

ENHANCEMENT OF THE EFFECT OF BRADYKININ ON MICROVASCULAR PERMEABILITY BY A PEPTIDE DERIVED FROM FIBRINOGEN.

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Some low molecular weight fibrin degradation products (LMW-FDP) cause increased microvascular leakage in the dorsal skin of the rat. Two peptides earlier termed 6A and 6D seem to cause most of this effect. As unfractionated LMW-FDP potentiate bradykinin we have investigated how the leakage due to bradykinin is affected by the peptides 6A and 6D.

Methods. Different concentrations of peptide 6A (Ala-Arg-Pro-Ala-Lys), 6D (Ser-Gln-Leu-Gln-Lys-Val-Pro-Pro-Glu-Trp-Lys), inhibitors of kininase I and II (arginine and SQ14,225) were tested alone as well as together with different concentrations of bradykinin. The substances were injected in the previously shaved dorsal skin of ^{125}I -albumin treated rats. The radioactivity content in the skin was measured in each injection site and the effect on microvascular permeability was calculated.

Results. Thirty minutes after an injection of 6A or SQ14,225 together with bradykinin the leakage due to each mixture was greater than the sum of each substance *per se*. The response was dose-dependant. Simultaneous application of 6A, bradykinin and SQ14,225 caused a further increase in microvascular permeability. 6A and arginine had only marginal action upon the leakage due to bradykinin. 6A did not potentiate the effect of bradykinin after 5 min, while SQ14,225 showed a slight potentiation, however less than after 30 min. SQ14,225 itself was without effect.

Discussion. As peptide 6A did not potentiate bradykinin after 5 min in contradiction to SQ14,225 it seems that their effects are not completely identical. The dramatic effect of SQ14,225 and the limited effect of arginine indicate that kininase II is the principle enzyme degrading bradykinin in rat skin. The effect of peptide 6A, even at low concentrations, indicate that this peptide, apart from its intrinsic effect on microvascular permeability, also can be of pathophysiological significance in inflammatory states as potentiator of bradykinin.

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COLLAGENS OF THE NORMAL AND ATHEROSCLEROTIC AORTIC WALL. STUDIES IN AUTHENTIC TISSUE AND IN ORGAN AND CELL CULTURES. M. Aumailley and H. Bricaud, Laboratoire de Recherches de Cardiologie, 33600 Pessac, France.

In the vessel wall collagen is present as genetically different types. During atherogenesis intimal smooth muscle cell (SMC) proliferation and collagen accumulation predispose to thrombosis. Therefore the aim of our study was first to characterize the authentic collagens in normal and atherosclerotic tissue, more specially type V collagen, and second to find out whether there was a change in atherosclerosis in the newly synthesized collagens. All the investigations were carried out using normal and atherosclerotic tissue derived from animal and human aortas minus adventitia.

From authentic tissue, pepsin solubilized collagens were submitted to ion-exchange chromatographies and identified using molecular sieve chromatography and electrophoresis (PAGE) as type I, III and V collagens. Using PAGE and studying SLS-crystallites type V collagen was found to be present in intima as well as in media of normal and atherosclerotic aortas.

After pulse-label experiments in organ culture of healthy and atherosclerotic specimens the newly synthesized collagens were isolated using pepsin treatment and subsequent molecular sieve chromatography. Interstitial collagens (type I and III) represented 80-85% of the total newly synthesized collagens. Type III accounted for 16-31% in explants from young specimens, for 30-36% when the explants were derived from older specimens and for 35-48% when the tissue were atherosclerotic. SMC were grown out from these tissue and investigated for their collagen synthesis in primary and secondary cultures. With respect to relative distribution of the interstitial collagens the data obtained were in the same range than in organ culture. Furthermore most of the interstitial collagens were secreted into the incubation medium whereas type V collagen was found to be preferentially associated with the cell layer. These results showed that type I, III and V collagen are present in normal and atherosclerotic aortas from various sources. In atherosclerosis a slight change occurs in the newly synthesized collagens. Furthermore with respect to these studies organ and cell culture systems lead to similar results.

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GLYCOSAMINOGLYCAN (GAG) DISTRIBUTION IN CATHETER INDUCED LESIONS IN RABBIT AORTAE IN RELATION TO LIPID ACCUMULATION. S. Moore and M. Richardson. Department of Pathology, McMaster University, Hamilton, Ontario, Canada, L8S 4J9.

Lipid-rich lesions develop in normolipemic rabbit aortae in response to continuing injury associated with an indwelling polyethylene catheter. Lipid disappears from these lesions when the catheter is removed. In contrast following removal of the endothelium by Fogarty balloon catheter, lipid accumulation, located mainly in areas of the neointima covered by regenerated endothelium, persists and increases over time. We have previously shown that this lipid accumulation, in areas of endothelial regeneration following balloon deendothelialization, is associated with an increase in GAG visualized by ruthenium red staining as large granules (RRG) seen on transmission electron microscopy in the interstitium of the neointima. Moreover there was a marked decrease in the concentration of large granules in the areas of neointima uncovered by endothelium where lipid accumulation was minimal or absent. In the present experiment, polyethylene catheters were placed in the aortae of rabbits and removed after 3 weeks. Early thrombi, raised lipid-containing lesions, flat lipid-free lesions and samples of normal aortae were examined at this time. Four weeks and 8 weeks following catheter removal, raised lesions, flat lesions and samples of normal aorta were examined. The GAG was quantitated by counting the RRG/square micron of intercellular space. Early, unorganized thrombi showed lipid-containing macrophages in the superficial part of the lesion and very few RRG ($7 \pm 3/\mu^2$). Thrombi showing early organization without lipid vacuoles in the smooth muscle cells showed the content of RRG to be ($52 \pm 19/\mu^2$) half that of raised lesions containing lipid ($115 \pm 13/\mu^2$). Lipid-free lesions at 4 and 8 weeks following catheter removal showed less RRG granules ($5 \pm 3/\mu^2$) than control tissue ($24 \pm 39/\mu^2$). Thus the accumulation and disappearance of lipid parallels the concentration of GAG in the interstitium of the neointima.

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MODELS OF GRANULOCYTE-MEDIATED ENDOTHELIAL INJURY IN VITRO. G. Vercellotti, P. Flynn, D. Weisdorf, C.J. Lammi-Keefe, H. Jacob, C.F. Moldow. Hennepin County Medical Center, University of Minnesota, Minneapolis, MN 55415, USA.

To determine the role of neutrophil (PMN) induced vascular injury during inflammation an *in vitro* model of endothelial damage was investigated. Injury to human umbilical vein endothelial cells (EC) labeled with ^{51}Cr or ^{14}C sodium arachidonate was monitored by specific release of these labels or their products. Several agents were capable of triggering PMN to induce significant EC injury: these include activated serum complement (C') opsonized particles, serotonin, phorbol myristate acetate, and the lipid A moiety of endotoxin. PMN must adhere closely to the EC for effective cytotoxicity, since agents which retard PMN adherence (cytochalasin B, methyl prednisolone) inhibit ^{51}Cr release.

Lysosomal proteases did not mediate PMN induced endothelial injury since there was no correlation between release and injury, and soluble stimuli which did not release lysosomal contents induced injury. Free radical scavengers such as SOD/catalase, and α -tocopherol significantly reduced PMN mediated endothelial injury implying that PMN generated reactive oxygen species were responsible for this damage.

To further study PMN mediated endothelial injury, other inflammatory agents were also utilized. C' activated PMN's exposed to Ibuprofen (I) (50 $\mu\text{g}/\text{ml}$) but not Aspirin (ASA) (200 $\mu\text{g}/\text{ml}$) induced no EC injury (^{51}Cr release). Furthermore, it was shown that I inhibits O_2^- production, blocks release of PMN lysozyme and glucuronidase, and inhibits aggregation of C' stimulated PMN's. ASA at doses of 500 $\mu\text{g}/\text{ml}$ (clinically toxic), failed to inhibit these *in vitro* activities. This data suggests that I's anti-inflammatory effect may be expressed through inhibition of PMN functions. Since I and ASA both inhibit cyclooxygenase, but only I modulated PMN induced endothelial injury, these agents may provide useful probes to elucidate PMN-endothelial interactions *in vivo*.