ANGIOTENSIN I [Phe^8-His^9] HYDROLASE - STUDIES WITH RENIN SUBSTRATES*

David Boaz, Stephen Wyatt, and Annette Fitz

Department of Internal Medicine, University of Iowa and
Veterans Administration Hospital, Iowa City, Iowa 52240

Received January 16,1975

SUMMARY

Angiotensin I [Phe^8-His^9] hydrolase produced a pressor substance (probably angiotensin II) from synthetic tetradecapeptide renin substrate but not from human renin substrate. The activity of angiotensin I [Phe^8-His^9] hydrolase was effectively inhibited by cupric ion. Tetradecapeptide renin substrate was also shown to competitively inhibit angiotensin I hydrolysis by angiotensin I [Phe^8-His^9] hydrolase.

INTRODUCTION

Boucher et al. have recently published studies of an atypical angiotensin I converting enzyme, which they have called tonin (1,2). The enzyme was extracted from rat submaxillary gland, but is believed to be present in other tissues as well. It was active in liberating angiotensin II from the parent angiotensin I molecule, but unlike previously reported enzymes it did not require chloride ion for activity, and was not inhibited by EDTA. An additional interesting property was its ability to transform synthetic tetradecapeptide renin substrate (TDP)* into angiotensin II, without initial formation of angiotensin I. The authors suggested a possible role for this enzyme in the regulation of tissue blood flow.

We have previously reported an atypical angiotensin I converting enzyme from human lung (3,4). We have more recently suggested this en-

---

* Supported by National Institutes of Health Grant #GMB-2-RO1-HL-12684, and by the Veterans Administration Hospital, Research and Education Division.

TDP = tetradecapeptide renin substrate
APHH = angiotensin I [Phe^8-His^9] hydrolase

zyme be termed angiotensin I [Phe^8-His^9] hydrolase (APHH)*. Although the apparent molecular weight of this enzyme is much larger (5) than that reported by Boucher, it shares many characteristics with "tonin." We wished, therefore, to determine its effect on TDP, and on natural human renin substrate extracted from plasma.

METHODS AND RESULTS

The extraction and purification of angiotensin I [Phe^8-His^9] hydrolase (APHH) has been previously reported in detail (3,4). Human cadaveric lung obtained at autopsy was cleared of blood with isotonic saline, homogenized in 0.25 M sucrose, and fractionally precipitated three times with ammonium sulfate between 25% and 60% of saturation. Further purification was accomplished by ultracentrifugation at 75,000 x g followed by application to a Sephadex G-200 descending flow column or to an ascending Sepharose 6-B column (2.5 x 100 cm). Enzyme fractions were eluted with 0.02 molar phosphate - 0.0315% sodium chloride buffer; pH 6.9. Two active APHH fractions were obtained from the columns. The molecular weights of these fractions were approximately 450,000 from the G-200 column and 600,000 and 450,000 from the Sepharose 6-B column.

Purified enzyme fractions were assayed for ability to form angiotensin II from angiotensin I by incubation of APHH aliquots (0.05 ml) for 60 minutes with 0.025 ml (2.0 nMole) [^{3}H]angiotensin I (New England Nuclear 250 mC/mM) and 0.75 ml of 0.02 M phosphate - 0.315% sodium chloride buffer (pH 6.9). Incubates were chromatographed on silica gel impregnated paper or were analyzed by high-voltage paper electrophoresis. APHH activity was calculated as the percentage of the total radioactivity which appeared in the histidylleucine position. The 450,000 $M\tilde{w}$ APHH fraction obtained from Sephadex G-200 gel filtration, and the 600,000 and 450,000 $M\tilde{w}$ fractions obtained from the Sepharose 6-B gel filtration, converted 35% to 50% of the angiotensin I to angiotensin II (3,4).

Biological activation of angiotensin I to angiotensin II by the

lung APHH fractions was demonstrated by assay of the incubate on superfused rat colon. The enzyme fractions were also shown to be free of angiotensinase activity by incubation with [^{14}C]angiotensin II followed by high-voltage electrophoresis.

Both the 600,000 $M\bar{w}$ and 450,000 $M\bar{w}$ APHH fractions were chemically assayed for their effect on synthetic [^{14}C] TDP. APHH (0.1 ml) was incubated for 60 minutes with 0.025 ml (1.25 nMole) of [^{14}C] TDP (Schwarz/Mann 40 mC/mM) and 0.1 ml of 0.02 molar phosphate - 0.0315% sodium chloride buffer. The incubation was stopped by quick freezing in dry ice. Alteration of the TDP by the action of APHH was measured by high-voltage paper electrophoresis and scintillation spectrometry. Quantitation of the end products was accomplished by comparison of the counts occurring in the TDP peak to all other counts on the electrophoresis strip. Most of the counts on the electrophoresis strip other than those appearing in the TDP peak migrated to the same position as angiotensin II. Incubations of TDP with the 600,000 $M\bar{w}$ APHH fraction resulted in modification of 61% of the TDP; 51% of the TDP was altered in incubations with the 450,000 $M\bar{w}$ APHH.

To test the possibility that the product of the reaction of TDP and APHH was angiotensin II, incubations of APHH and unlabeled TDP were carried out. The biological activity of the APHH - TDP incubation was then confirmed by application on a superfused rat colon (Figure 1). Since angiotensin I, produced by the action of renin on TDP does not constrict the superfused rat colon, this incubation was also performed, and the incubate applied to the superfused rat colon. No constrictor material was present in this incubate. This data suggests that the constrictor material produced by the APHH - TDP incubation is angiotensin II.

Boucher has indicated that "tonin" forms angiotensin II when incubated with TDP, but is inactive when incubated with human renin substrate. The reactivity of the 450,000 and 600,000 $M\bar{w}$ APHH fractions toward human

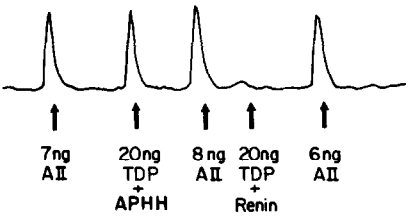

Fig. 1. Activation of tetradecapeptide renin substrate by angiotensin I [Phe^{8}-His^{9}] hydrolase. Incubation of 20 ng of TDP with APHH resulted in constrictor response equal to 7 ng of angiotensin II as measured on superfused rat colon. Incubation of 20 ng of TDP with human renin resulted in no detectable constrictor response.

---

renin substrate and TDP was studied. Human renin substrate was prepared by the method of Haas et al. (6) and when incubated with human renin was shown to produce the equivalent of 480 ng angiotensin II per ml after one hour as measured by the rat blood pressure bioassay. A one-hour incubation of renin with TDP (2 ug/ml) produced 390 ng of angiotensin II per ml. A similar incubation of the 600,000 APHH fraction with tetradecapeptide (2 ug/ml) produced the equivalent of 240 ng/ml angiotensin II. Similar results were obtained utilizing the 450,000 $M\bar{w}$ fraction. However, when the APHH fractions were incubated with human renin substrate, no angiotensin II or other pressor material was produced.

Preincubation of APHH with cupric chloride 1×10^{-4} M resulted in a 21% reduction in the amount of histidylleucine produced, as compared to control incubations of APHH and [^{3}H]angiotensin I without cupric chloride. Since cupric ion has also been reported to inhibit the activity of "tonin" a study was carried out to determine if cupric ion inhibited the APHH-TDP reaction. Incubation of 600,000 $M\bar{w}$ APHH and TDP in the presence of 1mM cupric chloride produced only 13% as much pressor material as APHH and TDP without added cupric ion.

Competitive inhibition of the APHH - angiotensin I reaction by tetradecapeptide was studied at TDP concentrations of 1mM and 0.1 mM. In control incubations of [^{3}H]angiotensin I and APHH, without added TDP,

54-60% of the angiotensin I was activated to angiotensin II. The addition of unlabeled TDP to this mixture resulted in a 65-89% decrease in the conversion of angiotensin I by APHH at 1mM TDP and a 42-58% decrease at 0.1 mM TDP.

DISCUSSION

Many reports have been published in recent years regarding studies of angiotensin I converting enzyme from both plasma and lung. While most of the enzymes studied to date have been shown to possess very similar characteristics (7), some exceptions have been noted. Two such exceptions are the beta converting enzyme or "tonin" from rat submaxillary gland (1,2) and converting enzyme or APHH from human lung previously reported from this laboratory (3,4). Neither "tonin" nor the lung APHH enzymes are inhibited by EDTA nor do they require chloride ion for activity. In addition, tonin has been reported (2) to produce angiotensin II directly from synthetic tetradecapeptide renin substrate but not from human renin substrate. Tonin activity was also inhibited by cupric ion and by plasma. The APHH fractions, in addition to their ability to convert angiotensin I to angiotensin II, were shown to produce a pressor material (probably angiotensin II) when incubated with synthetic tetradecapeptide renin substrate but not with human renin substrate. The activity of APHH toward both angiotensin I and TDP was effectively inhibited by cupric ion. In addition, TDP was shown to competitively inhibit the hydrolysis of angiotensin I by APHH. These data indicate that human lung APHH and tonin have very similar properties, and may perform the same function. One striking difference between these enzymes, however, is that tonin is reported to have a molecular weight of 31,400 (2) while human lung APHH is much larger in size (4,5).

It has been suggested that APHH may function to control angiotensin II production at local tissue sites, thus controlling blood flow and/or other hormone levels to these tissues. The exact role of human lung APHH

is as yet unclear, however, and awaits further study, particularly to determine the subcellular location of the enzyme and its activity toward other substrates.

REFERENCES

1. Boucher, R., Saidi, M., and Genest, J. (1972) Hypertension 1972, pp. 512-523, Springer-Verlag, New York.
2. Boucher, R., Asselin, J., and Genest, J. (1974) Supp. I to Circ. Res., 34 and 35, I-203-209.
3. Fitz, A., and Overturf, M. (1972) Hypertension 1972, pp. 507-511, Springer-Verlag, New York.
4. Boaz, D., Wyatt, S., and Fitz, A. (1974) Fed. Proc. 33, 1234.
5. Fitz, A., and Overturf, M. (1972) The Jl. Biol. Chem. 247, 581-584.
6. Haas, E., Goldblatt, H., Gipson, E.C., and Lewis, L. (1966) Circ. Res. 19, 739-749.
7. Bakhle, Y.S. (1973) Handbook of Experimental Pharmacology, pp. 41-80, Springer-Verlag, New York.