phosphoinositides (PIs) and
 protein phosphorylation in increased platelet activation in
 hypertension. Thrombin (0.05 U/ml) induced rapid hydrolysis of
 phosphatidylinositol-4,5-bis-phosphate (PIP₂), phosphatidyl-
 inositol-4-phosphate (PIP), and phosphatidylinositol (PI) in
 (³²P)-PO₄ labeled platelets. However, significantly greater
 hydrolysis of PIP₂ and PI was seen in SHR platelets than in WKY
 platelets (see Table). Thrombin also caused two- to three-fold
 increased accumulation of phosphatidic acid (PA) in SHR
 platelets than in WKY platelets (see Table).

	% CHANGE IN	TIME (SECONDS)				
		3	5	15	30	240
PIP ₂	WKY	-15±3	-13±5	-15±4	-4±5	+7±7
	SHR	-23±4	-32±2	-25±4	-8±5	+15±7
PIP	WKY	-14±3	-8±3	-4±6	+3±4	+7±4
	SHR	-18±3	+7±5	+5±3	+20±4	+22±5
PI	WKY	-4±4	-13±4	-8±4	-1±3	+9±5
	SHR	-7±4	-12±3	-24±7	-5±4	+0±4
PA	WKY		+8±2	+28±12	+42±6	+106±35
	SHR		+19±10	+89±9	+134±25	+219±39

Thrombin caused phosphorylation of 18,000 Dalton (P18) and 47,000
 Dalton (P47) proteins in SHR and WKY Platelets. Significantly
 increased phosphorylation of P47 was seen at 5, 15, 60 and 240
 seconds of incubation with thrombin in SHR platelets (60%, 68%,
 98% and 91%) than in WKY platelets (13%, 37%, 44% and 47%). The
 extent of P18 phosphorylation was same in both SHR and WKY
 platelets. Aspirin (500 μM) did not affect phosphorylation of
 P47 or P18 in SHR or WKY Platelets. These data lead us to
 suggest that increased turnover of PIs and increased
 phosphorylation of P47 are involved in abnormal platelet
 aggregation in SHR (Supported in part by the COHC grant #86-01-A
 and the Ohio University College of Osteopathic Medicine).

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