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Formation and metabolism of inositol 1,4,5 trisphosphate in human platelets.
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[^{3}H]Myo-inositol (1,4,5)trisphosphate ((1,4,5)IP_3), when added to lysed platelets, was rapidly converted to [^{3}H]inositol (1,3,4,5)tetrakisphosphate which was in turn converted to [^{3}H]inositol (1,3,4)trisphosphate. This result demonstrates that platelets have the same metabolic pathways for interconversion of inositol polyphosphates that are found in other cells. Labelling of platelets with [^{32}P]orthophosphate, followed by h.l.p.c. was used to measure thrombin-induced changes in the three inositol polyphosphates. Interfering compounds were removed by a combination of enzymatic and nonenzymatic techniques. [^{32}P]-(1,4,5)IP_3 was formed rapidly and reached its maximal level at about 4 sec. It was also rapidly degraded and was no longer detectable after 30-60 sec. Formation of (1,3,4,5)IP_4 was almost as rapid as that of (1,4,5)IP_3 and remained at detectable levels for a longer time. (1,3,4)IP_3 was formed after an initial lag and this isomer reached its maximal level that was ten-fold higher than that of (1,4,5)IP_3 at 30 sec. Comparison of the intracellular Ca^{2+} concentration as measured with fura-2 indicates that agents other than (1,4,5)IP_3 are responsible for the sustained maintenance of a high level of intracellular Ca^{2+}. It is proposed that either (1,3,4)IP_3 or (1,3,4,5)IP_4 may also be Ca^{2+}-mobilizing agents.

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THROMBOXANE-A_2 MEDIATES THE ACTION OF INOSITOL (1.4.5) TRISPHOSPHATE (1,4,5)IP_3 IN SAPONIN-PERMEABILISED PLATELETS.
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Inositol trisphosphate (IP_3) has now been identified as an important intracellular second messenger that can initiate the release of Ca^{2+} from intracellular stores in a variety of cells, including platelets. We have studied the effects of IP_3 on washed platelets permeabilised with saponin (12-14 µg/ml) which allows penetration into the cell of low M.Wt polar molecules. The permeabilised cells show normal responses to the agonists thrombin and collagen. The addition of IP_3 (1-20 µM) after saponin treatment induces shape change, aggregation and secretion of preloaded [^{14}C] 5HT. Concomitant with these responses, thromboxane is produced in a dose related manner. With 20 µM IP_3 thromboxane B_2 increases from basal levels of 5.4 ± 3.0 ng/ml to 140 ± 23 ng/ml. Both thromboxane production and the platelet responses induced by IP_3 are inhibited by pretreatment with the cyclooxygenase inhibitors, indomethacin (EC_{50} 50 nM) and aspirin (EC_{50} 30 µM). Aggregation and secretion responses to IP_3 are also inhibited by thromboxane B_2 receptor agonists; EPO 92 (R. Jones, Edinburgh) and AH 23848 (Glaxo Ltd.). If Ca^{2+} EGTA buffers are used with permeabilised platelets to "lock" the cytosolic [Ca^{2+}] at 0.1 µM, thromboxane production is reduced to the basal level. Intact platelets were labelled with $^{45}Ca^{2+}$ (4h incubation) and after washing, resuspension and saponisation, IP_3 induced the release of 20% of the cell associated ^{45}Ca. The release was unaffected by pretreatment with antimycin and oligomycin indicating an endoplasmic reticulum-like storage site for the sequestered Ca^{2+}. This IP_3-induced Ca^{2+} release was also not affected by pretreatment with either cyclooxygenase inhibitors or thromboxane receptor antagonists (EPO 92 and AH 23848). We believe these studies indicate that the action of IP_3 in saponised platelets involves release of intracellularly stored Ca^{2+}, activation of phospholipase A_2 and cyclooxygenase, and production of thromboxane A_2. The release of thromboxane mediates and/or attenuates platelet responses by acting upon platelet surface receptors.

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COMPARISON OF PLATELET PHOSPHOINOSITIDE HYDROLYSIS BY THROMBIN AND COMBINATIONS OF OTHER AGONISTS.
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The activation of human platelets is mediated by the generation of two distinct second messengers, inositol 1,4,5 trisphosphate (IP_3) and 1,2 Diacylglycerol (DG). Both may be derived from agonist-induced phosphoinositide (PI) hydrolysis. We have examined the effects of combinations of agonists on PI hydrolysis (monitored as {32P}-phosphatidate (PA) production). Supramaximal concentrations of PAF (180 nM), 5HT (10 µM) and the thromboxane analogue EP171 (100 nM), but not ADP (1 µM), elicited a 2-6 fold increase in {32P}-PA levels. In combination the above agonists stimulated formation of {32P}-PA to a level (6.6 fold) which was marginally, but significantly, more than each of the individual agonists. This is considerably less than that induced by IU/ml thrombin (approx. 15 fold), perhaps indicative of an additional mechanism for thrombin-induced PA formation. The serine protease trypsin (1 µM), elicits a 30 fold increase in {32P}-PA and potentiates the EP171-induced {32P}-PA formation from approx. 6 fold to approx. 35 fold. Thus, EP171 synergises with trypsin but not with other receptor directed agonists. Consequently, it is mechanistically possible that thrombin stimulates platelet PI hydrolysis via both receptor activated (typified by EP171) and proteolytic (typified by trypsin) processes.

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INHIBITION BY NEOMYCIN OF AGONIST-INDUCED POLYPHOSPHOINOSITIDE METABOLISM AND RESPONSES IN HUMAN PLATELETS
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Signal processing in platelets seems to involve polyphosphoinositide (PPI) metabolism, although direct coupling between PPI metabolism and responses has not been proved. Neomycin binds tightly to PPIs and has been used to probe the involvement of PPI metabolism and responses in platelets. Neomycin$(SO_4)_3$ powerfully inhibited ADP- and adrenaline-induced aggregation of platelets in PRP. This was partly due to the sulphate anion; the chloride form was therefore prepared. Platelets were prelabelled in PRP with ^{32}P-P_i and transferred by gel filtration to a calcium-free Tyrode's solution (GFP). Increasing concentrations (2-5 mM) of neomycinCl_6 caused progressive inhibition of thrombin-induced aggregation, dense granule secretion, acid hydrolase secretion and formation of ^{32}P-phosphatidic acid (PA); the inhibition was immediate, not affected by aspirin and counteracted by increasing thrombin concentrations. Incubation of neomycin (up to 5 mM) with this GFP or with ^{32}P-P_i in GFP prepared from unlabelled PRP had no effect on the ^{32}P content of ATP, phosphatidylinositol-4-phosphate (PIP) or phosphatidylinositol-4,5-bisphosphate (PIP_2). Increasing neomycin concentrations caused progressive inhibition of the thrombin-induced initial (10 sec) decrease, but not of the late (90 sec) increase in ^{32}P-PIP_2, while they enhanced the increase in ^{32}P-PIP. Similar results were obtained with collagen and PAF. Both the increase in cytosolic Ca^{2+} and pH (measured by INDO-I and BCECF, respectively) induced by thrombin were inhibited progressively by increasing concentrations of neomycin. These results are in support for a direct involvement of PPI metabolism in the stimulus-response coupling below the receptor level. However, the failure of neomycin to affect turnover of PIP and PIP_2 in nonstimulated platelets suggests that the aminoglycoside does not penetrate the membrane, and only become available to PPI during stimulation.