ON THE SPECIFICITY OF URINARY KALLICREINS*

Carlos R. Diniz, Anibal A. Pereira, Jader Barroso, Marcos Mares-Guia.

From the Departamento de Bioquímica, Faculdade de Medicina, Universidade de Minas Gerais, Belo Horizonte, Brazil.

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Kallicreins are tissue, plasma, or urine enzymes which liberate physiologically active peptides (kinins), from a globulin precursor (kininogen) that is prepared from plasma (see the first reference for a review). Trypsin (Rocha e Silva, Beraldo, Rosenfeld, 1949), snake venoms (Rocha e Silva et al., 1949; Deutsch, Diniz, 1955), and Nagarse (Prado, Prado, Jurkiewicz, 1964) are also able to liberate kinins from the same precursor. Kallicrein preparations have been shown to hydrolyze the methyl esters of benzoyl- and tosyl-L-arginine (Prado, Prado, 1961; Webster, Pierce, 1961), as well as to liberate bradykinin (L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-ArgOH), or kallidin (L-Lysyl-bradykinin) (Webster, Pierce, 1963) from a precursor that contains the sequence of another active peptide, L-Met-L-Lys-bradykinin (Elliott, Lewis, Smyth, 1963). The kallidin-releasing kallicreins seem, on one hand, to catalyze the hydrolysis of esters to the carboxyl group of N-alpha-acyl-L-arginine and, on the other hand, to catalyze the hydrolysis of a L-Met-L-Lys- peptide bond, two results that cannot be reconciled in view of the stringent specificity

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** Abbreviations used: BAEE: benzoyl-L-arginine ethyl ester: TAME: Tosyl-L-arginine methyl ester; TLCK: 1-chloro-3-tosylamido-7-amino-2-heptanone.

that the kallicreins apparently show.

The specificity properties of several kinin-releasing enzymes have been simply surveyed, according to a strategy that is now being developed, which consists in comparing the enzymes to a standard one of similar specificity requirements, trypsin in this case, through the use of small-molecular, exactly defined chemical compounds which are typical synthetic substrates, competitive inhibitors, such as benzamidine-HCl (Mares-Guia, Shaw, 1965), or irreversible inactivators, such as TLCK (Shaw, Mares-Guia, Cohen, 1965; Mares-Guia, Shaw, 1963) of the reference enzyme. In this way we hope to be able to study their mechanism of specificity, and its relation to their involvement in physiological processes.

Experimental:- Trypsin was a twice-crystallized, liophylized, salt-free preparation from Worthington Biochemical Corp, Lot ## 6223. Benzamidine-HCl.$2H_2O$ was a product from Aldrich Chemical Co. TLCK (1-chloro-3-tosyl-amido-7-amino-2-heptanone), was kindly supplied by Dr. Elliott Shaw*. Horse urine kallicrein was a purified preparation supplied by Dr. J.L. Prado. Rat urine extensively dialyzed against deionized water in the cold was used as source of kallicrein. Human urinary kallicrein was a partially purified preparation (Moriya, Pierce, Webster, 1963).

The methods for esterase and kinin-releasing activity determinations will be described in detail in a coming publication. In the experiments with TLCK the method used was similar to that described by Shaw, Mares-Guia, and Cohen (1965).

Results:- As the kinin-releasing enzymes catalyze the hydrolysis of typical synthetic trypsin substrates their susceptibility to benzamidine-HCl, a potent trypsin competitive inhibitor (Mares-Guia, Shaw, 1965) was also determined. From the data in Table I it is seen that benzamidine-HCl is able to inhibit the BAEE-esterase activity of

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TABLE I

The effect of benzamidine-HCl on the BAEE-esterase activity of kinin-releasing enzymes

(0.05 M TRIS, pH 8.0, 37.0ºC; initial rates in $M sec^{-1}$ $|mg\ protein/ml|^{-1}$)

ENZYME	BENZAMIDINE	RATE OF BAEE HYDROLYSIS	INHIBITION %
TRYPSIN	mM 0 3.92	1.15×10^{-3} 0.723×10^{-3}	 37.0
HORSE URINE KALLICREIN	0 1.52	1.88×10^{-4} 0.665×10^{-4}	 64.6
RAT URINE KALLICREIN	0 1.49 5.97	7.93×10^{-5} 5.06×10^{-5} 2.40×10^{-5}	 36.2 69.7
HUMAN URINE KALLICREIN	0 29.9	4.76×10^{-7} 0.90×10^{-7}	 81.1

TABLE II

The effect of benzamidine-HCl and TLCK on kinin-releasing enzymes

(0.2 M TRIS, pH 8.0, 37.0ºC; initial rates in ug bradykinin/min/mg protein)

	RAT URINE KALLICREIN	HORSE URINE KALLICREIN	HUMAN URINARY KALLICREIN
CONTROL	0.765	28.3	0.88
BENZAMIDINE-HCl	0.36[a]	20 [a]	0.80[a]
TLCK[b]	0.83	27.5	-

a- 8.85×10^{-4} M benzamidine-HCl;

b- TLCK in preincubations ranged from 0.5 to 1.0×10^{-3} M.

all enzyme preparations used. The rate of hydrolysis was expressed as $M sec^{-1}$ divided by the enzyme concentration in the incubation mixture, in mg/ml. The BAEE-esterase activity of human urinary kallicrein was always low, and one preparation lacked it completely, although its kinin-releasing activity was within the usually found values.

Experiments carried out with bradykininogen as substrate also show that benzamidine-HCl is able to inhibit the kinin-releasing activity of rat and horse urinary kallicreins (Table II). The human urine kallicrein preparation used in this set of experiments was devoid of BAEE-esterase activity and, as is evident from Table II, it was not affected by benzamidine-HCl. Even when a preparation containing BAEE-es-

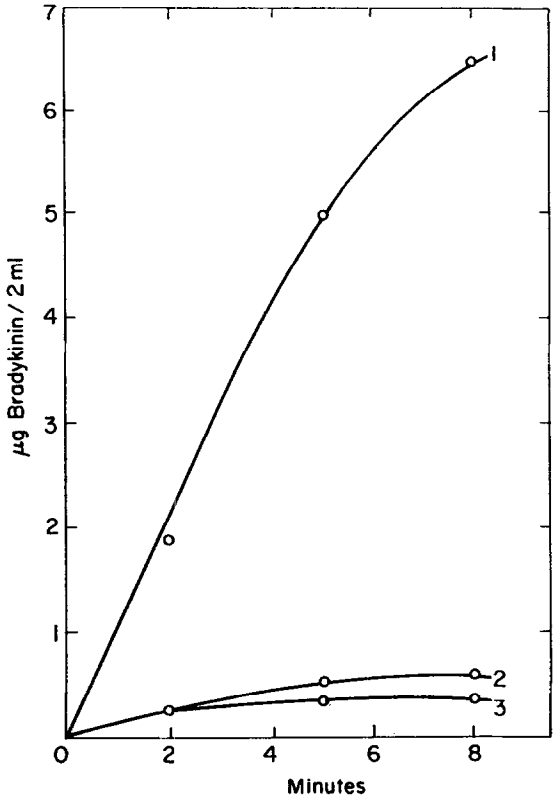

Fig. 1 - The effect of benzamidine-HCl and TLCK on the kinin-releasing activity of trypsin. TRIS, 0.2 M, pH 8.0, 37ºC; trypsin was 0.06 mg/ml. Curve 1: control; 2: trypsin + + 8.0×10^{-4} M benzamidine-HCl; 3: trypsin + + 1.0×10^{-3} M TLCK.

terase activity was used, no difference could be detected in the presence of benzamidine-HCl. When rat and horse urinary kallicreins were preincubated with TLCK at pH 8.0, no loss of PAEE-esterase activity was observed, even after one hour incubation at 37ºC. The kinin-releasing activities of the respective incubation mixtures were tested at the end of the experiment, and they were found essentially equal to the control experiments, which had no TLCK.

As shown in Fig. 1, the bradykinin-releasing activity of trypsin was inhibited by benzamidine-HCl, as expected. Included are also the data of a trypsin sample that had been 95% inactivated by reaction with TLCK, and whose kinin releasing activity was also reduced to the same extent.

Discussion:- The data in Table I indicate that the horse urine kallicrein BAEE-esterase activity is affected to a larger extent than the BAEE-esterase activity of the other kinin-releasing enzymes. Benzamidine-HCl inhibited both the BAEE-esterase and kinin-releasing activities of rat and horse urinary kallicreins, supporting the view that both are due to the same enzyme (Tables I and II). The BAEE- or TAME-esterase activity of human urinary kallicrein is very low, but in agreement with published values (Moriya, Pierce, Webster, 1963). However, we were able to show that it is not always present in the preparations, what indicates that the preparation described as human urinary kallicrein is indeed a mixture of two independent activities. This was tacitly demonstrated with a preparation entirely devoid of BAEE-esterase activity, but with the expected kinin-releasing activity, which was not affected by benzamidine-HCl. A comparison of the ability of benzamidine-HCl to inhibit the rat and horse urinary kinin-releasing activities shows the opposite effect observed with the BAEE-esterase activity. This is probably due to a higher affinity of the horse enzyme for bradykininogen.

An interesting result of these experiments is the finding that rat and horse urinary kallicreins, although hydrolyzing a typical trypsin substrate BAEE, and being inhibited by a competitive inhibitor of trypsin, benzamidine-HCl, are not at all affected by TLCK, a specific trypsin active-center reagent (Shaw, Mares-Guia, Cohen, 1965). This result will permit a clear-cut distinction between the trypsin-like kallicreins and trypsin. It also shows

that enzymes that have apparently identical specificity requirements might exhibit considerable differences in mechanism of action. A clear understanding of the lack of reaction of kallicreins with TLCK has not yet been reached, but the subject is now being actively pursued.

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