

IDENTIFICATION OF A CELL-SURFACE COFACTOR FOR ANTITHROMBIN III ON CULTURED MURINE ENDOTHELIUM. P.C. Busch and W.G. Owen. Departments of Pathology and Biochemistry, University of Iowa, Iowa City, IA, USA.

Cultures of mouse brain capillary endothelium grown on microcarrier beads were used as a model for the study of interactions of plasma proteins with the endothelium in the microcirculation, where the ratio of surface area to volume is large. Use of the beads increased the relative cell surface area about 100 fold over that in monolayer cultures. Cells obtained from capillary explants were grown to confluence on 140 μ (mean diameter) beads of cross-linked dextran. The cell-covered beads were packed into 0.3 ml columns in silicone-coated glass tubes, and were used within 2 hours. Cells remained viable during all experimental periods. Radioiodinated thrombin was bound to the cells, and unlabeled diisopropyl phosphoryl-thrombin eluted most of the bound radioactivity, which was identified by electrophoresis as α -thrombin. Likewise, bound thrombin was eluted with antithrombin III, and most of the radioactivity was identified as thrombin-antithrombin III complex. In contrast, when thrombin and antithrombin III were incubated for comparable intervals in the absence of cells, little complex formation occurred. Similar enhancement of complex formation could not be readily detected over monolayer cultures because of the low ratio of surface area to volume coupled with a significant reaction rate without cells. It is concluded that a substance on the cell surface catalyzes the reaction of thrombin with antithrombin III and that this catalyst becomes significant when cell surface concentration approaches that in the microcirculation.

0110

ENDOTOXIN INHIBITS PROSTACYCLIN RELEASE FROM RABBIT AORTA. J. Zahavi, A.C. Honey, J. Westwick and V.V. Kakkar. Thrombosis Research Unit, King's College Hospital Medical School, London, United Kingdom.

Released prostacyclin (PGI_2) activity has been studied in aortic rings of 19 New Zealand white female rabbits. These rings produced a potent inhibitor of platelet aggregation, identified as PGI_2 . All the rabbits were anaesthetized with pentobarbital and thereafter a solution of endotoxin (E. Coli, 0111, B_4 , Difco Lab.) was injected intravenously to 7 rabbits ($30 \mu\text{g/kg}$ every 15 min during 1 hour to achieve an estimated plasma level of 1-2 $\mu\text{g/ml}$). Another 5 rabbits served as controls and were injected with saline. After 1 hour the aorta was rapidly excised, cleaned, cut into small rings and the released PGI_2 activity studied at various time intervals (5-30 min) at 37°C . The mean release of PGI_2 (in pg/mg wet tissue) in the control rabbits was 201 (range 50-443). It decreased significantly to 104 (range 0-237) after 30 min. In the endotoxaemic rabbits, the initial PGI_2 release was only 73 (range 0-329) ($p < 0.008$ compared to control rabbits). This level did not change with time and was 71.9 (range 0-261) after 30 min suggesting that the "endotoxinemic" vessels were initially relatively exhausted and were not able to release PGI_2 . In the remaining 7 rabbits the aorta was removed immediately after anaesthesia and aortic rings incubated for 5-30 min Krebs-Henleit buffer or endotoxin 0.2-10 $\mu\text{g/ml}$ and the released PGI_2 activity studied. There was a dose dependent inhibition which was more pronounced after 30 min incubation.

The decrease in PGI_2 release from rabbit aorta following endotoxaemia removes the inhibitory effect on platelet aggregation of the arterial vessel wall and consequently may contribute to the development of a thrombogenic state.

0109

EFFECT OF TISSUE CULTURE IRRADIATION ON ORIENTATION OF CYTOSKELETAL FIBERS IN MIGRATING SHEETS OF ENDOTHELIAL CELLS. A. I. Gotlieb. Department of Pathology, University of Toronto, Toronto, Canada.

Irradiation of endothelial cells (EC) in tissue culture has been used to markedly reduce cell proliferation in order to study the effects of substances on the regulation of EC migration. Since irradiated EC (IRR-EC) migrate the same distance as do non-irradiated EC (NIRR-EC) over periods of up to six days, it is assumed that EC migration is not effected by irradiation. The purpose of this study was to examine the effect of irradiation on the cytoskeleton of migrating EC. The in-vitro experimental wound technique was used as a model system. A linear wound was made in confluent cultures of porcine thoracic aortic EC. EC were observed to migrate into the wound as a cohesive sheet of cells with only a few free unattached cells being present along the front edge of the wound. Cultures were irradiated one hour before wounding with 1500 rads. NIRR-EC migrated the same distance as did the IRR-EC although the latter were much flatter and each IRR-EC covered a larger surface than did the NIRR-EC. The orientation of cytoskeletal fiber bundles localized by immunofluorescence microscopy using antisera produced against electrophoretically purified porcine uterine myosin and chicken gizzard tropomyosin were different when comparing migrating IRR and NIRR-EC in the first row of the endothelial sheet. At 44 hours after wounding myosin localization showed that the main myosin stained fibers in 89% of the IRR-EC were roughly parallel to the wound edge while 11% were roughly perpendicular to the wound edge. In NIRR-EC the figures were 54% and 46% respectively. Tropomyosin localization showed a similar difference, 73% and 27% in IRR-EC and 45% and 55% in NIRR-EC. The data shows that there are differences in the extent of spreading of and in the orientation of cytoskeletal fiber bundles in migrating IRR-EC. These differences may reflect different cytoskeletal processes involved during migration of IRR and NIRR-EC.

0111

PROSTACYCLIN SYNTHESIS BY CULTURED VASCULAR SMOOTH MUSCLE CELLS: INFLUENCE OF GROWTH AND AGEING.

J. Larrue, M. Rigaud, C. Leroux, R. Crockett, H. Bricaud. Unité 8 de Cardiologie - INSERM - Pessac (France) and Laboratoire de Biochimie CHU Dupuytren - Limoges (France).

The ability of arterial smooth muscle cells (SMC) to produce prostacyclin (PGI_2) is well documented both in vivo and under culture conditions. Advanced atherosclerosis has been associated with a severe decrease in PGI_2 synthesis by arteries and we recently demonstrated that PGI_2 synthesis by cultured SMC from atherosclerotic aorta was significantly depressed. In the present experiments, changes in arachidonic acid (AA) cascade in cultured SMC were studied as a function of cell proliferation and ageing.

SMC derived from explants of the medial layer of 2 years old rabbit aorta were grown under standard conditions (HAM F10 medium + 10 % Foetal Calf Serum). Prostaglandin synthetic activity was investigated by cell free homogenate radiochemical assays and confirmed by gas chromatography - mass spectrometry determinations after 10 mn incubation with 2 nM AA.

An age related decrease in PGI_2 biosynthetic activity (expressed as $\text{pM}/10^6$ cells) was found in confluent cells from passages 5 to 22 (31.4 ± 3 vs 15.4 ± 5.6 respectively - $p \leq 0.01$ -). On the contrary, PGI_2 generation was enhanced during the proliferative phase (day 2-5) (55.5 ± 11.8) when compared to confluent cells at the same (5th) passage ($p \leq 0.01$).

These results are consistent with the enhanced PGI_2 formation in early arterial proliferative lesion and the inhibition of SMC proliferation by cyclooxygenase inhibitors recently reported and indicate that PGI_2 synthetic activity in arterial SMC may be a significant age-dependant endogenous factor involved in the pathogenesis of atherosclerosis.