

1719

Formation and metabolism of inositol 1,4,5 trisphosphate in human platelets. J. B. Smith, C. A. Dangelmaier & J. L. Daniel Temple University, Pharmacology Department and Thrombosis Research Center, Philadelphia PA, 19140, USA

[³H]Myo-inositol (1,4,5)trisphosphate ((1,4,5)IP₃), when added to lysed platelets, was rapidly converted to [³H]inositol (1,3,4,5)tetrakisphosphate which was in turn converted to [³H]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 [³²P]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. [³²P]-(1,4,5)IP₃ 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)IP₄ was almost as rapid as that of (1,4,5)IP₃ and remained at detectable levels for a longer time. (1,3,4)IP₃ was formed after an initial lag and this isomer reached its maximal level that was ten-fold higher than that of (1,4,5)IP₃ at 30 sec. Comparison of the intracellular Ca²⁺ concentration as measured with fura-2 indicates that agents other than (1,4,5)IP₃ are responsible for the sustained maintenance of a high level of intracellular Ca²⁺. It is proposed that either (1,3,4)IP₃ or (1,3,4,5)IP₄ may also be Ca²⁺-mobilizing agents.

1721

COMPARISON OF PLATELET PHOSPHOINOSITIDE HYDROLYSIS BY THROMBIN AND COMBINATIONS OF OTHER AGONISTS. A. McNicol and D.E. MacIntyre. Dept. of Pharmacology, Univ. of Glasgow, Glasgow G12 8QQ, Scotland.

The activation of human platelets is mediated by the generation of two distinct second messengers, inositol 1,4,5 trisphosphate (IP₃) 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.

465

1720

THROMBOXANE-A₂ MEDIATES THE ACTION OF INOSITOL (1,4,5) TRISPHOSPHATE (IP₃) IN SAPONIN-PERMEABILISED PLATELETS. K. S. Authi (1), B. J. Evenden (1), E. J. Hornby (2) and N. Crawford (1). Department of Biochemistry, Royal College of Surgeons of England, London, U.K. (1) and Department of Respiratory Pharmacology, Glaxo Ltd., Ware, Herts. U.K. (2).

Inositol trisphosphate (IP₃) has now been identified as an important intracellular second messenger that can initiate the release of Ca²⁺ from intracellular stores in a variety of cells, including platelets. We have studied the effects of IP₃ 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 IP₃ (1-20 μM) after saponin treatment induces shape change, aggregation and secretion of preloaded [¹⁴C] 5HT. Concomitant with these responses, thromboxane is produced in a dose related manner. With 20 μM IP₃ thromboxane B₂ 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 IP₃ are inhibited by pretreatment with the cyclooxygenase inhibitors, indomethacin (EC₅₀ 50 nM) and aspirin (EC₅₀ 30 μM). Aggregation and secretion responses to IP₃ are also inhibited by thromboxane B₂ receptor agonists; EPO 92 (R. Jones, Edinburgh) and AH 23848 (Glaxo Ltd.). If Ca²⁺ EGTA buffers are used with permeabilised platelets to "lock" the cytosolic [Ca²⁺] at 0.1 μM, thromboxane production is reduced to the basal level. Intact platelets were labelled with ⁴⁵Ca²⁺ (4h incubation) and after washing, resuspension and saponinisation, IP₃ induced the release of 20% of the cell associated ⁴⁵Ca. The release was unaffected by pretreatment with antimycin and oligomycin indicating an endoplasmic reticulum-like storage site for the sequestered Ca²⁺. This IP₃-induced Ca²⁺ 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 IP₃ in saponinised platelets involves release of intracellularly stored Ca²⁺, activation of phospholipase A₂ and cyclooxygenase, and production of thromboxane A₂. The release of thromboxane mediates and/or attenuates platelet responses by acting upon platelet surface receptors.

1722

INHIBITION BY NEOMYCIN OF AGONIST-INDUCED POLYPHOSPHO-INOSITIDE METABOLISM AND RESPONSES IN HUMAN PLATELETS. Holm Holmsen, Ole-Bjørn Tysnes, Adrie J.M. Verhoeven, Vidar M. Steen, Lindsey J. Moore and Sissel Rongved. Department of Biochemistry, University of Bergen, N-5000 Bergen, NORWAY.

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(SO₄)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 ³²P-P_i and transferred by gel filtration to a calcium-free Tyrode's solution (GFP). Increasing concentrations (2-5 mM) of neomycinCl₆ caused progressive inhibition of thrombin-induced aggregation, dense granule secretion, acid hydrolase secretion and formation of ³²P-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 ³²P-P_i in GFP prepared from unlabelled PRP had no effect on the ³²P content of ATP, phosphatidylinositol-4-phosphate (PIP) or phosphatidylinositol-4,5-bisphosphate (PIP₂). Increasing neomycin concentrations caused progressive inhibition of the thrombin-induced initial (10 sec) decrease, but not of the late (90 sec) increase in ³²P-PIP₂, while they enhanced the increase in ³²P-PIP. Similar results were obtained with collagen and PAF. Both the increase in cytosolic Ca²⁺ 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 PIP₂ in nonstimulated platelets suggests that the aminoglycoside does not penetrate the membrane, and only become available to PPI during stimulation.