Monday, July 13, 1981

Oral Presentations

Endothelium – I

08:00–09:30 h

Platelets – I

Release, Aggregation

09:45–11:00 h

Grand Ballroom West

0045

08:00 h

EFFECTS OF BAY g 6575 ON PLATELETS AND ON VASCULAR PGI_2 PRODUCTION. D.E. MacIntyre, E.W. Salzman. Department of Surgery, Beth Israel Hospital and Harvard Medical School, Boston, U.S.A., Department of Pharmacology, Univ. Glasgow, Scotland.

Bay g 6575 (1-[2-(β-naphthyloxy) ethyl]-3-methyl-2-pyrazolin-5-one) exerts a protective effect in several animal models of thrombosis. To elucidate its mechanism of action, we examined the effects of Bay g 6575 on platelets and on vascular PGI_2 production. In vitro addition of Bay g 6575 (≤200 μM) to human citrated platelet rich plasma (PRP) did not inhibit aggregation induced by ADP or U44069, or augment inhibition of ADP-induced aggregation by PGD_2, PGE_1, PGI_2 or papaverine. When added to isolated human or rat vascular rings, Bay g 6575 (≤200 μM) did not stimulate production of PGI_2 or 6-oxo-$PGF_{1\alpha}$. Ex vivo studies one hour after administration of Bay g 6575 to rabbits (10 mg/kg, i.a.) or rats (100 mg/kg, p.o.) revealed no inhibition of ADP-induced aggregation or enhancement of the level of "circulating" PGI_2 as measured by bio-immunoassay. When production of anti-aggregating activity by vascular rings from Bay g 6575 treated (B) and Control (C) rats were compared, in 6 of 8 experiments B inhibited more than C and B produced more 6-oxo-$PGF_{1\alpha}$ than C (mean increase in B ± s.d.=74.3 ± 35.7%, range 42-135%). Production of anti-aggregatory activity by "exhausted" C rings was enhanced by B>C platelet free plasma. In all cases, the inhibitor of aggregation produced by B and C rings acted on both human and rat PRP, and its effects could be reversed by anti-PGI_1 antibodies that neutralize PGI_2>6-oxo-PGE_1>PGD_2. When exogenous PGI_2 was incubated with (exhausted) aspirin treated vascular rings, the duration of action of PGI_2 was longer in the presence of B rings than C rings.

Bay g 6575 has no direct effects on platelets or on vascular tissue. Its antithrombotic activity appears to be caused by regulation of PGI_2 synthesis and metabolism, an effect mediated by factors, possibly Bay g 6575 metabolites, present in plasma after in vivo administration.

0046

08:15 h

STIMULATION OF PROSTACYCLIN PRODUCTION IN AORTIC ENDOTHELIAL CELLS BY A NON-DIALYSABLE SERUM FACTOR. E.R. Hall, M. Rafelson and K. Wu. Departments of Medicine and Biochemistry, Rush Medical College, Chicago, IL, U.S.A.

The production of prostacyclin (PGI_2) by vascular endothelial cells is thought to be of primary importance in maintaining normal hemostasis. We have investigated the production of prostacyclin in bovine arterial endothelial cells maintained in Dulbecco's modified Eagle's medium (DMEM) containing 30% fetal calf serum. Intact, confluent monolayers of endothelial cells ($3x10^6$ cells) in passages 2 through 6 were used. The growth medium was removed and the cells were washed in DMEM that did not contain serum. 3 mls of medium alone or containing normal plasma or serum was then added and incubated at 37°C for 15 min. Then, 1 mg of arachidonic acid was added and the cells incubated for an additional hour. The test medium was removed, centrifuged to remove any loose cells and stored at -70°C. To determine the production of PGI_2 by the endothelial cells, the medium was assayed for 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2, by radioimmunoassay. The synthesis of prostacyclin by bovine aortic endothelial cells was significantly increased in a concentration dependent manner by both normal platelet poor plasma and normal serum. This increase in prostacyclin production was inhibited by both aspirin and indomethacin, indicating an increase in synthesis rather than the release of PGI_2. Furthermore, this increase could be demonstrated in the presence or absence of added arachidonic acid. The active component in plasma and serum was non-dialysable, eliminating the possibility of a small compound such as bradykinin or angiotensin II. This active factor was present after freezing and thawing the plasma and serum and was heat stable (60°C, 5 min). The presence of an endogenous prostacyclin stimulating factor may be significant in the in vivo regulation of prostacyclin production.

0047

08:30 h

PROSTACYCLIN RELEASE FROM EX VIVO VASCULAR ENDOTHELIUM: COMPARATIVE STUDIES WITH IONOPHORE A23187, ARACHIDONIC ACID, BRADYKININ, AND THROMBIN. J.C.Goldsmith and C.T.Jafvert, Department of Medicine, University of Iowa, Iowa City, IA.

Prostacyclin (PGI_2) release from systemic bovine, sheep, canine, and fetal lamb arterial segments has been evaluated in a template device and in cultured cell monolayers employing a radioimmunoassay for 6-keto-$PGF_{1\alpha}$.

Canine segments released the greatest concentration of PGI_2 and fetal sheep the lowest under unstimulated conditions. After exposure of the various vascular segments to the ionophore A23187, PGI_2 release was increased 5-20 fold over baseline. Similar responses were observed following stimulation with arachidonic acid. Bradykinin induced a dose-dependent release of PGI_2 from bovine vascular segments. Pulmonary arterial segments were more responsive than aortic. When exposed to thrombin, all tissues examined except canine did not demonstrate an increase in PGI_2 release over baseline. After thrombin stimulation, canine segments slightly increased their PGI_2 release from 91.4 ± 15.7 pmoles/ml/15 min to 152.7 ± 21.8 pmoles/ml/15 min. The other fetal and adult systemic vascular segments were unresponsive to thrombin as a releasing agent for PGI_2 as were cultured monolayers of bovine and sheep systemic endothelium. This feature contrasted to prior studies with cultured human umbilical venous endothelial cell monolayers in which thrombin released PGI_2. Cultured bovine endothelial cell monolayers were employed to examine thrombin-cell interactions more extensively. High affinity binding sites for thrombin were demonstrated on cultured bovine aortic and pulmonary endothelium. Examination of vascular tissue from diverse species has confirmed the presence of PGI_2 in supernatents obtained from these vessel segments. Studies with the template model suggest that the vasodilator effects of bradykinin could be a consequence of the release of PGI_2 from the endothelium. Our results also suggest that either thrombin binding is causally unrelated to PGI_2 release from systemic animal endothelium or that this endothelium lacks a mediator required for thrombin to exert its effect.