
Rapid infusion of plasma protein fraction (PPF) containing prekallikrein activator (PKA) may cause severe hypotension. It has been suggested that the decrease in blood pressure is due to PKA-mediated activation of the kallikrein system. We studied both in patients and in animals, whether a relationship exists between PKA activity in PPF, the plasma bradykinin (BK) level and changes in mean arterial pressure (MAP), during rapid infusion of PPF and albumin. Plasma BK was measured by radioimmunoassay, using blood collected in a mixture of kallikrein and kininase-inhibitors. PKA was determined spectrophotometrically, PPF and albumin were infused (250 ml, 30 sec.) in the bypass circuit of patients undergoing open heart surgery. After infusion of PPF (29 U PKA/L), MAP decreased within 1.5 min. from 76 ± 11 (SD) to 49 ± 9 mm Hg; concurrently the venous BK concentration increased from 0.7 ± 0.1 to 1.7 ± 0.2 ng/ml (n = 6). After albumin infusion (3 U PKA/L) MAP dropped from 71 ± 14 to 66 ± 13 mm Hg; the BK concentration did not increase significantly (n = 6). In rats, pretreated with the kininase II inhibitor C1INH, the hypotensive effect of the infusion of different lots of PPF on arterial BK concentration and MAP were compared. Both the decrease of MAP and the increase of arterial BK concentration were proportional to the amount of PKA infused. Infusion of synthetic BK provoked a similar fall in MAP at corresponding arterial BK concentrations. After complete inhibition of PMA in PPF by C1-INH, no effect on arterial BK level were observed for several lots of PPF to generate BK (ng/ml/10 min.) in Hageman factor deficient plasma closely correlated with the PKA content of PPF (r = 0.95, p < 0.005). From these observations we conclude that the hypotensive reactions after PPF infusion are mainly generated by the PKA mediated generation of BK in the recipient.

RELATIVE IMPORTANCE OF PLASMA PROTEASE INHIBITORS IN THE INACTIVATION OF KALLIKREIN IN HUMAN PLASMA. H. Schapira, C.F. Scott, and R.W. Coleman. Thorombosis Research Center, School of Medicine and Dentistry, University of California, Irvine, CA.

Human plasma contains several inhibitors of plasma kallikrein (KAL), including CI-inhibitor (CI-INH), α2-macroglobulin (α2M), antithrombin III (ATIII), and α1-antitrypsin (α1AT). Studies in purified systems revealed no kinetic advantage for the quantitation of KAL by inhibition of α2M. When purified inhibitors and HMK are present at plasma concentrations, it can be calculated that CI-INH, α2M, ATIII and α1AT account respectively for 49%, 49%, 0.8% and 0.2% of the KAL activity. To assess if this prediction derived from purified system adequately describes the inhibition of KAL under more physiological conditions, we incubated KAL with normal human plasma (NHP), CI-INH-deficient plasma (CI-INH-D), and α2M-deficient plasma (α2M-D). KAL activity was assessed using H-D-Pro-Phe-Arg-Nan as a substrate. CI-INH-D was obtained from a patient with hereditary angioedema. CI-INH in CI-INH-D was 15% of the normal value as assessed by radial immunodiffusion. α2M-D was obtained by selective and complete inactivation of α2M by methylamine. The pseudo-first-order rate constants for the inactivation of KAL by NHP, CI-INH-D, α2M-D, and plasma deficient in both CI-INH and α2M were respectively 8.8, 5.0, 0.15 and 0.2 s(x10^-2). The results indicate that CI-INH and α2M are the only important inhibitors of KAL in NHP.

THE KINETICS OF THE INHIBITION OF HUMAN PLASMA KALLIKREIN BY PLASMA PROTEASE INHIBITORS: ROLE OF HIGH MOLECULAR WEIGHT KININOGEN. M. Schapira, A. James, C.T. Scott, F. Koppes, R.L. James, A.S. Cohen, and E.W. Coleman. Center for Thrombosis and Deleterious Medicine, Temple University Hospital, Philadelphia, PA, USA.

Plasma kallikrein (KAL) is inhibited by several plasma protease inhibitors, including C1-inhibitor (CI-INH), antithrombin III (ATIII), α1-antitrypsin (α1AT), and α2-macroglobulin (α2M). To assess the mechanism of action and the relative importance of these inhibitors, we have undertaken inhibition studies with purified proteins, using H-D-Pro-Phe-Arg-Nan as KAL substrate. Inhibition was competitive with CI-INH, AT III, and α1AT and noncompetitive with α2M. KAL retained 14% of its catalytic efficiency when complexed with CI-INH, AT III, and α1AT, and α2M were 28, 0.18, 0.001, and 6.9 nM-L-1(x10^3), respectively. Michaelis-Menten kinetics was observed for the inhibition by CI-INH, AT III, α1AT, and α2M. The rate constants for inhibition by CI-INH, AT III, α1AT, and α2M were 28, 0.18, 0.001, and 6.9 nM-L-1(x10^3), respectively. Michaelis-Menten kinetics was observed for the inhibition by AT III, α1AT, and α2M. The constants for the rate-limiting formation of the irreversible complexes were 16, 0.27 and 2.0 nM-L-1(x10^3), while the K_s for the reversible complex were 86, 63, and 0.25 nM, respectively for AT III, α1AT and α2M. In contrast, no Michaelis-Menten complex was observed when CI-INH inhibited KAL. These results indicate that (a) CI-INH is the most efficient inhibitor of KAL, (b) α2M is a significant inhibitor of KAL, (c) both AT III and α1AT are noncompetitive inhibitors of KAL. We have shown that high molecular weight kininogen (HMWK) decreases the inactivation rate of KAL by CI-INH by forming a reversible complex with KAL. We now report that the reaction rates of KAL with AT III and α1AT, which are competitive inhibitors, were decreased by 50%, when HMWK was 1 U/ml on KAL inhibited by CI-INH but not by a noncompetitive inhibitor, the inactivation rates were identical in the presence or absence of HMWK. Since HMWK protects KAL from being inhibited by competitive inhibitors but not by a noncompetitive inhibitor, these results confirm our previous observation indicating that the binding site for HMWK on KAL is closely linked to its catalytic site.