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Hydrolysis of Bradykinin and its Higher Homologues by Angiotensin-Converting Enzyme

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The hydrolysis of bradykinin and its higher homologues by angiotensin-converting enzyme has been investigated by using an automated ninhydrin technique. The results show an inverse relationship of hydrolysis rate with size and charge of the peptide, which parallels the inactivation in the pulmonary circulation and offers an explanation for the selectivity of metabolism of these kinins by the lungs.

Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is largely eliminated from the blood by a single passage through the pulmonary circulation (Ferreira & Vane, 1967). Its elimination is accounted for by hydrolytic degradation, bradykinin undergoing no fewer than five hydrolytic reactions during the 3–4s required to pass from the pulmonary artery to the pulmonary vein in the rat (Ryan *et al.*, 1968, 1970*a*). However, higher homologues of bradykinin, which contain the same eight peptide bonds, are not inactivated by intact lungs as quickly as is bradykinin itself. In fact, there is an inverse progression with size and charge such that Lys-bradykinin is inactivated at about one-half of the rate, Met-Lys-bradykinin is inactivated at one-tenth to one-twentieth of the rate, and Lys-Lys-bradykinin and Polistes kinin (an 18-residue homologue having bradykinin as its *C*-terminal moiety) are not inactivated at all (Ryan *et al.*, 1970*a*; Roblero *et al.*, 1973).

The apparent selectivity of metabolism of bradykinin and its homologues may be due to high orders of specificity of the relevant lung enzymes. Alternatively, the enzymes may be located within a cellular microenvironment such that circulating bradykinin has ready access, whereas the larger, more highly charged, kinins do not (Ryan *et al.*, 1970*a*; Smith & Ryan, 1970, 1973). It is also possible that the larger kinins assume conformations that 'protect' their bradykinin moieties. However, results obtained so far on solution conformations of the kinins offer little support for this last hypothesis (Brady *et al.*, 1971; Cann *et al.* 1973).

Recently there has been a convergence of evidence indicating that one of the enzymes likely to be involved in the pulmonary degradation of bradykinin is also capable of cleaving angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) to yield angiotensin II and the dipeptide His-Leu (Alabaster & Bakhle, 1972, 1973; Igic *et al.*, 1972; Dorer *et al.*, 1974). *In vitro*, the purified enzyme degrades bradykinin in two steps: first, releasing the *C*-terminal dipeptide Phe-Arg, and, secondly, releasing the new *C*-terminal dipeptide Ser-Pro (Dorer *et al.*, 1974). The reactions observed *in vitro* parallel those observed when intact lung is used since the venous effluent of lungs perfused with [[^{3}H]Phe^8]bradykinin contains radioactive Phe-Arg (Ryan *et al.*, 1970*a*). In a corresponding manner, radioactive His-Leu is found in the effluent of lungs perfused with [[^{14}C]Leu^{10}]angiotensin I (Ryan *et al.*, 1970*b*).

In the present study, we have examined the possibility that the selective metabolism of the kinins might be accounted for, in part, by the specificity of action of the pulmonary angiotensin-converting enzyme. The preparation of a homogeneous enzyme from hog lung has been described by Dorer *et al.* (1972). All of the peptides listed in Table 1 were synthesized by the solid-phase technique (Stewart & Young, 1969) and were purified by chromatography on Sephadex G-25 and/or countercurrent distribution. Peptide concentrations for reactions with the enzyme were calculated on the basis of peptide content as determined by quantitative amino acid analysis. Thus hydrates and salts were eliminated from consideration.

Rates of hydrolysis of the peptides by hog lung angiotensin-converting enzyme were measured by the automated quantitative ninhydrin technique described by Dorer *et al.* (1974). Each substrate was used at a concentration of 4μM except angiotensin I, which was at 10μM. Incubations were carried out at

Table 1. *Rates of hydrolysis of bradykinin and related peptides and angiotensin I by hog lung angiotensin-converting enzyme*

See the text for details.

Substrate	Concn. of chloride ...	Rate of hydrolysis (μmol of product/min per mg of enzyme) 0.0M	0.01M	0.1M
Bradykinin		0.41	1.10	0.71
Lys-bradykinin		0.18	0.73	0.66
Met-Lys-bradykinin		0.10	0.61	0.57
Lys-Lys-bradykinin		0.02*	0.14	0.22
Gly-Arg-Met-Lys-bradykinin		0.02*	0.05	0.10
Polistes kinin		0.02*	0.04	0.05
[D-Pro^7]Bradykinin		0.0	0.0	0.0
Angiotensin I		0.03	0.5	1.2

* Values of 0.02μmol/min per mg are at the limit of detection by this method.

37°C in 0.05M-sodium Hepes (*N*-2-hydroxyethylpiperazin-*N'*-2-ylethanesulphonic acid) buffer, pH7.5, with NaCl at the indicated concentration. It was assumed that the hydrolysis products in all cases were Phe-Arg or Phe-Arg and Ser-Pro; Phe-Arg and Ser-Pro have identical ninhydrin colour constants, 83% of that of leucine.

The results are shown in Table 1. As observed previously (Dorer *et al.*, 1974), the hydrolysis of bradykinin proceeds in the absence of chloride. Addition of chloride further increases the rate of hydrolysis with a maximum rate at 0.01M-NaCl. The hydrolysis of angiotensin I has a nearly absolute requirement for chloride (Dorer *et al.*, 1972), and the rate is at a maximum at 0.1M. The hydrolysis rates for Lys-bradykinin and Met-Lys-bradykinin are lower than that of bradykinin, but show a similar pattern of chloride activation with an optimum at 0.01M. Lys-Lys-bradykinin and the higher homologues are hydrolysed at much lower rates at all chloride concentrations and do not show the decrease in rate at 0.1M-NaCl. [D-Pro^7]Bradykinin, an analogue that retains some biological activity and that is not eliminated by the lungs (Roblero *et al.*, 1973), was not hydrolysed at any chloride concentration. This last observation parallels the finding by Oparil *et al.* (1973) that [D-Phe^8]angiotensin I is not hydrolysed by angiotensin-converting enzyme.

These findings suggest that part of the selectivity of processing of kinins by intact lungs may be accounted for by the specificity of action of a single enzyme. The selectivity of action of angiotensin-converting enzyme is surprising in view of the fact that the enzyme can hydrolyse a variety of peptides having few or no structural similarities to bradykinin. Thus angiotensin I, the B-chain of insulin, BPP_{5a} (pGlu-Lys-Trp-Ala-Pro), hippuryl-Gly-Gly and t-Boc-Phe(NO_2)-Phe-Gly are substrates (for review see Bakhle, 1974). It should be emphasized, however, that, since these substrates have not been tested under identical reaction conditions, one cannot compare reaction rates. Nonetheless, the most surprising feature of our results is that *N*-terminal substitutions impart such profound effects on the reactivity of the kinins at the *C*-terminus. Chain length and charge appear to be important factors.

Previous investigators have reported that the hydrolysis of angiotensin I requires chloride whereas the hydrolysis of bradykinin does not. Alabaster & Bakhle (1972, 1973) have taken this finding, along with the observation that angiotensin I and bradykinin are mutual competitive inhibitors, to suggest that the enzyme has two active sites with allosteric interactions, or alternatively to indicate that the enzyme has one active site alterable by chloride. In view of our finding that rates of hydrolysis of these kinins are influenced by chloride, the latter hypothesis would seem more attractive.

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Alabaster, V. A. & Bakhle, Y. S. (1972) *Circulation Res.* **30–31**, *Suppl.* **2**, 72–81

Alabaster, V. A. & Bakhle, Y. S. (1973) *Brit. J. Pharmacol.* **47**, 799–807

Bakhle, Y. S. (1974) in *Angiotensin* (Page, I. H. & Bumpus, F. M., eds.), pp. 41–80, Springer-Verlag, Heidelberg

Brady, A. H., Ryan, J. W. & Stewart, J. M. (1971) *Biochem. J.* **121**, 179–184

Cann, J. R., Stewart, J. M. & Matsueda, G. R. (1973) *Biochemistry* **12**, 3780–3788

Dorer, F. E., Kahn, J. R., Lentz, K. E., Levine, M. & Skeggs, L. T. (1972) *Circulation Res.* **31**, 356–366

Dorer, F. E., Kahn, J. R., Lentz, K. E., Levine, M. & Skeggs, L. T. (1974) *Circulation Res.* **34**, 824–827

Ferreira, S. H. & Vane, J. R. (1967) *Brit. J. Pharmacol.* **30**, 417–424

Igic, R., Erdos, E. G., Yeh, H. S. J., Sorrells, K. & Nakajima, T. (1972) *Circulation Res.* **30–31**, *Suppl.* **2**, 51–61

Oparil, S., Koerner, T., Tregear, G. W., Barnes, B. A. & Haber, E. (1973) *Circulation Res.* **32**, 415–423
Roblero, J., Ryan, J. W. & Stewart, J. M. (1973) *Res. Commun. Chem. Pathol. Pharmacol.* **6**, 207–212
Ryan, J. W., Roblero, J. & Stewart, J. M. (1968) *Biochem. J.* **110**, 795–797
Ryan, J. W., Roblero, J. & Stewart, J. M. (1970*a*) *Advan. Exp. Med. Biol.* **8**, 263–271
Ryan, J. W., Stewart, J. M., Leary, W. P. & Ledingham, J. G. (1970*b*) *Biochem. J.* **120**, 221–223
Smith, U. & Ryan, J. W. (1970) *Advan. Exp. Med. Biol.* **8**, 249–261
Smith, U. & Ryan, J. W. (1973) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **32**, 1957–1966
Stewart, J. M. & Young, J. D. (1969) *Solid Phase Peptide Synthesis*, W. H. Freeman, San Francisco