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Communications to the Editor

A Uniquely Potent Renin Inhibitor and Its Unanticipated Plasma Binding Component

Sir:

The search for inhibitors of renin has intensified in recent years, fueled in part by an awareness of the key role of this enzyme in the blood pressure regulating renin-angiotensin cascade.[1] Numerous approaches to the design of renin inhibitors have been investigated, and their successful products can be classified by structure into three groups: (1) peptide analogues of segments of the natural substrate;[2-5] (2) peptides combining the substrate sequence with statine, the key residue of the naturally occurring inhibitor pepstatin;[6-9] (3) peptides having new chemical groups in place of the scissile bond of substrate.[10,11]

A common theme of these efforts has been an adherence to the peptidal structure of renin substrate, with nonpeptidal modifications focused at the site of substrate cleavage. This conservative approch to the substrate peptide can be justified by the reported high specificity of renin.[12] However, it may also limit the utility of the resulting compounds as drugs by burdening them with the liabilities common to many peptides, most notably metabolic instability.

In attempting to prepare therapeutically useful renin inhibitors, we have concentrated on altering this peptide backbone throughout its length, with the goal of producing small, potent, nonpeptidal renin inhibitors. We report here a singular achievement of this approach in the form of the pentapeptide analogue 1.

Compound 1 is a renin inhibitor of new structural type. It is an analogue of the tetrapeptide 2 in which the C-terminal amide bond has been reduced to the corresponding amine. This reduced analogue is the most potent renin inhibitor (K_I vs. human kidney renin = 2.6×10^{-11} M) yet reported. It is more than 3 orders of magnitude more potent than the precursor amide 2 and 200 times more potent than the peptidal lead structure for its class, compound 3.[13] The source of this exceptional potency is not obvious when examined against the background of previously reported renin inhibitors.

In addition to its unique potency, compound 1 showed what appeared on the surface to be a high specificity for purified human kidney renin, with a markedly reduced ($10^4\times$) potency vs. the corresponding hog enzyme. However, it showed a reduced ($10^5\times$) potency vs. human renin as well when measured in the commonly used whole plasma assays. Addition of plasma to the purified kidney enzyme assay also greatly diminished potency in that system, leading us to believe that some unknown complicating factor was at work, perhaps a binding to some plasma component or degradation by plasma proteases.

Further investigation revealed that 1, rendered undetectable (<1%, HPLC) in solution by addition of human plasma, could be completely (98%) regenerated by precipitation of the plasma protein with acetone. When the 1–plasma mixture was first incubated under the conditions of the human kidney renin assay (37 °C, 64 min), the outcome was virtually identical (97% recovery). These results show that 1 is bound effectively by some unidentified plasma component and that it survives intact in this form at least for the duration of the incubation experiment. The affinity of this binding is sufficient to make the inhibitor undetectable by HPLC.

Another potent renin inhibitor, one that did not show the large potency discrepancy between the purified human kidney renin and crude human plasma renin assays, was used as a control. This compound, 4, remained almost fully (87%, HPLC) detectable in solution in the presence of plasma.

The plasma binding was observed subsequently in a number of these smaller renin inhibitors. One of these, compound 5, was found to be only weakly bound (K_d = 3.8×10^{-4} M) to fatty acid free bovine serum albumin, suggesting albumin is not the binding component in question. The molecular features determining plasma binding efficiency remain unclear. The binding may be

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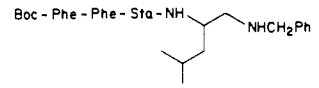
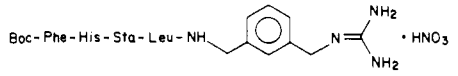
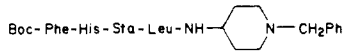
Table I. Potencies of Renin Inhibitors

no.		K_I, nM: purified human kidney renin[a]	I_{50}, nM: human plasma renin[b]	I_{50}, nM: hog kidney renin[b]
1		0.026 ± 0.008	37% I; ≈1000 nM	858 (731–1050)
2	Boc-Phe-Phe-Sta-Leu-$NHCH_2Ph$	43 ± 8.6	1900 (1750–2100)	18 (17.1–18.8)
3	Boc-Phe-Phe-Sta-Leu-Phe-NH_2	4.8 ± 1.0		88 (83.0–93.4)
4		3.6 ± 1.1	9.0 (7.4–10.1)	19 (17.4–21.2)
5		0.064 ± 0.020	25 (6.2–100)	269 (244–296)

[a] Values from triplicate determinations, with standard deviation. Values from duplicate or triplicate determinations, with 95% confidence limit.

related in part to the high lipophilicity of compounds such as the well-bound 1 since, in at least some cases, the effect appears to be modulated by the addition of hydrophilic functionality (cf. 4). A detailed account of these studies will be reported elsewhere.

The dramatic effect of plasma binding on the apparent potency of 1 as a renin inhibitor underscores the importance of enzyme purity in such studies. Against the crude human renin preparation, 1 appeared to be simply a weak inhibitor. It was only the high purity of the enzyme used in the human kidney renin assay that unmasked the unique potency of 1, leading in turn to discovery of the heretofore unsuspected plasma binding component. Whether the reduced potency seen in the hog renin assay was due to an actual reduction in enzyme affinity or a binding to impurities in this crude enzyme preparation was not established.

All peptides were prepared by coupling N^{α}-Boc amino acids to the appropriate amino terminal segment using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HBT) in dimethylformamide. N-Deprotection, where necessary, was carried out with HCl(g) in ethyl acetate at 0 °C. For preparation of compound 1, N^{α}-Boc-Leu-benzylamide was reduced with borane–THF according to the procedure of Roeske and co-workers.[14] The resulting amide was N-deprotected and further coupled as above. Extraneous coupling to the secondary amine was not significant. All final products were >95% pure by HPLC and exhibited NMR spectra consistent with the assigned structures; all gave satisfactory elemental and/or amino acid analyses. Several intermediates in the synthesis of 1 were additionally characterized by satisfactory NMR and mass spectra.

Compound 1 (0.13 mM) in reconstituted human plasma was not observed (<1%) by HPLC either at zero time or after incubation at 37 °C for 64 min. However, after precipitation of the plasma by an equal volume of acetone, 1 was observed in the supernatant at zero time (98%) and after incubation for 64 min (97%).

Compound 4 (0.12 mM) in reconstituted human plasma was observed by HPLC at zero time (87%) and after incubation at 37 °C for 64 min (83%).

For the human kidney renin assay, pure human kidney renin was prepared according to Slater and Strout.[15] The fluorimetric assay of Poe et al.[16] was used, measuring cleavage for 16–24 h of 5700 nM synthetic tetradecapeptide renin substrate at 37 °C and pH 7.2 (0.1 M citrate phosphate) by 0.5 ng/mL (10 pM) human kidney renin. Experimental data were fit to a standard competitive inhibition equation with no correction for renin-bound inhibitor.[16] Results are summarized in Table I. The K_I value obtained for 1 as an inhibitor of the cleavage of hog angiotensinogen (Sigma) by the purified human renin was identical within experimental error to the value reported in the table for inhibition of the human kidney enzyme acting upon the tetradecapeptide substrate.

The hog renin assay measured the inhibitory potency of peptides vs. hog kidney renin in accordance with the procedure of Rich et al.,[17] except that pH 7.3 was used. The results of the assay, presented in the table, are expressed as I_{50} values, which refer to the concentration of inhibitor necessary to produce 50% inhibition of renin activity. This I_{50} value was obtained typically by plotting data from four inhibitor concentrations. Pepstatin was used as an active control.

The human plasma renin assay was carried out as described in Boger et al.[7]

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N-(1,3,4,6,7,12b-Hexahydro-2*H*-benzo[*b*]furo[2,3-*a*]quinolizin-2-yl)-*N*-methyl-2-hydroxyethanesulfonamide: A Potent and Selective α_2-Adrenoceptor Antagonist

Sir:

α-Adrenoceptors exist as two distinct subclasses that have been characterized both pharmacologically and functionally. The evidence for this classification has been

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