0853

10:00 h

THE EFFECT OF PROSTACYCLIN ON THE PARTICIPATION OF PLATELETS IN X-ACTIVATION AND THROMBIN FORMATION. E.Bevers, G.v.Dieijen, J.Rosing, G.Hornstra and R.F.A.Zwaal. Department of Biochemistry, Limburg University, Maastricht, the Netherlands.

Damaged vascular tissue triggers the intrinsic and extrinsic clotting system, resulting in the clotting of plasma. Platelets, especially after their activation, promote this clotting response, which is inhibited by prostacyclin (PGI_2). The present experiments were devised to characterize this anticoagulant effect of PGI_2.

Using specific chromogenic substrates, the intrinsic activation of factor X and the conversion of prothrombin into thrombin (II_a) was measured in reaction mixtures containing highly purified clotting factors. Phospholipids were added as vesicles, platelet lysates, or as whole platelets, either or not activated with a mixture of collagen and thrombin. Phospholipid vesicles and lysed platelets greatly promoted X_a and II_a formation, which was not affected by PGI_2. The very low formation of X_a and II_a occurring in the presence of non-activated platelets was not inhibited by PGI_2 either. Platelets activated with a mixture of collagen and thrombin stimulated X_a and II_a formation considerably. PGI_2 inhibited this effect in a dose-dependent way.

These results demonstrate that prostacyclin does not interfere with X_a and II_a formation as such, but specifically inhibits the process by which a mixture of collagen and thrombin stimulates the participation of platelets in these reactions.

The inhibiting effect of PGI_2 is only partial. This is most probably explained by the fact that prostacyclin can only inhibit but not block platelet activation by a mixture of collagen and thrombin.

0854

10:15 h

THE EFFECT OF PROSTACYCLIN (PGI_2) ON THE PROCOAGULANT ACTIVITY OF HUMAN PLATELETS. J. Brox and B. Østerud. Inst. of Clin. Med. and Inst. of Med. Biol., Univ. of Tromsø, Tromsø, Norway.

Platelets from healthy donors were isolated by albumin-gradient centrifugation and gelfiltration. The platelets were exposed to thrombin, collagen, and ADP separately, and thrombin and collagen in combination. The concentrations used were the lowest that gave maximal aggregation. The following parameters were assayed: aggregation, platelet factor 3(PF 3), Factor V-Va(F.V-Va), total procoagulant activity (TPA, which measures the combined activity of PF 3 and F.V-Va), and serotonin release. The effect of various concentrations of PGI_2 on these parameters was examined.

Thrombin was more potent than collagen, and collagen was more potent than ADP in stimulating the procoagulant activity and serotonin release (i.e. thrombin generated 33%, collagen 14%, ADP 3% TPA as compared to 100% for lysed platelets). Thrombin alone was equally strong as thrombin and collagen in combination in regard to TPA. The platelet aggregation was maximal in all these experiments.

PGI_2 (1.4×10^{-8}M) inhibited very efficiently aggregation, serotonin release, TPA, PF 3 and F.V-Va activity when the platelets were stimulated with thrombin, collagen or ADP. When platelets were exposed to thrombin and collagen simultanously, the inhibitory effect of PGI_2 on TPA decreased. PGI_2 concentration of 1.4×10^{-7}M in such platelet mixtures inhibited TPA by 30-40% whereas the same PGI_2 dose inhibited TPA by 80-90% when thrombin and collagen were used separately. In these experiments platelet aggregation was less than 20%.

This study demonstrates that PGI_2 strongly inhibits the availability of the platelet procoagulant activity, and PGI_2 may therefore also slow down the generation of thrombin.

0855

10:30 h

DOES EPINEPHRINE ACTIVATE PLATELETS BY BINDING TO A RECEPTOR AND THEN REDUCING HEME IN A MEMBRANE ENZYME TO TRANSMIT THE ACTIVATING SIGNAL?
D.A. Peterson*, J.M. Gerrard+, G.H.R. Rao**, J.G. White**
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It is well established that epinephrine binds to an α-receptor on platelets. How the signal for cell simulation is transmitted is uncertain. Reduction of Fe^{3+}-heme to Fe^{2+}-heme was evaluated as described previously (Prost. Med. 4:73,1980). Compounds which are known to interact with the platelet α-receptor reduced heme in the same rank order as their effectiveness as agonists epinephrine > norepinephrine >> dopamine > phenylephrine. Phentolamine which binds to the platelet receptor but is an inhibitor not an agonist was ineffective at reducing heme. 1,10 phenanthroline, 3-chloropyridine, 2,2'-dipyridyl and 4,4'-dipyridyl which can bind to the Fe in heme all inhibited first wave epinephrine aggregation at lower levels than they inhibited first wave ADP aggregation (IC50s to epinephrine 0.31-0.95; IC50s to ADP 0.93-4.8). The results are consistent with the concept that epinephrine binds to its receptor primarily through interactions involving the methyl-amine and beta-hydroxy groups along with hydrophobic bonds involving the aromatic ring, while intrinsic activity is a function of the catechol moiety which reduces Fe^{3+} heme to Fe^{2+} heme in a membrane enzyme.

0856

10:45 h

ARACHIDONATE METABOLISM AND PLATELET DISAGGREGATION (DA) - REAGGREGATION (RA). Gundu H.R. Rao and James G. White. The Department of Laboratory Medicine & Pathology and The Department of Pediatrics, University of Minnesota Health Sciences Center, Minneapolis, MN 55455

Previous work has shown that platelets irreversibly aggregated by ADP or thrombin (T) can be dissociated by various agents and that the refractory state of disaggregated cells can be reversed immediately by treatment with epinephrine (E). In the present study we have evaluated the influence of drugs which affect different steps in the process of prostaglandin (PG) synthesis on platelet DA-RA. Aspirin and indomethacin did not cause DA of platelets in the process of aggregation nor did they prevent reversal of the refractory state by E and subsequent RA of previously dissociated platelets. Imidazole, which inhibits conversion of endoperoxide to thromboxane A_2, also failed to influence DA or restoration of sensitvity and RA of disaggregated platelets. On the other hand, chemicals which interfere with release of AA from the membrane of activated platelets, such as mepacrine, chlorpromazine and trifluoperazine, caused rapid DA. Products of PG synthesis, such as PGE_1, PGD_2 and PGI_2, which usually inhibit platelet aggregation, also caused rapid DA. The refractory state of platelets dissociated from aggregates by most of these agents could be reversed by E treatment. However, trifluoperazine disaggregated platelets could be reaggregated only by the combination of E and AA. Agents which block the α-adrenergic receptors did not cause dissociation of aggregating platelets, but prevented correction of the refractory state of dissociated platelets by E. Thus interference with AA release, even after aggregation, can cause DA of clumped platelets, but blockade of peroxidase, cyclo-oxygenase and thromboxane synthetase do not cause reversal once it is in progress. A membrane linked mechanism associated with AA availability, but not metabolism, regulates DA and restoration of membrane sensitivity for RA.