0240
08:45 h

MULTIPLE MOLECULAR FORMS OF PLASMINOGEN ACTIVATOR PRODUCED BY CULTURED BOVINE ENDOTHELIAL CELLS. Eugene G. Levin and David J. Loskutoff. Research Institute of Scripps Clinic, La Jolla, Ca. USA

The production of plasminogen activator (PA) by the vascular endothelium has been implicated in the maintenance of vessel patency. Cultured bovine aortic endothelial (BAE) cells were employed to study and compare the cell associated and secreted forms of PA. Samples were fractionated by polyacrylamide gel electrophoresis in the presence of SDS. PA activity in the gel was localized by the fibrin-overlay technique. Cellular PAs were found to be membrane associated and to consist of a major form of M_r 48,000 (C48) and minor forms of 53,000 (C53), 74,000 (C74), and 100,000 (C100). Incubation of the cell extracts at 37°C resulted in the appearance of two additional forms of M_r 41,000 and 33,000 suggesting that these forms were degradation products. Serum-free conditioned medium (CM) contained secreted PAs of M_r 52,000 (S52), 55,000 (S55), 74,000 (S74) and 100,000 (S100). In addition, a broad zone of fibrinolytic activity was observed in the region between M_r 80,000 and 95,000. Cellular PAs have isoelectric points of pH 8.5-8.6 and 7.5 while secreted PAs demonstrate activity at pH 8.6, 8.5, 8.0, and 7.5. The forms showed differential sensitivities to DFP with S74 and C74 being inactivated by 1mM DFP within one Hr at 37°C while S52 and C48 were still partially active after treatment with 10mM for two Hrs. S100 was completely refractile to treatment with 40 mM DFP. The addition of fresh serum to confluent cultures resulted in the disappearance of C48 and C74, and of S52 and S74, but caused an increase in C100 and S100. These studies indicate that several forms of PA are produced by endothelial cells and suggest that the production of each may be independently regulated.

0241
09:00 h

MEASUREMENT OF PROSTAGLANDINS THAT ACTIVATE PLATELET ADENYLATE CYCLASE IN RABBIT ARTERIAL BLOOD; STUDIES ON PGI_2 AND 6-KETO-PGE_1. R.J. Haslam and M.D. McClenaghan. Dept. of Pathology, McMaster University, Hamilton, Ont., Canada.

The increases in platelet cyclic [^{3}H]AMP on addition of washed rabbit platelets containing ^{3}H-labelled adenine nucleotides to fresh arterial blood samples mixed with 1 mM 3-isobutyl-1-methyl xanthine permitted assay of any activators of adenylate cyclase present. Blood PGI_2 was calculated from the difference between the increases in cyclic [^{3}H]AMP over 30 s in the presence and absence of antibody that bound PGI_2 and the effects of PGI_2 standards on cyclic [^{3}H]AMP formation in preincubated blood. The PGI_2 found in rabbit blood assayed within 2.5 min of arterial puncture (0.05 ± 0.01 pmol/ml in 10 males and 0.07 ± 0.02 pmol/ml in 8 females (means ± S.E.M.)) was far less than that needed to inhibit platelet function (>1 pmol/ml). The antibody used also bound 6-keto-PGE_1, a metabolite of PGI_2 that was one fifth as effective, both as an activator of rabbit platelet adenylate cyclase and as an inhibitor of platelet aggregation. PGI_2 and 6-keto-PGE_1 were distinguished by their half-lives in citrated blood at 37°C (10 and 44 min, respectively). Fresh arterial blood contained no assayable activity after incubation for 30 min, confirming that the material measured was PGI_2.

After an intravenous bolus injection of 1 nmol PGI_2/kg, arterial PGI_2 averaged 1.7 pmol/ml after 2 min (i.e. 90% had been removed) and returned to preinjection levels after 10 min. 6-Keto-PGE_1, injected intravenously at 5nmol/kg, was cleared at a similar rate. After a bolus injection of 5 µg angiotensin II/kg, the PGI_2-like material in arterial blood amounted to 7 pmol/ml after 2 min, but declined rapidly to control levels within 10-30 min. This PGI_2-like material had the same half-life in vitro as authentic PGI_2.

The results show that though physiologically significant PGI_2 does not normally circulate in rabbits, angiotensin II can raise blood PGI_2 to inhibitory levels. Formation of 6-keto-PGE_1 is unlikely to contribute to the effects of PGI_2 on platelets in rabbits

0242
09:15 h

LACK OF CIRCULATING PROSTACYCLIN (PGI_2) IN GUINEA PIG AND RAT. J.M. Fisher, A.L. Willis, D.L. Smith, D. Donegan. Department of Physiology, Institute of Biological Sciences, Syntex Research, Palo Alto, Ca. 94304

Under light ether anesthesia, blood of male guinea pigs (Hartley strain, 330-410 g, Simonsen, Gilroy, CA) or rats (Sprague-Dawley, 230-575 g, Simonsen) was withdrawn into sodium citrate (0.38% w/v). Platelet rich plasma (PRP) was then prepared by a rapid centrifugation procedure. Aggregation induced by ADP (1.6 µg/ml) was then examined in 0.15 or 0.25 ml aliquots of PRP at various time intervals from 5-60 min after blood withdrawal. A spontaneous time-dependent rise in aggregation response occurred that was similar to that observed when PGI_2 (0.5-3ng) was added to PRP in vitro. In both species, intraperitoneal administration of indomethacin (100 mg/kg, 1h previously) failed to interfere with the spontaneous rise in aggregation although vascular PGI_2 formation was shown to be virtually abolished. Similar results were seen in essential fatty acid (EFA) deficient rats that had been chronically maintained on a fat free diet. In these animals, vascular PGI_2 production was less than 15% that of controls. These results clearly indicate that any basal levels of PGI_2 present in the arterial circulation are less than those (0.3-1ng/ml) necessary to appreciably inhibit aggregation. This conclusion coincides with that of Steer, et al. (Nature, 283, 194, 1980) who failed to detect biologically active amounts of PGI_2 in human venous blood and of several other groups who have failed to detect significant blood levels of the PGI_2 breakdown product, 6-keto-$PGF_{1\alpha}$.

0243
09:45 h

STRUCTURE OF PLASMA FIBRONECTIN. GENE MULTIPLICATION. T.E. Petersen, H.C. Thøgersen, K. Skorstengaard, K. Vibe-Pedersen, P. Sahl, R. McDonagh (deceased), J. McDonagh, S. Magnusson and L. Sottrup-Jensen. Department of Molecular Biology, University of Aarhus, DK-8000, Aarhus C, Denmark.

Plasma fibronectin (cold insoluble globulin) is a multiple domain glycoprotein composed of two nearly identical disulphide bridged polypeptide chains with molecular weight of 220,000. It is a substrate for factor $XIII_a$ and binds to gelatin and heparin. After digestion of bovine fibronectin with plasmin, four fragments with M_r 29,000, 170,000, 23,000 and 6,000 have been isolated. The N-terminal 29,000 M_r fragment (259 residues) has 10 disulphide bridges within five mutually homologous domains, called "fingers". The sequence <Gln-Ala-Gln-Gln-Ile-Val-Gln-Pro-Gln- contains the acceptor site for factor $XIII_a$ at position 3. The 170,000 M_r fragment contains both the gelatin and the heparin binding site. After further digestion with chymotrypsin a fragment (M_r 45,000) which binds to gelatin, and a fragment (M_r 30,000) which binds to heparin, have been isolated. The M_r 45,000 fragment consists of at least one more "finger" plus two other mutually homologous domains each with two disulphide bridges. The 23,000 M_r fragment (178 residues) consists of three "finger"- domains and has an N-terminal sequence Val-Arg-. The M_r 6,000 (C-terminal fragment) is a dimer of two identical 26-residue peptides linked by two disulphide bridges. 820 of the expected approx. 1800 residues have been placed in sequence.

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