PHARMACOLOGICAL SIGNIFICANCES OF PEPTIDASE
AND PROTEINASE IN THE BRAIN
REPORT 3: SUBSTRATE SPECIFICITY AND AMINO ACID
COMPOSITION OF PARTIALLY PURIFIED ENZYME
INACTIVATING BRADYKININ IN RAT BRAIN

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We have already reported the existence and nature of an enzyme which inactivates bradykinin in rat brain (1). We also reported on its purification procedure from rat brain by ammonium sulfate precipitation and column chromatography. Although, in starch-gel electrophoresis, the final preparation did not migrate as a single band, it behaved as a homogeneous protein on analytical ultracentrifugation with a sedimentation constant of 4.55S (2). When bradykinin was incubated with this enzyme preparation, free arginine and phenylalanine were released (2). This paper deals with the substrate specificity and amino acid composition of this partially purified enzyme.

In the assay of the hydrolysis, the standard assay procedure was somewhat modified from the original ones (6-16): Incubations were carried out at 37°C using 0.04 M Tris-HCl (pH 7.6) buffer. Metal ions were omitted from the assay mixture, for using bradykinin as substrate, our enzyme preparation was inhibited by metal ions other than calcium (2).

TABLE 1. Substrate specificity.

No.	Substrate	Incubation time (min)	Concentration (mM)	Hydrolysis rate (mμ moles/mg prot./min)	Method
1	L-Leucine β-naphthylamide	120	0.25	0	(6)
2	Carbobenzoxy-glycyl-L-phenylalanine	120	20	2	(7)
3	Benzoyl-glycyl-L-lysine	5	1	0	(8)
4	Carbobenzoxy-L-glutamyl-L-tyrosine	120	20	3	(7)
5	Benzoyl-L-arginine amide	30	25	2	(9)
6	Glycyl-L-phenylalanine amide	30	25	2	(9)
7	Benzoyl-L-tyrosine ethyl ester	10	0.5	0	(10)
8	Carbobenzoxy-L-tyrosine <i>p</i> -nitrophenyl ester	10	0.001	0	(11)
9	Benzoyl-L-tyrosine <i>p</i> -nitroanilide	10	0.1	1	(12)
10	Benzoyl-L-arginine ethyl ester	5	1	0	(13, 14)
11	Tosyl-L-arginine methyl ester	10	1	0	(10)
12	Casein, denatured	30	0.5%	1	(15, 16)
13	Bovine hemoglobin, denatured	30	0.5%	7	(15, 16)
14	Bradykinin		0.002	30*	(2)

Hydrolysis of various substrates by purified kininase from rat brain at pH 7.6 in Tris-HCl buffer

* Hydrolysis rate measured by biological assay. Numbers in "Method" show the Reference number.

Amino acid analysis was performed in an automatic amino acid analyser (KLA-3B type, Hitachi Ltd.) by the method of Spackman et al. (3).

As shown in Table 1, denatured casein and bovine hemoglobin, trypsin- and chymotrypsin-type substrates such as substrate No. 7-11, were not hydrolysed to any appreciable extent. Moreover, we found previously (2), that ovomucoid trypsin inhibitor and TPCK (N-tosylphenylalanine chloromethylketone, a specific chymotrypsin inhibitor) had no effect on the enzyme activity. These facts suggest that the enzyme has not proteinase activity like trypsin and chymotrypsin. In the present experiment, hippuryl-L-lysine was not hydrolysed. Zinc and cobalt ions and EDTA also inhibited the enzyme activity. These results imply that our enzyme preparation differs from the carboxypeptidase B and carboxypeptidase N (4, 5). The analysis of amino acid composition of partially purified enzyme showed that the preparation contained much amount of aspartic acid (12%) and glutamic acid (14%) but little cystine (2%). Glutathione and cysteine inhibited our enzyme preparation and its activity was increased by SH-reagents (2). These observations suggest that the SH-residue plays an important role in the mechanism of the enzyme action.

To obtain an ultracentrifugally and electrophoretically homogeneous preparation, further purification are now in progress.

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INFLUENCE OF METHYLAMPHETAMINE ON BLOOD
LACTIC ACID FOLLOWING EXERCISE

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It has been shown by Weiss and Latices¹⁾ that amphetamines, besides being central stimulants, not only improve physical performance but also a motivation to perform the work better, and more so when fatigue is setting in. Prolonged exercise with the concomitant relative muscular anoxia causes accumulation of lactic acid in blood and tissues because its uptake by liver is slower than its rate of production by the muscle. Since amphetamines delayed fatigue, it looked desirable to study the effect of methylamphetamine on the