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PROPERTIES OF PHOSPHATIDYLINOSITOL KINASE IN HUMAN PLATELETS.
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We have reported the specific ^{32}P-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 elucidatedyet.Thereby, attempts were made to assay and to characterize PI-kinase of human platelets.Fresh lysed platelets prelabelled with ^{32}P in cold Tris-HCl buffer containing 2mM EGTA were incubated at 37 C in the presence of $MgCl_2$ for designated times and the phospho-lipids were extracted and analyzed by thin layer chromatography. ^{32}P-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 ^{32}P-ATP was added to non-labelled lysed platelets upon incubation, ^{32}P 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 Mg^{2+} strictly for the activity and the maximal activity was obtained in the presence of 30mM Mg^{2+}.In contrast,it was markedly inhibited in the presence of Ca^{2+} (as low as 2mM Ca^{2+} 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 Ca^{2+}.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 ^{32}P-ATP. 2)It requires Mg^{2+} absolutely and is inhibited by a very low concentration of Ca^{2+}. 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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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 ^{32}P-P_i, 2) by replacement of ^{32}P-P_i 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 (PIP_2) had the same specific radioactivity as the γ-phosphate of metabolic ATP. The specific radioactivity of the 1-phosphates of phosphatidylinositol, PIP and PIP_2 was similar, but only 4-13 % as compared to the γ-phosphate of ATP. When ^{32}P-P_i pre-labelled platelets were incubated with up to 25 mM of unlabelled phosphate, the displacement of the ^{32}P-label from PIP, PIP_2 and metabolic ATP followed similar kinetics. Inhibition of ATP regeneration in ^{32}P-P_i pre-labelled platelets resulted in a rapid fall in metabolic ATP with much slower fall in ^{32}P-PIP_2. ^{32}P-PIP increased initially and decreased thereafter in parallel with PIP_2. However, ATP turnover was not abolished, as indicated by the marked (25 % of the control) incorporation of extracellular ^{32}P-P_i into PIP and PIP_2 in metabolically inhibited platelets. This low phosphate turnover may explain the relative resistance of PIP and PIP_2 to metabolic inhibition.
We conclude that PIP and PIP_2 are present as a single metabolic pool in human platelets. Turnover of the 4- and 5-phosphates of PIP and PIP_2 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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PLATELET-FIBRIN CLOTS FORMED BY THROMBIN SELECTIVELY RETAIN PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP_2).
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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 PIP_2 compared with unstimulated controls (4.7 ± 0.24 nmol/10^9 plat.^{2} vs 3.83 ± 0.14 nmol/10^9 plat., p<0.01, n=8). However, stimulation with thrombin for 60 s in the presence of fibrinogen causes a large decrease in the amount of PIP_2 that can be extracted with acidified chloroform/methanol compared with unstimulated controls (1.62 ± 0.39 nmol/10^9 plat. vs 3.84 ± 0.44 nmol/10^9 plat., p<0.001, n=6). Stimulation of rabbit platelets with thrombin in the presence of fibrinogen also decreases the amount of extractable PIP_2 (60% at 60 s, p<0.001, n=8). Similar decreases in amount can not be demonstrated for phosphatidylinositol 4-phosphate, phosphatidylinositol, phosphatidic acid or phosphatidylcholine under the same conditions, indicating that the decrease is specific for PIP_2. With rabbit platelets, polymerized fibrin formed by reptilase, which does not stimulate platelets or induce clot retraction, does not cause the decrease in extractable PIP_2 (3.06 ± 0.05 nmol/10^9 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 PIP_2 (to 2.54 ± 0.19 nmol/10^9 plat., p<0.05, n=4) than is caused by ADP and fibrinogen alone (to 2.87 ± 0.06 nmol/10^9 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 PIP_2 (4.37 ± 0.30 nmol/10^9 plat. were extracted) from human platelets. These results indicate that the interaction of polymerizing fibrin with stimulated platelets is required for the decrease in PIP_2. The decrease in extractable PIP_2 seen with polymerizing fibrin can not be explained by increased degradation of PIP_2 to IP_3 or PIP. Thus, when human or rabbit platelets are stimulated with thrombin in the presence of fibrinogen, an association of polymerizing fibrin with the stimulated platelets occurs that leads to decreased extractability of PIP_2. This may mean that PIP_2 forms a specific association with platelet proteins that are involved in clot retraction.

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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.
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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 (PIns) and protein phosphorylation in increased platelet activation in hypertension. Thrombin (0.05 U/ml) induced rapid hydrolysis of phosphatidylinositol-4,5-bis-phosphate (PIP_2), phosphatidyl-inositol-4-phosphate (PIP), and phosphatidylinositol (PI) in (^{32}P)-PO_4 labeled platelets. However, significantly greater hydrolysis of PIP_2 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 uM) did not affect phosphorylation of P47 or P18 in SHR or WKY Platelets. These data lead us to suggest that increased turnover of PIns 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).