III were isolated as by-products of the kininogen purification. They were further purified by affinity chromatography on Concanavalin A. Complete separation of the two inhibitors occurred in the final step, isoelectric focusing. Antithrombin III inhibited the arginine esterase (BAEe) and kinin-generating activities of both kallikrein and plasmin. Both the degree and rate of inhibition was markedly enhanced by physiological concentrations of heparin. No inhibition was detected upon incubation of plasma kallikrein with α_1 -antitrypsin. However, α_1 -antitrypsin was a weak inhibitor of plasmin. Like plasma kallikrein, plasmin cleaved bradykinin from both LMW and HMW-kininogens. The peptide was isolated and identified by CM-cellulose, Sephadex G-15 and high voltage electrophoresis. (Supported by the Ontario Heart Foundation and the Medical Research Council of Canada).

A VASODEPRESSOR PEPTIDE IN COHN FRACTION III-0 of HUMAN PLASMA PROTEINS

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Human plasma contains a protein of MW approximately 100,000 which exhibits vasodilator properties in intact vascular beds and induces vasocontriction in the isolated perfused central vein of the rabbit ear, a vessel which is highly sensitive to plasma kinins. Similar activity is also exhibited by Cohn fraction III-0 of human plasma proteins. However in this fraction there are at least two vasoactive materials both inactivated by chymotrypsin and carboxypeptidase B, and both dialysable. The material accounting for the major part of the vasoactivity is a peptide of approximate MW 1,500, which passes readily through Amicon UM 10 membranes, but is retained by UM 02 membranes.

A purification procedure involving column chromatography on Sephadex CM25 and G25 columns, followed by countercurrent distribution in a 2-butanol: acetic acid system and high voltage electrophoresis, has yielded small quantities of highly purified material. Preliminary amino acid analysis suggests that the active material may be a duodecapeptide, containing 4 proline, 3 glycine, 3 glutamic acid, 1 alanine and 1 arginine residues.

BOVINE PLASMA HMW and LMW KININOGENS: ISOLATION AND CHARACTERIZATION OF THE STRUCTURAL FRAGMENTS PRODUCED BY KALLIKREINS

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Bovine HMW kininogen is a monomeric plasma glycoprotein with a molecular weight of 76,000. The kininogen locates bradykinin in its inner polypeptide

region bridged by a disulfide bond. From this kininogen, bovine plasma kallikrein liberated two peptide fragments, in addition to bradykinin. One of the fragments was a biologically active histidine-rich peptide (fragment 2) consisting of 41 amino acid residues (Han et al., J. Biochem., 77, 55 (1975), and the other (fragment 1) was a glycopeptide containing also high level of histidine. The N-terminal sequence of Ser-Val-Gln-Val-Met-Lys-Thr-Glu-Gly--- of the glycopeptide was the same as C-terminal sequence of the CNBr-fragment involving kallidin (Komiya et al., J. Biochem., 76, 833 (1974)).
The release of these fragments in the course of digestion of HMW kininogen with plasma kallikrein was examined by SDS-polyacrylamide gel electrophoresis. At initial stage of the digestion, a large fragment was liberated in parallel with the release of bradykinin, producing kinin-free kininogen, and this large fragment was subsequently hydrolyzed into two fragments, histidine-rich peptide (fragment 2) and glycopeptide (fragment 1) mentioned above. These structural fragments derived from HMW kininogen were isolated preparatively and identified chemically. The kinin-free kininogen consisted of two chain polypeptides, heavy and light chains, which are held together by a disulfide bond.
On the digestions of HMW kininogen with other tissue kallikreins, they also liberated a few of peptide fragments, in addition to the kinin, but the chemical properties of these fragments seemed to slightly differ from those released by plasma kallikrein. As previously reported, LMW kininogen was resistant to the action of plasma kallikrein. On SDS-gel electrophoresis, no peptide grafments could be detected on the digest of LMW kininogen with plasma kallikrein. Comparison of the chimical structure of HMW and LMW kininogens will be also discussed.

ACCUMULATION OF MONONUCLEAR CELLS AND GROWTH-SLOWING OF SV-40 HAMSTER FIBROSARCOMAS FOLLOWING INTRA-TUMOR INJECTION OF BRADYKININ

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Two sites on male LSH inbred hamsters were injected with 10^5 male LSH SV-40 transformed fibrosarcoma cells. When the non-metastasizing subcutaneous tumors reached 10 mm diameter (at 5-5 1/2 weeks) tumors (one per hamster) were injected with 0.2 ml saline or bradykinin in 0.2 ml saline daily for four days and on days 5, 7, 10, 14, 18 and 21, with tumor diameters being measured daily prior to any injection. Animals with tumors reaching 10 mm diameter prior to or after 5-5 1/2 weeks were excluded for tumor growth kinetic comparison reasons. Splenic HDC activity was assayed by the 14-C