THE HORMONE ANGIOTENSIN: A CHEMICAL AND PHARMACOLOGIC SURVEY

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WITH diseases of multiple origin, such as hypertension, much time is often required for a detailed study of only one facet of the entire problem. Our interests have been broadly directed toward hypertensive disease originating with the kidney, more specifically toward the study of the renin-angiotensin pressor system, and its relationship to normal and abnormal blood pressure.

Reduction of blood flow through the kidney, as illustrated by a Goldblatt clamp on the renal artery,[1] can lead to the release of the enzyme renin by the kidney. In the blood, renin reacts with an α-2 globulin, called renin-substrate, to liberate a decapeptide angiotensin I. Neither renin nor angiotensin I is a pressor agent. However, an enzyme present in blood rapidly converts angiotensin I to an octapeptide, angiotensin II, which is the most potent pressor substance known. This process is summarized by the chemical reactions indicated in *Figure 1.*

The sequence of the first 14 amino acids in renin-substrate protein was determined by Skeggs and his group[2] in 1957. Angiotensins I and II from horse blood were shown by Skeggs, Lentz, Kahn, Shumway, and Woods[3] to have the sequence shown in *Figure 1.* Angiotensin I from beef blood has the same sequence of

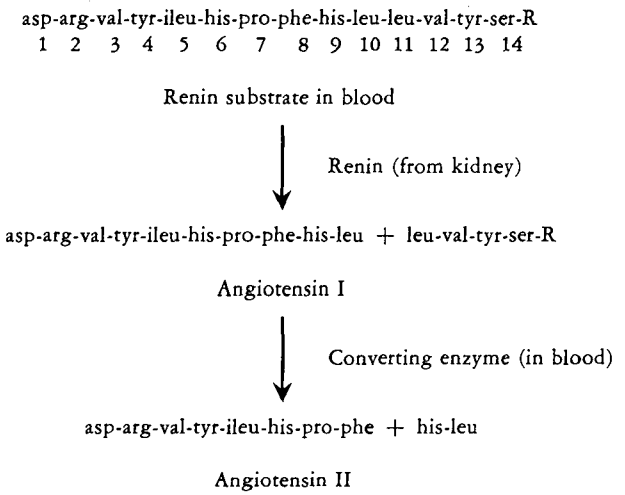

Fig. 1. Sequence of reactions that yield angiotensin II in blood.

amino acids except that valine is substituted for isoleucine in position 5,[4] while angiotensin from hog blood is identical with that from horse blood.[5] The final proof for the sequence of amino acids in angiotensin II came with its synthesis by Bumpus and the group at the Cleveland Clinic[6] and by Rittel, Iselin, Kappeler, Riniker, and Schwyzer.[7]

To delve more deeply into the problem of the renal pressor system one must search for answers to the following questions. What role does angiotensin II play in regulating normal blood pressure or in causing hypertensive disease? How is this peptide able to transmit a "biological message" to a muscle and cause a contraction? What parts of this molecule are responsible for its reaction with a muscle or other target cell? Can a molecule be designed that will specifically compete with angiotensin II for a "receptor site" on a muscle? Progress made toward answering these difficult questions is summarized in this report.

To investigate the angiotensin molecule as to which portion is responsible for its biological activity, peptides have been prepared in which one group at a time has either been blocked or has been omitted. Biological activities of these altered molecules are then determined. The results of these studies are summarized in *Figure 2*, and the biological significance of the functional groups of each amino acid in the peptide is discussed below.

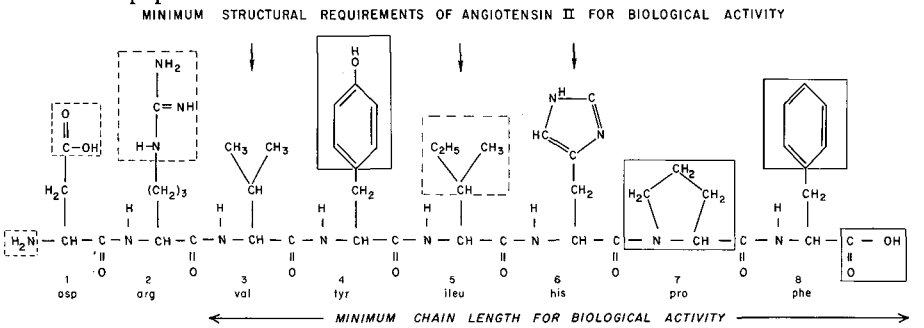

Fig. 2. Minimum structural requirements of angiotensin II for biological activity. Boxes indicate points of substitution: solid line denotes area necessary for biological activity; broken lines denote areas of minor or no significance. Arrows point to groups that require further investigation of their exact roles.

Aspartic acid. Removal or blocking of either the free N-terminal amino group[8,9] or the free carboxyl group[10] does not significantly alter biological activities of the peptide. Complete removal of aspartic acid reduces the pressor activity to 30 per cent of angiotensin II, and the oxytocic activity to 70 per cent of angiotensin II.[11]

Arginine. The destruction of the basicity of the guanidine group or removal of this group does not significantly alter biological activity[10]. Removal of this amino acid with aspartic acid, leaving a hexapeptide, reduces the pressor activity to 2 to 3 per cent of that of the parent peptide hormone. However, the hexapeptide has about the same pressor activity as noradrenaline.[11]

Valine. Removal of aspartyl-arginyl-valine leaves a pentapeptide that is completely devoid of biological activity.[11] No substitutions have been made in this position.

Tyrosine. Removal of the aromatic ring of tyrosine in the hexapeptide destroys biological activity[11] while removal of the phenolic-OH reduces biological activity.[10]

Isoleucine. Substitution of valine for isoleucine does not affect biological activity, while substitution of leucine causes a slight reduction in activity.[10]

Histidine. From studies on the photooxidation of angiotensin II it has been concluded that histidine is essential for biological activity.[12]

Proline. Rupture of the aliphatic ring of proline greatly reduces the biological activity.

Phenylalanine. Removal of phenylalanine,[13] removal of the aromatic ring of phenylalanine,[14] or blockage of the C-terminal carboxyl group of phenylalanine[10] all destroy biological activity.

From these results the following conclusions on the groups required for angiotensin II activity can be reached: (1) there must be at least six amino acids in the peptide, (2) the aromatic rings of tyrosine and phenylalanine must be present, (3) proline must be next to the C-terminal amino acid, (4) the C-terminal carboxyl group must be free, and (5) an imidazole ring may be required in position six.

The angiotensin molecule is not a two-dimensional structure but a three-dimensional arrangement of atoms. The three-dimensional structure or conformation of a peptide of this type would be stabilized by hydrogen bonds. (A hydrogen bond is largely an ionic bond formed between an "active" hydrogen atom and an unshared electron pair of a strongly electronegative atom such as oxygen.) Destruction of such bonds should destroy a specific peptide conformation. When tested under conditions that would destroy hydrogen bonding, the peptide lost nearly all myotropic activity.[11] Thus, the conformation of angiotensin II is important to the biological activity of the peptide.

Recently, a conformation was suggested for angiotensin II based on the assumption it would form an α-helix to the greatest extent possible.[15] In this conformation, three of the groups required for biological activity, the two aromatic rings and the C-terminal carboxyl group, are all close together and on the same side of the molecule. Rupture of the aliphatic ring of proline completely changes the position of these groups relative to one another. Thus, the area occupied by these groups may represent the "active site" of the molecule.

With this knowledge of the chemistry of angiotensin, let us briefly review some of the physiological actions of angiotensin.

In 1939, Page and Helmer[16] and Braun-Menendez, Fasciolo, Leloir, and Munoz[17] independently discovered angiotensin. Shortly thereafter, this peptide was demonstrated to contract isolated rabbit intestines.[18] In 1940, Luduena[19] showed that angiotensin acted on all smooth-muscle preparations but to varying degrees. Rat and rabbit uteri were the most sensitive preparations, and the gallbladder, retractor

penis, ureter, vas deferens, and bronchi of the dog were less sensitive. The vascular pressor response is certainly due in part to contraction of the vascular smooth muscle.

Response of isolated smooth-muscle preparation has recently been shown to be due to two different mechanisms.[20] The isolated rat uterus, which is essentially smooth muscle, contracts in the presence of angiotensin because the peptide reacts with the smooth-muscle fibers. Isolated guinea pig intestines, on the other hand, respond to angiotensin, but the contraction is the result of two different mechanisms. Intestinal strips have their own intrinsic nerve supply made up of ganglion cells of Auerbach's and Meissner's plexuses with preganglionic and postganglionic fibers. The latter innervate the smooth muscle fibers. The response of an intestinal strip to angiotensin is partially blocked by atropine or morphine. Since atropine blocks receptor sites of acetylcholine at the postganglionic nerve endings, angiotensin then must act through the parasympathetic innervation of the intestinal smooth muscle. Ganglion-blocking agents, except tetraethyl ammonium chloride, have no effect on the response of intestines to angiotensin, but paralyzing doses of nicotine partially block this response. Thus, with the intestinal strips, angiotensin must act both by direct effect on smooth muscle (25 to 30 per cent) and indirectly by stimulating the ganglion cells, secondarily causing release of acetylcholine which in turn causes the muscle to contract.

In 1940, [21,22] impure angiotensin preparations were shown to have an effect on myocardial muscle. Recently, Fowler and Holmes[23] using synthetic angiotensin demonstrated an increase in cardiac output, coronary flow, and contractile force of the heart in a dog heart-lung preparation after a single injection of from 10 to 40 μg. Initially there was a temporary decrease in all three because of increased coronary vascular resistance and a decreased oxygen supply to the heart. However, Page and Olmsted showed that cardiac output decreased after an infusion of synthetic angiotensin in an intact, unanesthetized and unrestrained dog. This could be explained by a continuous coronary vasoconstriction due to the infusion. Thus, by increasing the force of myocardial contraction, angiotensin has an effect somewhat similar to that of the cardiac glycosides.

More recently angiotensin has been shown to have an effect on the adrenal gland. Genest, Koiw, Nowaczynski, and Sandor,[24] and Laragh, Angers, Kelly, and Lieberman[25] found a significant increase in aldosterone secretion in patients with malignant hypertension. Carpenter, Davis, and Ayers[26] offer an explanation for this phenomenon by their findings that synthetic angiotensin stimulates the secretion of aldosterone and 17-hydroxycorticoids in hypophysectomized nephrectomized dogs.

From these studies, we now know that although angiotensin is primarily considered to be a pressor peptide it stimulates a number of different tissues and cell types including smooth muscle, cardiac muscle, ganglion cells, and cells of the zona glomerulosa of the adrenals. Now more information is needed concerning

localization of action of angiotensin on the cell. A study of large molecular weight angiotensin derivatives suggest that angiotensin does not permeate into the cell, but functions outside the cell wall and possibly upon the membrane itself. These large polymers, which were made by combining the amino group of angiotensin with poly-O-acetyl serine[18] or with serum albumin or serum globulin, have molecular weights from about 10,000 to 200,000 and probably are too large to pass through a semipermeable membrane, yet they are almost as active as the parent angiotensin, which is 1/10 to 1/200 the molecular size.

The inhibitory action of catecholamines is not yet fully understood, but theories for its mechanism of action postulate an action on the outer surface of the cell membrane. The inhibitory effects of the catecholamines on the responses of the intestinal strips to angiotensin suggest by analogy that angiotensin acts in a similar area.

Angiotensin is the most potent naturally occurring pressor agent. We have learned which groupings on the amino acid chain are necessary for biological activity, and have suggested that a three-dimensional structure of the molecule is important. It is extremely interesting that we can construct a three-dimensional model consistent with all physical and biological data which places all essential groups close together and on one side of the molecule which we can assume to be a reactive site.

Angiotensin acts on different tissues and cells with varying biological functions and seems to be acting on the outside of the cell membrane. Alteration of flux of monovalent and divalent ions across the cell membrane is responsible for biological phenomena as muscle contraction and nerve excitation. Experimental results suggest to us that possibly angiotensin is bound to a receptor site on the cell membrane, causing some deformation of the proteins or activation of an enzyme system and thereby altering the flux of ions. It is this mechanism that ultimately leads to the biological response.

Even though much progress has been made toward a better understanding of the basic actions of the peptide angiotensin, much work still remains to be done. It has not yet been learned how extensively the renin-pressor system is involved in renal hypertensive disease, or whether or not the effect of angiotensin can be specifically blocked by a competitive antagonist. This important aspect of the problem is being studied at the present time.

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