Synthesis and Properties of Angiotonin

By F. Merlin Bumpus, Ph.D., Hans Schwarz, Ph.D., and Irvine H. Page, M.D.

Angiotonin has been shown to be formed by a converting enzyme in blood by the splitting off of the 2 amino acids histidine and leucine from the inactive decapeptide (asp-arg-val-tyr-ileu-his-prol-phe-his-leuc). The synthesis of the active octapeptide is described and its pharmacologic resemblance to the natural product is demonstrated.

Angiotonin (hypertensin) was discovered in 1939, but it was not until 1956 that its structure was reported. In 1954 we published an amino acid analysis and a determination of the end amino acids of angiotonin. We know now that the preparation used was about 75 per cent pure, which accounts for the additional amino acids reported; the end amino acids were later shown to be correct.

During the purification of hog angiotonin, it was assayed on two separate preparations. One of these, the intact animal measured pressor activity only; the other, the rat’s uterus, measured oxytocic activity.

In figure 1 is shown what we thought to be two separate factors, a pressor and an oxytocic-pressor substance. The material shown by the large peak has predominantly pressor activity with very little ability to stimulate smooth muscle. The second peak indicates a substance with high oxytocic and high pressor activity. In several preparations we have noted a third pressor substance, but always in small amounts. Numerous angiotonins are possible in a preparation made from proteolytic enzymes.

In figure 2 is shown the structure of angiotonin. The complete structure of the decapeptide isolated from beef as first demonstrated by Elliott and Peart has isoleucine replaced by valine. The structures of horse decapeptide and hog angiotonin determined in our laboratory are identical and are shown in this figure. The structure of angiotonin octapeptide3 (hypertensin II) and the point of attack by the converting enzyme are shown.

We inferred the structure of the oxytocicpressor principle to be the same as that of hypertensin II and consequently carried out the synthesis of the octapeptide to prove it. Angiotonin contains 3 amino acids with multiple reactive groups: histidine and arginine with 2 basic groups and aspartic acid with 2 acid groups. Histidine peptides can be made without protecting the imidazole group, but a nitro group must be added to the guanidine group of arginine to prevent further reaction. The beta-carboxyl group of aspartic acid had also to be esterified to prevent further reaction.

Because these protecting groups were removed by hydrogenolysis or hydrolysis, it was necessary to add these two amino acids as a dipeptide at the last step. It was first thought that the hexapeptide should be made from the 2 tripeptides: val-tyr-ileu and his-prol-phe, however, the yields during the preparation of the latter were so low that a new approach was needed.

From the Research Division, Cleveland Clinic Foundation, and the Frank E. Bunts Educational Institute, Cleveland, Ohio.
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Fig. 2. The structural formula of angiotonin derived from action of hog renin on horse and hog substrates. Note point of attack of enzyme changing the decapeptide (angiotonin I) from the inactive form to the octapeptide (angiotonin II) which possesses oxytocic-pressor activity.

METHODS

Initially the four dipeptides were made and condensed in the following order:

1. First cbzo-val-tyr-me ester was formed by the mixed anhydride method with 74 per cent yield. This was easily transformed into the hydrazide by heating in alcoholic hydrazine and finally to the azide with an over-all yield of 80 per cent:

\[
\begin{align*}
\text{cbzo-val} & \rightarrow \text{tyr-me} \\
74 \text{ per cent} & \rightarrow \text{EtOCOC(} \text{Bu})_3N \text{H}_2\text{NNH}_2 \rightarrow \text{H}_2\text{NNH}_2 \\
\text{cbzo-val-tyr-me} & \rightarrow \text{EtOCOC}(\text{Bu})_3N \\
80 \text{ per cent} & \rightarrow \text{[(1) } \text{H}_2\text{NNH}_2 \text{]} \\
\text{cbzo-val-tyr - } N_3 & \rightarrow \text{[(2) HONO]} \\
\end{align*}
\]

2. The condensation of cbzo-ileu with his-me ester was also carried out by the mixed anhydride method, but in a lower yield than that for the previous dipeptide. This was obtained as the crystalline hydrochloride. The yield was increased, however, by recovering the unreacted cbzo-isoleucine and recondensing it with more histidine methyl ester:

\[
\begin{align*}
\text{cbzo-ileu} & \rightarrow \text{his-me} \\
58 \text{ per cent} & \rightarrow \text{EtOCOC(} \text{Bu})_3N \rightarrow \text{cbzo-ileu-his-me-HCl} \\
\text{cbzo-ileu-his-me-HCl} & \rightarrow \text{Pd (charcoal)} \\
\text{ileu-his-me-HCl} & \rightarrow \text{HONO} \\
\end{align*}
\]

3. The tetrapeptide cbzo-val-tyr-ileu-his-me ester was made from the dipeptide acids derived from steps 1 and 2 and ileu-his-me ester. This ester was insoluble in solvents except dimethyl formamide; the azide prepared from it was too in-
soluble in all solvents to be used for condensations and because of this another means of condensation had to be used:

\[
\begin{align*}
\text{cbzo-val-tyr-N}_3 & \quad \text{ileu-his-me} \\
75 \text{ per cent} & \quad \downarrow \\
\text{cbzo-val-tyr-ileu-his-me} & \quad \text{NaOH} \\
\text{cbzo-val-tyr-ileu-his} & \\
\end{align*}
\]

4. Cbz-proline was condensed with phenylalanine methyl ester by the mixed anhydride method to yield cbzo-prol-phe-me ester. This could not be crystallized. After removal of both protecting groups the free dipeptide was crystallized easily and the crystalline prol-phe-me ester-HCl was obtained by re-esterifying with methanol and thionyl chloride. The over-all yield through all steps was 56 per cent:

\[
\begin{align*}
\text{cbzo-prol} & \quad \text{phe-me} \\
\downarrow & \quad \text{EtOCOCl} \\
\text{cbzo-prol-phe-me} & \quad (\text{Bu})_4\text{N} \\
(1) & \quad \text{Hydrolize} \\
(2) & \quad \text{Pd (charcoal)} \text{ H}_2 \\
(3) & \quad \text{Re-esterify} \\
\text{prol-phe-me-HCl} & \\
\end{align*}
\]

5. The tetrapeptide free acid was condensed with prol-phe-me ester by the amide modification of the diethylchlorophosphite method. This hexapeptide was reprecipitated from acetone to give an analytically pure sample.

\[
\begin{align*}
\text{cbzo-val-tyr-ileu-his} & \quad \text{62 per cent} \\
\text{prol-phe-me} & \quad (\text{EtO})_2\text{POCl} \\
\downarrow & \quad (\text{Et})_2\text{N} \\
\text{cbzo-val-tyr-ileu-his-prol-phe-me} & \quad \text{H}_2 \quad \text{Pd} \\
\text{val-tyr-ileu-his-prol-phe-me} & \\
\end{align*}
\]

6. Cbz-\(\beta\)-me-aspartic acid was condensed with nitroarginine to yield crystalline cbzo-\(\beta\)-me-asp-\(\text{NO}_2\)-arg. This dipeptide was condensed with the reduced hexapeptide shown in step 5 by the mixed anhydride procedure. The carboxbenzoxyl-nitro-

angiotonin dimethyl ester thus obtained was difficult to purify, however, after removal of the 2 ester groups the resulting dibasic acid was easier to purify by reprecipitation. Reduction of this acid in the presence of palladium black catalyst yielded a biologically active preparation. Hydrolysis and chromatography showed 8 amino acids present in almost equal quantities:

\[
\begin{align*}
\text{cbzo-\(\beta\)-me-asp} & \quad \text{\(\text{NO}_2\)-arg} \\
\downarrow & \quad \text{EtOCOCl} \\
\text{cbzo-\(\beta\)-me-asp-\(\text{NO}_2\)-arg} & \quad (\text{Bu})_4\text{N} \\
\text{hexapeptide-me} & \\
\downarrow & \quad \text{EtOCOCl} \\
\text{cbzo-\(\beta\)-me-asp-\(\text{NO}_2\)-arg-val-tyr-ileu-his-prol-phe-me} & \quad (\text{Bu})_4\text{N} \\
(1) & \quad \text{NaOH} \\
(2) & \quad \text{H}_2 + \text{Pd} \\
\text{angiotonin (octapeptide)} & \\
\end{align*}
\]

Fig. 3. Pressor responses in dog under pentobarbital anesthesia to intravenously injected noradrenaline (5 \(\mu\)g.), natural and synthetic angiotonin and serotonin (60 \(\mu\)g.). (Reprinted from Science 125: 886, 1957.)
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Results

In figure 3 are shown responses in a dog to both natural and synthetic angiotonin. The response curves are almost identical and are significantly different from that of noradrenaline and serotonin.

When assayed against noradrenaline, synthetic angiotonin is 4 to 8 times as active by weight. It has a specific activity of more than 50,000 units per mg. nitrogen, which is similar to that of the natural material. It also possesses strong uterine stimulating activity. This evidence suggests that the synthetic octapeptide is identical to the oxytocic-pressor principle angiotonin or hypertensin II.

The asparaginyl octapeptide corresponding to angiotonin octapeptide has been synthesized and reported to have a similar pressor activity, but no data on its oxytocic activity has been reported.

REFERENCES

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F. MERLIN BUMPUS, HANS SCHWARZ and IRVINE H. PAGE

Circulation. 1958;17:664-667
doi: 10.1161/01.CIR.17.4.664
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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