Short communications

Inhibition of angiotensin I converting enzyme by venom peptides

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The synthetic analogues of three naturally occurring peptides from the venom of *Bothrops jararaca* have been tested as inhibitors of angiotensin converting enzyme activity in two systems. They have widely differing absolute potencies but the relative potencies are similar in the two systems. The high inhibitory potency of the nonapeptide is compatible with a non-competitive type of inhibition.

The venom of *Bothrops jararaca* contains a mixture of peptides (bradykinin potentiating factor, BPF; Ferreira, 1965) which potentiate the actions of bradykinin *in vivo* and *in vitro* and which inhibit the peptidases inactivating bradykinin in tissues (Camargo & Graeff, 1969) and in blood (Ferreira, 1966). BPF also inhibits the peptidases catalysing the formation of angiotensin II from angiotensin I—'converting enzyme' (Bakhle, 1968; Bakhle, Reynard & Vane, 1969). BPF has been separated into its component peptides (Ferreira, Bartelt & Greene, 1970a) and several of these are potent inhibitors of converting enzyme (Ferreira, Greene, Alabaster, Bakhle & Vane, 1970b). Recently, the structure of some of these peptides has been established and their synthesis accomplished (Greene, Stewart & Ferreira, 1970; Ondetti, Williams, Sabo, Plusec, Weaver & Kocy, 1970). This report is concerned with the effects on angiotensin converting enzyme of the synthetic forms of three of these peptides from BPF; a pentapeptide, Pca-Lys-Trp-Ala-Pro; a nonapeptide, Pca-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro; and a decapeptide, Pca-Ser-Trp-Pro-Gly-Pro-Asn-Ile-Pro-Pro. The converting enzyme activity in two systems was studied: that in a particulate fraction from homogenates of dog lung, and that in the guinea-pig isolated lung perfused with Krebs bicarbonate solution.

Methods.—The preparation of converting enzyme from dog lung and its assay have been described (Ferreira *et al.*, 1970b). The concentration of substrate (angiotensin I) in all these experiments was 5 μg/ml (4×10^{-6}M). The venom peptides were dissolved in sterile saline and added to the incubation mixture before the substrate was added to start the reaction.

Perfused lungs. The isolated lungs of guinea-pigs were perfused at a rate of 10 ml/min via the pulmonary artery with Krebs bicarbonate solution at 37° C as described previously (Bakhle *et al.*, 1969). The effluent from the lung superfused a rat isolated colon and rectum arranged in series. The rat isolated rectum is less sensitive than the colon to prostaglandins (Bakhle, unpublished experiments). Both assay tissues received an infusion of a combination of antagonists (methysergide bimaleate, 200 ng/ml; phenoxybenzamine hydrochloride, 100 ng/ml; propranolol hydrochloride, 2·3 μg/ml; hyoscine hydrochloride, 100 ng/ml; mepyramine maleate, 140 ng/ml) at 0·1 ml/min in the Krebs superfusate. The conversion of angiotensin I to angiotensin II in the pulmonary circulation of the perfused lung was measured by comparing the contractions of the assay tissues to angiotensin I injected either into the pulmonary arterial cannula or directly to the assay tissues. The tissues were calibrated by injections of angiotensin II given directly. After conversion under control conditions had been measured, the venom peptides, dissolved in isotonic saline, were infused into the pulmonary arterial cannula at a rate of 0·1 ml/min and the conversion measured again during this infusion. Inflow pressure was monitored by a pressure transducer (S.E. Laboratories) connected via a T-piece to the arterial cannula, and never increased by more than 2 mmHg (1 mmHg≡1.333 mbar) during injections of angiotensin I or II into the pulmonary circulation. The contractions of the assay tissues were transduced via auxotonic levers attached to Harvard smooth muscle transducers and all responses displayed on a Watanabe multi-channel recorder. The composition of the Krebs bicarbonate solution was as follows: Na HCO_3, 25 mM; NaCl, 120 mM; KCl, 4·7 mM; $CaCl_2$, 2·5 mM; KH_2PO_4, 1·2 mM; $MgSO_4$, 1·2 mM; glucose, 5·6 mM. This solution was gassed with 95% O_2; 5% CO_2. Synthetic Ileu^5-

angiotensin I (Schwarz Biochemicals Ltd.) and the three synthetic venom peptides were generously provided by Drs. D. Cushman and M. Ondetti of the Squibb Institute for Medical Research (the penta-, nona- and deca-peptides have designations SQ 20, 475; 20, 881 and 20, 859 respectively). Angiotensin II was Asn^1-Val^5-angiotensin II (' Hypertensin ', Ciba).

Results.—The three synthetic venom peptides will be referred to in this report as P5, P9 and P10 (for the penta, nona- and deca-peptides, respectively). All three peptides inhibit the converting enzyme from dog lung and the results of these experiments are summarized in Fig. 1a. Each point is the average of two–four estimations. The concentration of peptide producing 50% inhibition of the reaction (ID50) was 0·025 μg/ml for P9, 0·5 μg/ml for P5 and 3·4 μg/ml for P10. The conversion of angiotensin I to angiotensin II by the isolated perfused lungs is also inhibited and Fig. 1b summarizes the results in this system. The points represent the mean (± standard error) of the results of four–six lungs using injections of 50 ng of angiotensin I in most cases. The contractions of the assay tissues in response to the injections of angiotensin II were unaffected by the infusion of the venom peptides. The ID50 values in these experiments were: P9, 0·009 μg/ml; P5, 0·18 μg/ml; P10, 0·8 μg/ml. The converting enzyme activity recovered rapidly (about 2–3 min) after an infusion of P5, but the inhibition by P9 and P10 persisted for a longer time (about 10–15 min) after the infusion of these peptides had been stopped.

Discussion.—The natural peptides corresponding to P5, P9 and P10 are the peptides V-3-A, IV-1-D and IV-1-A of the original separation procedure of Ferreira *et al.* (1970a) and their potency as inhibitors of converting enzyme from dog lung have been reported (Ferreira *et al.*, 1970b). For two pairs of peptides the ID50 values are in reasonable agreement: P5, 0·5 μg/ml; V-3-A, 0·8μg/ml; and P9, 0·025 μg/ml; IV-1-D, 0·02 μg/ml. The synthetic and natural deca-peptide, however, differ in potency: thus P10 has an ID50 of 3·4 μg/ml, almost 4 times that obtained for IV-1-A, 0·9 μg/ml. The explanation for this discrepancy may lie in the fact that the natural peptides tested were those prepared by Ferreira *et al.* (1970a) whereas the synthetic peptides used here were synthesized by Ondetti *et al.* (1970). The structure of P10 was elucidated by Ondetti *et al.*, and although it has the same amino-acid composition as the natural peptide IV-1-A, there may be differences in sequence between the two peptides. The experiments

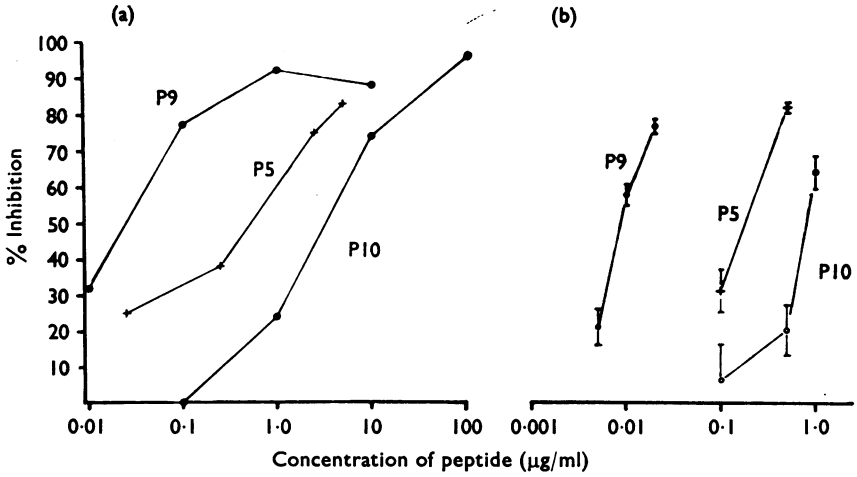

FIG. 1. Inhibition of angiotensin I converting enzyme by venom peptides.
(a) *In vitro* experiments. The percentage inhibition of the conversion of angiotensin I by extracts of dog lung is plotted against the concentration of peptide. Substrate concentration 5 μg/ml.
(b) Perfused lungs. The percentage inhibition of the conversion of angiotensin I in the pulmonary circulation of guinea-pig isolated lungs perfused with Krebs solution is plotted against the concentration of peptide infused through the lung. Standard injection of angiotensin I, 50–100 ng.

with the perfused lung are more expensive in terms of the amount of peptide used and the natural peptides were not tested in this system.

It is interesting that the order of inhibitory potency is the same in both systems and furthermore that the relative potencies of the peptides are similar despite differences in the absolute values of the ID50. Thus the ID50 for P5 is twenty times that for P9 in both systems although the absolute values differ by about threefold. The lower ID50 values measured in the perfused lung system are probably due to the lower amounts of angiotensin I used in this system, 50–100 ng compared with the 5 μg/ml used in the *in vitro* assay. P10 is the least potent peptide in both systems showing that size is not the only determinant of potency. A more critical requirement could be a basic amino-acid residue such as Lys in P5 or Arg in P9, and which is absent from P10.

However, from this study of only three peptides firm structure-activity relationships may not be deduced. What is interesting is the high potency of P9. With the *in vitro* assay, the ID50 value corresponds to about 2×10^{-8}M in the presence of 4×10^{-6}M substrate, and in the perfused lung an ID50 value of 9×10^{-9}M is reached with a substrate level (per injection) of 4×10^{-8} mol. It is not possible to decide from these data what type of inhibition the nona-peptide produces, but it is clear that the binding constant of P9 to the enzyme is large compared with that of the substrate. Cushman & Cheung (personal communication) have shown that the inhibition by P5 of a converting enzyme preparation from rabbit lung is of the non-competitive type when analysed by the method of Lineweaver and Burk, and the strong binding of P9 would be compatible with such a mode of action.

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