0809
10:00 h

BRADYKININ MEDIATED HYPOTENSION AFTER INFUSION OF PREKALLIKREIN ACTIVATOR CONTAINING PLASMA PROTEIN FRACTION. R.F. van Rosevelt, W.K. Bleeker, J.C. Bakker, J.G.R. Ufkes, D. Sinclair, J.A. van Mourik, W.G. van Aken. Central Lab. of the Netherlands Red Cross Blood Trans. Serv. and Parmacol. Lab., Univers. of Amsterdam, Amsterdam, The Netherlands.

Rapid infusion of plasma protein fraction(PPF) containing prekallikrein activator (PKA) may cause severe hypotension. It has been suggested, that such changes in blood pressure are 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 BPP9A the effect of rapid 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 PKAin PPF by $C\bar{1}$-inhibitor, no effects on MAP or Bk level were observed. The ability of several lots of PPF to generate Bk (ng/ml/10 min.) in Hageman factor deficient plasma closely correlated with the PKA content of PPF (n=25, r 0.95, p 0.005). From these observations we conclude that the hypotensive reactions after PPF infusion are mainly caused by the PKA mediated generation of Bk in the recipient.

0810
10:15 h

RELATIVE IMPORTANCE OF PLASMA PROTEASE INHIBITORS IN THE INACTIVATION OF KALLIKREIN IN HUMAN PLASMA. M. Schapira, C.F. Scott, and R.W. Colman. Thrombosis Research Center, School of Medicine. Philadelphia, PA USA

Human plasma contains several inhibitors of plasma kallikrein (KAL), including $C\bar{1}$-inhibitor ($C\bar{1}$INH), α2-macroglobulin (α2M), antithrombin III (ATIII), and α1-antitrypsin (α1AT). Studies in purified systems have allowed us to quantitate the kinetic constants of each isolated inhibitor. We also have demonstrated that high molecular weight kininogen (HMWK) is an important regulator since it decreases the inactivation rate of KAL by all inhibitors except α2M. When purified inhibitors and HMWK are present at plasma concentrations, it can be calculated that $C\bar{1}$INH, α2M, AT-III and α1AT account respectively for 49%, 49%, 0.8% and 0.2% of the KAL inhibitory 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), $C\bar{1}$INH-deficient plasma ($C\bar{1}$INH-D), and α2M-deficient plasma (α2M-D). KAL activity was assessed using H-D-Pro-Phe-Arg-Nan as a substrate. $C\bar{1}$INH-D was obtained from a patient with hereditary angioedema. $C\bar{1}$INH in $C\bar{1}$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, $C\bar{1}$INH-D, α2M-D, and plasma deficient in both $C\bar{1}$INH and α2M were respectively 8.8, 5.0, 5.5, and 1.8 $s^{-1}(x10^3)$. Therefore, CIINH accounted for 63% of the observed inhibition, α2M for 35%, and all the other inhibitors for 2%. When these values were corrected for the concentration of HMWK, $C\bar{1}$INH accounted for 47% of the inhibition, α2M for 51%, and all the other inhibitors for <2%. Thus, there is an excellent agreement between the results obtained when KAL is inhibited in purified systems and those obtained when it is inhibited in plasma. Moreover, these results indicate that $C\bar{1}$INH and α2M are the only important inhibitors of KAL in NHP.

0811
10:30 h

THE KINETICS OF THE INHIBITION OF HUMAN PLASMA KALLIKREIN BY PLASMA PROTEASE INHIBITORS: ROLE OF HIGH MOLECULAR WEIGHT KININOGEN. M. Schapira, A. James, C.F. Scott, F. Kueppers, H.L. James, A.B. Cohen, and R.W. Colman. Center for Thrombosis Research and Department of Medicine, Temple University Hospital, Philadelphia, PA, USA.

Plasma kallikrein (KAL) is inhibited by several plasma protease inhibitors, including $C\bar{1}$-inhibitor ($C\bar{1}$-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 $C\bar{1}$INH, ATIII, and α1AT and noncompetitive with α2M. KAL retained 14% of its catalytic efficiency when complexed to α2M. The rate constants for inhibition by $C\bar{1}$INH, ATIII, α1AT, and α2M were 28, 0.18, 0.003, and 6.9 $M^{-1}s^{-1}(10^{-3})$ respectively. Michaelis-Menten kinetics was observed for the inhibition by ATIII, α1AT, and α2M. The constants for the rate-limiting formation of the irreversible complexes were 16, 0.27 and 2.0 $s^{-1}(x10^2)$, while the K_I's for the reversible complex were 86, 63, and 0.29 μM, respectively for ATIII, α1AT and α2M. In contrast, no Michaelis-Menten complex was observed when $C\bar{1}$INH inhibited KAL. These results indicate that (a) $C\bar{1}$INH is the most efficient inhibitor of KAL, (b) α2M is a significant inhibitor of KAL, (c) both ATIII and α1AT are probably not significant inhibitors of KAL. We have shown that high molecular weight kininogen (HMWK) decreases the inactivation rate of KAL by $C\bar{1}$INH by forming a reversible complex with KAL. We now report that the reaction rates of KAL with ATIII and α1AT, which are competitive inhibitors, were decreased by 50%, when HMWK was 1 U/ml or 0.73 μM. When KAL was inhibited by α2M, 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 one, these results confirm our previous observation indicating that the binding site for HMWK on KAL is closely linked to its catalytic site.

0812
10:45 h

KALLIKREIN-LIKE ACTIVITY OF CROTALASE, A SNAKE VENOM ENZYME WHICH CLOTS FIBRINOGEN. FS Markland,[1,2] E Shaw,[3] C Kettner,[3] S Schiffman,[1,4] SS Bajwa,[5] KNN Reddy,[1,2] H Kirakossian,[1,2] and H Pirkle. Departments of Biochemistry[1], Medicine [4] and Pathology[5] and Comprehensive Cancer Center[2], University of Southern California, School of Medicine, Los Angeles, CA, 90033, Biology Department[3], Brookhaven National Laboratory, Upton, NY, 11973 and University of California, Irvine, CA.

During amino acid sequence determination of crotalase, a thrombin-like enzyme from Crotalus adamanteus (eastern diamondback rattlesnake) venom, we found that in addition to the expected structural homology with bovine thrombin, there was even greater homology with porcine pancreatic kallikrein. In exploring further the similarity between crotalase and kallikrein, several completely unexpected and interesting observations were made. First, crotalase was rapidly and specifically inhibited by the tripeptide, affinity-labelling chloromethyl ketone inhibitor Pro-Phe-Arg-CH_2Cl, which is known to be a specific inhibitor of urinary kallikrein. Further, crotalase exhibits significant activity not only with the thrombin chromogenic substrate S-2238 (H-D-Phe-pipecolyl-Arg-p-nitroanilide) and the general serine protease substrate S-2160 (N-benzoyl-Phe-Val-Arg-p-nitroanilide) but also with the plasma kallikrein substrate S-2302 (H-D-Pro-Phe-Arg-p-nitroanilide) and the glandular kallikrein substrate S-2266 (H-D-Val-Leu-Arg-p-nitroanilide). Additionally, SDS polyacrylamide gel electrophoresis reveals that crotalase cleaves the plasma kallikrein susceptible bonds in human high molecular weight kiniogen (HMWK) producing intermediates with procoagulant activity. Analyses for bradykinin release from HMWK are presently in progress. Interestingly, one of the normal activities of plasma kallikrein, the activation of human plasminogen, was not one of the activities possessed by crotalase.

In summary, it would appear that crotalase has significant kallikrein-like activity. Whether this will prove to be of importance in the ongoing clinical application of fibrinogen clotting snake venom enzymes, such as ancrod and batroxobin, remains to be shown.