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A SUBSTRATE ANALOG INHIBITOR OF RENIN THAT IS EFFECTIVE IN VIVO

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SUMMARY

A potent inhibitor of primate renin effective in vivo has been synthesized. The peptide, Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys, is an analog of the amino acid sequence found between residues 6 and 13 of renin natural substrate (angiotensinogen). The inhibitor is eight times more soluble at physiologic pH and is cleared from circulation about two-orders of magnitude more slowly than earlier substrate analog inhibitors which were not active in vivo. Infusion of the inhibitor into the monkey (M. fascicularis) blocks the rise in mean arterial pressure caused by exogenous human renin, but not by angiotensin I or II. When injected into a salt-depleted monkey, the peptide lowers blood pressure in a dose-dependent manner about as effectively as the converting enzyme inhibitor, teprotide.

INTRODUCTION

The renin angiotensin system plays a central role in the maintenance of blood pressure. Various stimuli, including hypotension, cause the release of renin (E.C. 3.4.99.19) from the kidney. Renin cleaves circulating natural substrate (angiotensinogen) to generate angiotensin I which is then converted to angiotensin II by removal of the C-terminal dipeptide. Angiotensin II has many actions including vasoconstriction, renal sodium conservation, and stimulation of aldosterone release. The renin angiotensin system may be blocked with either angiotensin antagonists such as Saralasin (1) or converting enzyme inhibitors such as teprotide or Captopril (2). A practical renin inhibitor is lacking. Pepstatin and its soluble analogs (3,4) inhibit all acid proteases and do not specifically block renin.

Several in vitro inhibitors of renin based on the amino acid sequence around the cleavage site of natural substrate have been reported (5,6,7). Use of this sequence is attractive because the protein binds both specifically and tightly to renin (K_M 0.5 μM).

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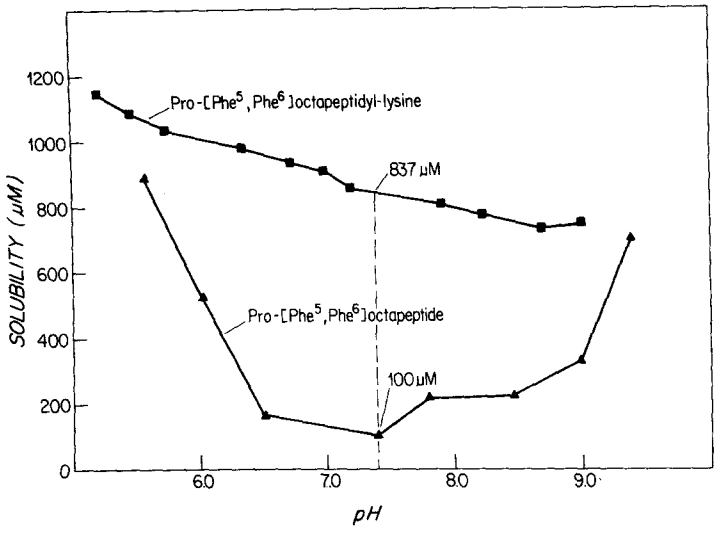


Figure 1: Solubility (µM) of substrate analog renin inhibitors as a function of pH at 20°.

Modifications of the minimal effective sequence, which is an octapeptide occurring between positions 6 and 13 (5), that increase binding and solubility have been reported (7). One peptide, Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr, strongly and specifically inhibits the generation of angiotensin I in human plasma but is ineffective at lowering blood pressure in vivo (8).

MATERIALS AND METHODS

All peptides were synthesized by solid phase techniques (7,9). On completion of the chemical synthesis, the peptides were deprotected with HF and purified by chromatography on Sephadex G-25 (M acetic acid) and Biogel P-2 (0.01 NHCl). The products are homogeneous by TLC (three systems), high voltage electrophoresis, and HPLC (10). Physical properties of the two peptides reported in this study are:

Pro-[Phe^5,Phe^6]octapeptide (Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr): Tyr, 0.99; Phe, 3.01; Val, 1.01; His, 2.05; Pro, 1.94 (13.3%). Specific activity [^{3}H] 1.08 Ci Mol^{-1} $[M]^{20°}_{589}$ nM, -815° (750 µM; 50% ethanol) ε_{280} nM, 906 (50% ethanol)

Pro-[Phe^5,Phe^6]octapeptidyl-lysine (Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys): Lys, 1.09; Tyr, 0.94; Val, 1.06; Phe, 2.83; His, 2.07; Pro, 2.01 (100%). Specific activity [^{3}H] 0.35 Ci Mol^{-1} ε_{280} nM, 940 (saline)

The inhibitory constants (Ki) are calculated from measurements of the decrease in the formation of angiotensin I from either tetradecapeptide or human natural substrate as outlined previously (7). Solubility of the peptide is measured by dissolving the peptide at low pH and adding NaOH until a precipitate forms. The suspension is centrifuged and an aliquot of the clear supernatant counted to determine peptide concentration at that pH. After resuspension of the precipitate, the pH is raised with more NaOH and the procedure repeated to give data shown in Figure 1. Half life of the peptide in circulation was obtained by withdrawing samples of blood from an indwelling catheter in the abdominal aorta of a male M. fascicularis after a bolus injection of the labeled peptide into the

inferior vena cava. Concentrations of peptide were determined by counting samples of plasma. High voltage electrophoresis of deproteinized plasma was used to show that the peptide was not degraded in the time studied. In vivo inhibition was measured in male M. fascicularis (4-6 kg) having chronic catheters in the inferior vena cava and aorta. The animals were fed regular chow (Purina) containing 1.05 mEq Na^+ $kg^{-1}day^{-1}$, supplemented with fruit and given water ad lib. Sodium depletion was achieved using a fruit for chow exchange and giving furosemide (1 mg $kg^{-1}day^{-1}$) for one week before the study. Sodium depletion was confirmed by urinalysis. The studies were performed on conscious, unanesthetized animals placed in a sitting position. Internal catheters were connected to Statham transducers interfaced with a Grass Multichannel recorder.

RESULTS AND DISCUSSION

Solubility of the in vitro inhibitor Pro-[Phe^5,Phe^6] octapeptide at various pH values is shown in Figure 1. Addition of a lysyl residue to this peptide both increases solubility (840 vs 100 μM, pH 7.4) and eliminates the broad minimum seen between pH 6-9. Presumably addition of the positive charge to the C-terminus of the inhibitor shifts the isoelectric point to a more alkaline pH.

Inhibitory constant (Ki) for the Pro-[Phe^5,Phe^6] octapeptide is 1 μM at pH 7.4. Addition of a lysyl residue increases this to 2 μM.

The practical effectiveness of an in vivo inhibitor is enhanced by increasing circulating half life. As shown in Figure 2, Pro-[Phe^5,Phe^6] octapeptide is cleared from circulation very rapidly with nonexponential kinetics. At 0.8 minutes after injection, more than 90% of this peptide has disappeared from circulation. By high voltage electrophoresis the radioactive label remaining in plasma was shown to be intact peptide. Because of the rapid disappearance, half life of the peptide cannot be calculated accurately but is probably less than 15 seconds. Addition of the lysyl-residue to the C-terminus increases half life in circulation to 3.78 minutes. In addition, the data can be fitted to a monoexponential expression with high accuracy ($r = 0.997$) which allows the circulating volume for the monkey to be calcualted as 454 mL. This is in excellent agreement with a value of 450 mL obtained on this monkey using labeled dextran (11).

Specificity of the renin inhibitors was tested by showing that the inhibitors would specifically block the rise in mean arterial pressure caused by administration of exogenous human renin, but not that caused by angiotensins I or II. The renin inhibitor thus does not interfer with either the angiotensin receptor or converting enzyme. Data summarized in Figure 3 show that the 22 Torr rise in pressure induced by administration of 0.5 GU

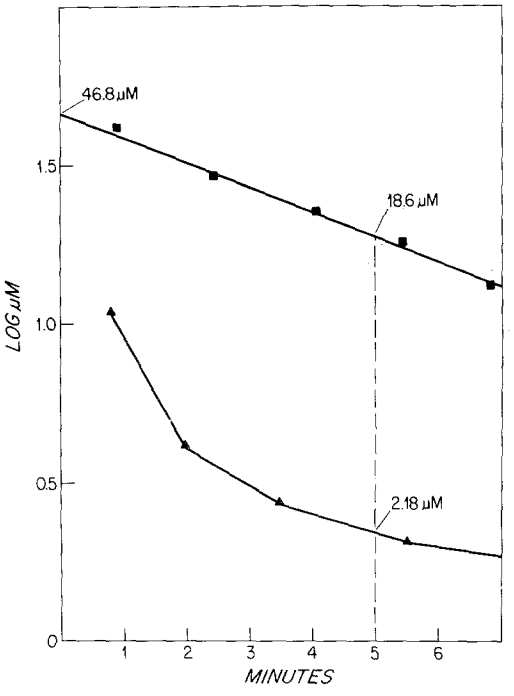
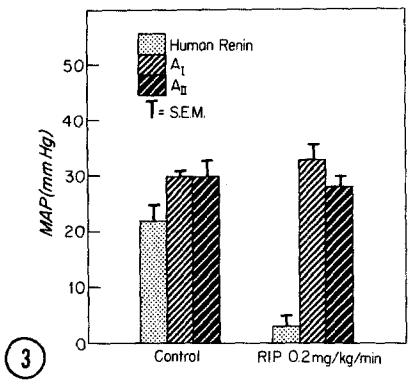


Figure 2: Concentration of substrate analog renin inhibitors in the plasma of M. fascicularis at various times after a single injection of 16.9 mg peptide in 0.9% N saline.

purified human renin to the salt replete monkey is reduced to less than 4 Torr by infusion (0.2 mg $kg^{-1}min^{-1}$) of Pro-[Phe^5,Phe^6] octapeptidyl-lysine. Rises in pressure of 28 Torr induced by administration of angiotensins I and II are unaffected by infusion of the renin inhibitor. Similar results are also obtained when Pro-[Phe^5,Phe^6] octapeptidyl lysine is infused at the higher dose of 0.6 mg $kg^{-1}min^{-1}$. Further evidence for specificity comes from the observation that the inhibitor does not affect pressure in the salt replete monkey.

Effect of the in vivo inhibitor on blood pressure of the salt depleted macaque is shown in Figure 4. A bolus injection of Pro-[Phe^5,Phe^6] octapeptidyl-lysine (2.0 mg kg^{-1}) shows mean arterial pressure drops on average from 103 to 74 Torr with a slow return to the first pressure over a 30 minute period. The converting enzyme inhibitor teprotide (12) causes a similar decline in pressure at doses of 1 mg kg^{-1}. In the normotensive salt replete monkey, pressure is not significantly changed by either drug.

A decapeptide analog of the amino acid sequence around the cleavage site of renin



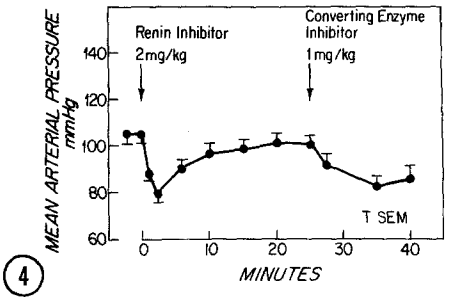


Figure 3: Pressor response to injections of 2.5 Goldblatt units human renin, 0.3 μg angiotensin I, and 0.3 μg angiotensin II in the salt-replete M. fascicularis in the presence and absence of a constant infusion of Pro-[Phe^5,Phe^6] octapeptidyl-lysine (0.2 mg kg^{-1} min^{-1}).

Figure 4: Mean arterial pressure in salt-depleted M. fascicularis given furosemide (1 mg $kg^{-1}day^{-1}$ for 1 week) after an injection of Pro- [Phe^5,Phe^6]octapeptidyl-lysine (2 mg kg^{-1}) in saline and following injection of 1 mg kg^{-1} converting enzyme inhibitor, teprotide. Mean arterial pressure is measured through an indwelling catheter in the abdominal aorta via a Statham transducer interfaced with a Grass multichannel recorder.

natural substrate has been shown to be a potent and specific inhibitor of renal renin which is effective in vivo.

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