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# COMMENTARY

# INTERRELATIONS BETWEEN VARIOUS BLOOD PRESSURE REGULATORY SYSTEMS AND THE MOSAIC THEORY OF HYPERTENSION *

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In 1949, Page [1] proposed the mosaic theory of hypertension (Fig. 1). According to this concept, a number of regulatory factors work in an interrelated manner to control blood pressure and tissue perfusion. These factors could be chemical, neural, vascular or volumic in nature. A derangement in any of these factors could disturb this equilibrium, resulting in hypertension.

For a young generation this concept may seem self-evident, as, indeed, do most generalizations. In the 1930s it was far from evident, since most of the biomedical community had been taught that a disease had a single cause. Multiple causes were looked upon as fuzzy thinking. Sooner or later, a single cause would be found!

This made for an atmosphere of claims and counter-claims with protagonists for single causes ranging from psychosomatic, environmental, neural to humoral—in retrospect, all correct when viewed as participants but wholly wrong as "the" cause. Clinically, failure to appreciate the interrelatedness of the multiple regulators led to severe limitations in thinking, with the result that many causes which are currently accepted as obvious were overlooked, or ignored.

After 29 years since this theory was proposed, it is now realized that none of the existing antihypertensive drugs "alone" has proved effective in the long-term control of blood pressure, and treatment with combination therapy may last for years. These results have aroused fresh interest in the mosaic theory of hypertension.

The concept now points the way to deeper approaches to problems of diseases of regulation, through molecular biology, a step which is now being timidly taken by a few. The present review aims to discuss the mosaic theory in relation to the progress made to study the etiology of hypertension and to find its treatment.

*Evolution of the renin–angiotensin system*

In 1934, Goldblatt *et al.* [2] produced persistent hypertension in dogs by constricting the main renal artery with a silver clamp. Later studies by Kohlstaedt *et al.* [3], Braun-Menendez *et al.* [4], and Leloir *et al.* [5] indicated that reduced blood flow to the kidney released renin. This suggested that perhaps renin was responsible for hypertension. However, subsequent work by Leloir *et al.* [5] and by Page and Helmer [6] showed that renin by itself is not a pressor substance. It is a proteolytic enzyme which acts on the $\alpha_2$-globulin fraction of plasma to release angiotensin.

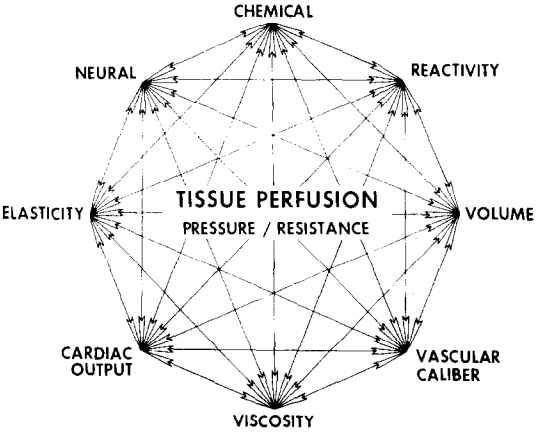


Fig. 1. Mosaic theory of hypertension of Page [1].

In 1954, Bumpus *et al.* [7] and Skeggs *et al.* [8] isolated angiotensin in the pure form. In the same year, Skeggs *et al.* [9] reported that angiotensin existed in two forms. One was a decapeptide and was called angiotensin I, while the other was an octapeptide called angiotensin II. In 1956, Elliot and Peart [10] reported the structure of bovine angiotensin I (Asp-Arg-Val-Try-Val-His-Pro-Phe-His-Leu). In 1957, Bumpus *et al.* [11] synthesized hog angiotensin II, while Rittel *et al.* [12] synthesized an analog of bovine angiotensin II (Asn-Arg-Val-Tyr-Val-His-Pro-Phe). It is now known that during pulmonary circulation, the carboxyl-terminal dipeptide, histidylleucine, is cleaved, and in this way angiotensin I is converted into angiotensin II. Human, horse, mice, rat, rabbit and dog angiotensin are similar to hog angiotensin, while sheep angiotensin is similar to ox angiotensin (Fig. 2).

In 1953, Simpson *et al.* [13] isolated and characterized aldosterone, and it was soon recognized that angiotensin II plays a key role in the regulation of blood pressure by its direct vasoconstrictor effect as well as through the regulation of aldosterone secretion. Aldosterone, produced exclusively in the zona glomerulosa of

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| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MAMMALS: Human, horse, pig, mice, rat, rabbit, dog | | Asp | -Arg- | Val- | Tyr- | Ile | - His- | Pro- | Phe- | His | - Leu |
| Sheep, ox | | Asp | -Arg- | Val- | Tyr- | Val | - His- | Pro- | Phe- | His | - Leu |
| TELEOSTS: Japanese goosefish | | Asn | -Arg- | Val- | Tyr- | Val | - His- | Pro- | Phe- | His | - Leu |
| AVIANS: White leghorn fowl | | Asp | -Arg- | Val- | Tyr- | Val | - His- | Pro- | Phe- | Ser | - Leu |
| AMPHIBIANS & REPTILES: Frog, snake | Y- | Asx | -Arg- | Val- | Tyr- | Val | - His- | Pro- | Phe- | Tyr | - Leu |
| Frog skin | Ala-Pro-Gly- | Asp | -Arg | [Ile] | Tyr- | Val | - His- | Pro- | Phe | | |
| | | ANGIOTENSIN II | | | | | | | | | |

Fig. 2. Variations in the structure of angiotensin I during the course of evolution.

the adrenal cortex, was shown to modulate volume homeostasis and sodium metabolism. There is some evidence to suggest that angiotensin II also has a direct effect on sodium transport. These aspects have been reviewed recently [14, 15].

These pioneering investigations formed the basis for the evolution of the renin–angiotensin–aldosterone system which appeared to be the mechanism responsible for regulating blood pressure in the normal and the diseases states. It was hoped that, if the renin–angiotensin–aldosterone system could be controlled, hypertension would disappear. However, further investigations in the mammalian as well as in the non-mammalian vertebrates changes this concept, and the complexity of blood pressure regulation became apparent.

*Phylogenetic studies in the non-mammalian vertebrates*

Phylogenetic studies [14–25] indicate that ACTH and adrenocortical steroids are present in almost all primitive vertebrates including agnatha, cyclostomes and elasmobranchs (Fig. 3). It has been observed that the vertebrates living in salt water (or sea water) mainly produced cortisol and corticosterone, while those living in fresh water, or on land, produced aldosterone and corticosterone. In contrast, renin activity has been found to be absent in the kidneys of jawless vertebrates (e.g. lamprey and hagfish) or in other most primitive living vertebrates including cyclostomes and elasmobranchs [16, 17]. It has been postulated that renin activity first appeared during the early evolution of bony fishes and its presence has been detected in all

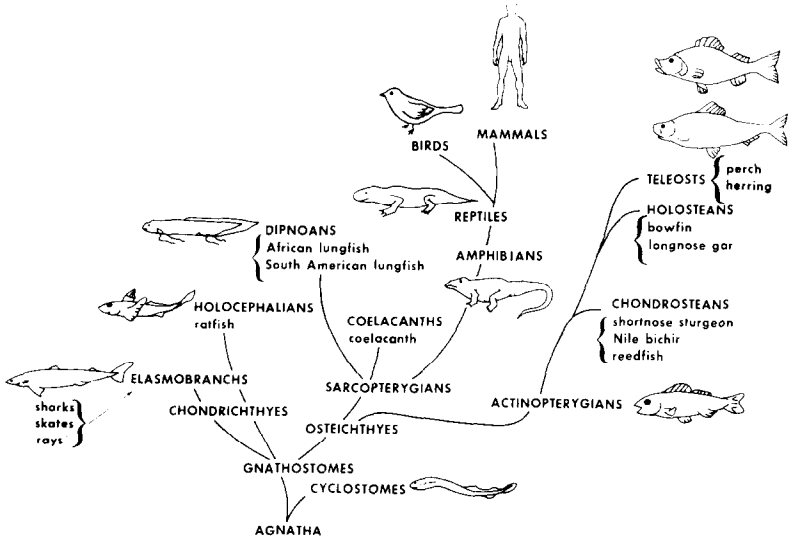



Fig. 3. The phylogenetic tree of vertebrates (modified from the figures reported by Nishimura *et al.* [22] and Sawyer [26].)

living groups of *actinopterygians, sarcopterygians, holocephalians*, and *dipnoans* [16, 22]. However, in some cases the criteria for the presence of renin (e.g. juxtaglomerular granules and macula densa) have not been fully satisfied and a fully developed juxtaglomerular apparatus is found only in mammals [17]. In the lower vertebrates, a macula densa is absent and, therefore, juxtaglomerular cells alone may regulate the secretion of renin. Birds show a transitional type macula densa and, as regards the control of glomerular filtration rate, birds may be considered intermediate between mammals and reptiles (or anamniotes) [23].

Thus, it appears that ACTH and adrenocortical steroids were present before the emergence of the renin–angiotensin system. There is no evidence that these steroids acquired an important physiological role during the early evolution of bony fishes. Similarly, there is no evidence that the renin–angiotensin system stimulated the biosynthesis of aldosterone when it first appeared.

Sharks, skates and reys are members of a very old class of fishes the *elasmobranchs*, that live in a sodium chloride environment. In order to combat the increasing salinity of the ocean water, these creatures maintain a plasma osmolality slightly hyperosmotic to sea water [27]. This is accomplished by a special mechanism by which *elasmobranchs* can retain high concentrations of urea, sodium chloride and a product of protein metabolism, trimethylamine oxide [28]. And, in spite of a high salt concentration in their plasma and in the sea water in which they live, these creatures have been able to maintain a relatively low arterial blood pressure. For example, the mean blood pressure of a contemporary shark, *Squalus acanthias* ("dog-fish") is 15–20 mm Hg [29]. *Elasmobranchs* do not possess the means of producing significant amounts of renin since they lack renal juxtaglomerular cells [16, 30].

Studies by Opdyke *et al.* [31–33] suggested that acute expansion of plasma volume in *S. acanthias* with hypertonic saline or urea solution increased plasma osmolality without a significant increase in blood pressure. In contrast, plasma volume expansion with hyperosmotic dextran solution increased blood pressure significantly without increasing plasma osmolality. Further, this species showed a strong pressor response to angiotensin I and II and norepinephrine. The response to angiotensin I was completely abolished by pretreatment with angiotensin I-converting enzyme inhibitor, SQ 20881, while an adrenergic blocking agent, phentolamine, completely abolished the pressor responses to both angiotensin I and II. However, the pressor response to angiotensin I and II was not abolished by pretreatment with a ganglion blocking agent, hexamethonium.

These results suggest that *S. acanthias* possesses a type of receptor which, when stimulated with angiotensin II, causes release of catecholamines which, in turn, produce a pressor response. Further, an enzyme capable of converting angiotensin I into angiotensin II is present in effective amounts in this species, although it is not known whether this enzyme is identical, or related, to the enzyme that potentiates the effect of bradykinin in higher forms. Lastly, modulation of blood pressure by change in blood volume does not appear to be a primitive method of vascular control.

Similar studies on the American eel (*Anguilla rostrata*) [34] have indicated that: (a) an enzyme capable of converting angiotensin I into angiotensin II may exist in this species, and (b) at least a part of the pressor action elicited by angiotensins in this species may be ascribed to the release of catecholamines from chromaffin tissue or adrenergic nerve endings.

Thus, one may "speculate" that in the more primitive stage, when these vertebrates lived in salt water, neurogenic or simply salt water regulatory mechanisms may have been predominant. When these species migrated into fresh water, they suffered from ecological stress and the need for a hormone arose that would stimulate the retention of sodium—perhaps this hormone was aldosterone. We may, therefore, assume that the reduction of sodium ion concentration or an increase in potassium ion concentration acted as a stimulus for the biosynthesis of aldosterone in the adrenal cortex. There is some evidence to support this hypothesis: (a) sodium deficiency gradually facilitates conversion of corticosterone to aldosterone [35–37]; and (b) wombats and kangaroos living in the snowy mountains of Australia, an area where very little sodium is available, have higher plasma levels of aldosterone than wombats and kangaroos living where sodium is more plentiful [38].

One may further "speculate" that at an intermediate stage the renin–angiotensin system was evolved. This system perhaps acted as an alternate or a supporting mechanism for the regulation of blood pressure. This is evident from the fact that the (renal) renin content in fresh water teleosts is usually higher than that of marine species [14, 39]. Similar results were obtained with marine teleosts adapted to fresh water [23]. By longer adaptation, renal activity in *Anguilla japonica* was decreased 3 weeks after transfer from fresh water to sea water [40, 41]. Juxtaglomerular granularity also decreased after 4 weeks [41]. Renal renin decreased in *Tilapia mossambica* within 1 week after transfer from fresh water to sea water [41].

However, there are a number of points that do not fit into this hypothesis. As an example, angiotensin II does not appear to regulate aldosterone secretion in teleosts and in birds, while conflicting results have been obtained with reptiles and amphibians [24]. Bern [18] postulated that the extremely small amounts of aldosterone occurring in fish raise doubt as to a significant physiologic role for this steroid and that "it may be invention of landliving vertebrates", suggesting that the physiologic role of the renin–angiotensin system antedated that of aldosterone in the phylogenetic scale [14]. It is likely that the interpretation of some of these results may be misleading due to two reasons: (a) angiotensin used in most of these studies is native to higher vertebrates, viz. mammals, rather than being native to the species being studied, and (b) the sophisticated procedures for isolation and bioassay of renin, angiotensin and mineralocorticoids have been developed only recently.

### *Characterization of angiotensin-like substances from the non-mammalian species*

Nakajima *et al.* [42, 43] examined angiotensin-like substances of avian, reptilian and teleostean origin by: (a) the elution pattern of SE-sephadex column chromatography, (b) the ratio of oxytocic and pressor activity,

and (c) the susceptibility to proteases. Most of these angiotensins were eluted in the multiple peaks of activity by the chromatographic separation. Some were then sequentially analyzed and the amino acid sequence of the major active peptide obtained. The results indicated that most of these angiotensins differ from mammalian angiotensin (Fig. 2).

Fowl (white leghorn) angiotensin I, on the basis of its amino acid composition and characteristics of its 1-dimethylamino-napthaline-5-sulfonyl (DNS) derivative, was identified as a decapeptide, [1-aspartic acid, 5-valine, 9-serine] angiotensin I [44]. Khosla *et al.* [45] confirmed this structure through the synthesis of this peptide. Although this is the first example of species variation in position 9, another report indicates that kangaroo angiotensin I also differs from the known sequence near the C-terminus [46].

Snake (*Elaphe climocophora*) angiotensin I has tyrosine in position 9, valine in position 5, and an *N*-blocked apartic acid or asparagine in position 1 [47]. The nature of the *N*-terminal blocking group is presently not known. In addition, the corresponding 10-des-Leucine nonapeptide and [1-Aspartic acid, 5-valine] angiotensin II have also been isolated. There is some evidence to suggest that frog (*Rana catesbeiana*) angiotensin I may also have a similar structure [43]. An angiotensin II-like peptide from the skin of the Australian frog *Crinia georgiana* has been characterized recently (T. Nakajima *et al.*, unpublished results). The structure of this angiotensin differs from the conventional angiotensins in two respects (cf. Figure 2): (a) it has a tripeptide, Ala-Pro-Gly attached to the *N*-terminus, and (b) valine in position 3 is replaced with an isoleucine residue. The structure of angiotensin I from the aglomerular Japanese goosefish (*Lophius litulon*) was reported as [1-asparagine, 5-valine, 9-histidine] angiotensin I [48]. This is the only example of species variation in position 1.

A preliminary study of the comparative pressor activity and aldosterone releasing effects of these angiotensins in dispersed rat adrenal zona glomerulosa cells (with less than 5% fasciculata cells) indicates that there is considerable variation in their biological activities [49, 50]. For example, pressor assays in vagotomized ganglion-blocked rats indicate that [5-valine] angiotensin II (bovine) is more potent than either [5-isoleucine]-(human) or (1-asparagine, 5-valine] angiotensin II (teleost or Ciba Hypertensin). However, the potency varied when the assays were carried out by alternating the dose–response curves of the two angiotensins or when similar doses of the two angiotensins were injected in an alternate fashion. The mechanism of this variation in potency is presently not understood.

More dramatic results were obtained when these angiotensins were tested for their ability to stimulate the secretion of aldosterone. [5-isoleucine] angiotensin II showed about 60 per cent of the aldosterone releasing effect of [5-valine] angiotensin II in the dispersed rat adrenal zona glomerulsa cell [49, 51].

With the present knowledge, we do not know why tyrosine in position 9 of angiotensin I was replaced with serine or histidine during the evolutionary cycle. What was the need for this change? How did this change affect man? Was there a parallel change in the nature of converting enzyme?

*Biological effects of the mammalian angiotensin II*

The complex hormonal action of angiotensin II has been attributed to the multiplicity of direct or indirect physiological actions of angiotensin II. For example, it contracts vascular smooth muscle, uterus, intestine and aorta. It enhances myocardial contractility, and stimulates the release of catecholamines, aldosterone and prostaglandins.

In kidney, at low dose level, angiotensin II decreases renal blood flow, glomerular filtration rate, sodium excretion, urine volume and renin release. At high concentrations, however, its action is biphasic and with the increasing dose the initial antidiuresis and antinatriuresis change to diuresis and natriuresis.

In the central nervous system it stimulates thirst, causes increased central adrenergic discharge and stimulates the release of the antidiuretic hormone, vasopressin. In the autonomic nervous system it stimulates the release of norepinephrine at sympathetic nerve terminals and it also inhibits its re-uptake.

These diversified biological effects of angiotensin II posed two main questions: (a) How does this hormone invoke such a variety of biological effects? and (b) What is the mechanism of its involvement in the pathogenesis of high blood pressure?

To answer the first question, chemists and pharmacologists studied structure–activity relationships. The outcome of this effort was that by 1970 over 100 analogs of angiotensin II were synthesized and tested for their pressor and myotropic activities. To answer the second question, biologists and clinicians studied the various humoral mechanisms. However, it was soon realized that the mechanism of involvement of angiotensin II in the pathogenesis of high blood pressure was not as simple as had been expected.

*Structure–activity relationship with the analogs of angiotensin II*

Structure–activity relationship with the analogs of angiotensin II [52–54] indicated that (a) the essential features of this hormone are present in the C-terminal hexapeptide which is composed of amino acid residues with alternate aliphatic and aromatic side chains, (b) all aromatic residues (tyrosine, histidine and phenylalanine) are essential for biological activity, and (c) the carboxyl group at the C-terminus must be free.

For the past several years investigations have been undertaken to define the three-dimensional structure of angiotensin II and its analogs [55–61]. An important aspect of these studies is to attempt to relate the changing biological action, as the structure of the backbone or side-groups is changed, to the observed changes in physical or binding properties. It was hoped that this study would be helpful in understanding the mode of action of angiotensin II on its receptor(s). However, a universally acceptable model for the conformation of angiotensin II has not emerged and no single model proposed so far is consistent with all the physical data obtained with circular dichroism and n.m.r. spectra, or with studies related to deuterium or tritium exchange [57]. This is partly due to the fact that angiotensin II is a short linear peptide, and it is expected that its secondary structure will change in a specific environment with changes in temperature, solvent, concentration, pH, etc.

It has been observed that in aqueous solution angio-

tensin II is characterized by an equilibrium of several closely similar conformations, whereas in organic solvents a certain conformational selection is introduced [55, 60]. Circular dichroism spectra in the amide and aromatic spectral regions of angiotensin II and analogs in fluorinated alcohols (e.g. 2,2,2-trifluoroethanol) have suggested a $\beta$-structure for angiotensin II with a folding at the amino and the carboxyl terminal part of this peptide [55]. The titration data indicate that the two ends are positioned near each other to allow electrostatic interactions, while all side groups are free to interact with solvent [55, 59]. Substitutions in position 1 (aspartic acid), position 7 (proline) and position 8 (phenylalanine) of angiotensin II entail changes in the backbone conformation while substitutions in the side-chain of position 3 (valine), position 5 (isoleucine) and position 6 (histidine) serve mainly to correctly align the phenolic group of tyrosine in position 4 [61]. The phenolic hydroxyl group may be involved in the intermolecular bonding since it is not involved in the intermolecular conformation but is essential for the biological activity of this hormone [52, 55, 61].

These studies, however, afforded little information about the nature of its receptor sites or how this hormone is able to invoke a spectrum of biological effects. It was realized that the missing link was an antagonist of angiotensin II that should be useful in studying the mechanism of action of angiotensin II, in identification of its receptors, in studying the etiology of renovascular hypertension, and in finding possible diagnostic and therapeutic agents.

*Regulation of blood pressure, blood volume and sodium balance in normal and diseased states*

In situations such as hemorrhage or sodium loss, the arterial blood pressure falls and the effective blood volume contracts. This acts as a stimulus for renal juxtaglomerular cells to secrete renin into the systemic circulation. Renin, being a proteolytic enzyme, acts enzymatically on the $\alpha_2$-globulin fraction of plasma to release angiotensin I. Converting enzyme, particularly in the lung, cleaves the C-terminal dipeptide histidyl-leucine from angiotensin I to generate the vasopressor octapeptide angiotensin II. Angiotensin II acts on adrenal cortex to stimulate the biosynthesis of aldosterone. Aldosterone in turn acts on renal tubules to increase the retention of sodium ions and to promote the elimination of potassium ions. Increased retention of sodium leads to retention of water thus causing extracellular volume expansion. Angiotensin II also has a direct action in constricting the arterioles, and weight for weight it is the most potent pressor substance known today.

Some investigators believe that both angiotensin II and aldosterone act together to raise blood pressure to an optimum level to restore renal perfusion. At this stage, any further increase in blood pressure, blood volume or sodium concentration triggers a feedback mechanism in which the release of renin is reduced, leading to reduced levels of angiotensins or aldosterone. This opinion is by no means universal.

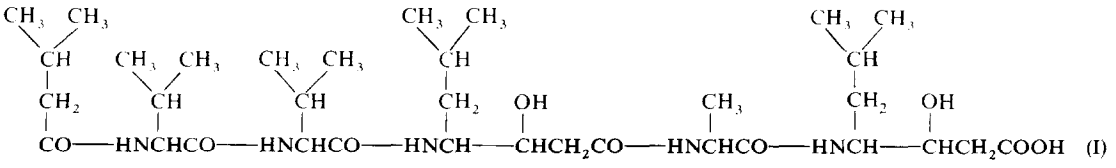
Recent investigations have shown that the mechanisms regulating blood pressure are not as simple as the above postulated negative feedback mechanism. The regulation of aldosterone secretion, renin release, and sodium retention or excretion may be altered by a number of factors [14, 15]. For example: (a) besides angiotensin II, ACTH and plasma concentration of potassium appear to play an important role in the regulation of aldosterone secretion, (b) apart from aldosterone, a number of other hormones may alter sodium concentration by direct or indirect mechanisms; these include estrogen, vasopressin, cortisol and other adrenocortical steroids, (c) angiotensin II has been shown to have a direct effect on the transport of sodium and this effect appears to be independent of aldosterone, (d) the secretion of renin is affected by a number of factors such as vasopressin, adrenocortical steroids, epinephrine and norepinephrine, and (e) renin-like enzymes have been extracted from a variety of organs thereby suggesting local generation and destruction of angiotensins.

In the diseased state, however, the above equilibria are disrupted. For the sake of illustration one may take an extreme (or rather hypothetical) case in which there is an excess of aldosterone and very low renin activity. In this case hypertension is due to extracellular volume expansion and is characterized by edema. This type of hypertension, known as "volume" hypertension [62], can be controlled by volume depletion through diuretics. On the other hand, when there is prolonged excess of angiotensin II, hypertension may be primarily due to vasoconstriction, and is characterized by severe vascular injury. Hypertension can be controlled by vasodilators such as hydralazine, diazoxide and sodium nitroprusside. Between these two extremes a whole spectrum of hypertensions would occur with various combinations of abnormal volume and vasoconstriction. Obviously depending upon the situation, one could break this vicious cycle by synthesizing compounds that would block the release of renin, converting enzyme, angiotensin II or aldosterone. However, it is expected that due to numerous variations in the combination of vasoconstriction, cardiac output and blood volume no one drug would be effective for the individual patients.

*Renin inhibitors*

Renin inhibitors of peptide origin may be broadly classified into two categories: (a) pepstatin, and (b) sequence variants of renin substrate. Of the renin inhibitors isolated from kidney or other natural sources, phospholipid inhibitors appear to be of interest. However, the roles of extrarenal renin, isorenin, and brain renin are presently not known. These aspects have been reviewed recently [54, 63–66].

Pepstatin (I) has been isolated from various strains of *streptomyces*. It is a pentapeptide with an acylated amino terminus. It is composed of hydrophobic amino acids (valine, alanine and 4-amino-3-hydroxy-7-methyl-heptanoic acid) with two hydroxyl groups in positions 4 and 6 and a free carboxyl group at the C-terminus. It is a powerful inhibitor of several acid proteases and its action on renin has been demonstrated in several species such as hog, rat and rabbit. It has been shown to inhibit the reaction of renin on its substrate and thus the formation of angiotensin I. However, its clinical usefulness as an antihypertensive agent is still uncertain and no definite conclusions have been reached [67–69]. This may be attributed to the hydrophobicity of the molecule which prevents its solubilization at concentrations necessary to achieve complete



H-Asp-Arg-Val-Tyr-Ile-*His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OH* (II)

blockade of renin. Further, the peptide is many-fold less efficient at physiologic pH (7.4) than at pH 5.7.

Inhibitors of renin derived from a renin substrate are those that have an unnatural amino acid at the Leu-Leu bond (II) [64, 65]. For example, replacement of either of the L-leucine residues with a D-leucine residue prevents cleavage by renin. Similar results have been obtained by replacing the Leu-Leu linkage with Leu-Phe or Leu-Tyr linkages. These peptides also suffer from the same limitations as pepstatin. At physiologic pH (7.0) the inhibitory constant (*Ki*) increases and solubility decreases to the point that inhibition cannot be demonstrated with these peptides. Recently, however, it has been reported that attachment of $(Pro)_3$ or $(Pro)_5$ sequences to the amino terminus of His-Pro-Phe-His-Leu-Leu-Val-Tyr increases the solubility of this peptide to some extent, but these compounds are also not much more active at physiologic pH [64].

A renin inhibitor of phospholipid origin was reported by Sen *et al.* [70]. This inhibitor reduced blood pressure in acutely hypertensive rats that had elevated renin levels and pressures of 200 mg Hg. The blood pressure, however, did not return to normotensive levels. After cessation of injection of this inhibitor, the pressure again rose to the pre-injection hypertensive levels. Likewise, the renin activity increased after the cessation of injection to levels even higher than the pre-injection level. Similar results were also obtained when this pre-inhibitor was injected into animals made hypertensive by Page's method [see Refs. 70 and 71]. This substance is active in rats but has little activity in dogs. The reason for its activity in rats is perhaps related to the presence of high levels of phospholipase A, which is necessary to convert the phospholipid pre-inhibitor into an active form.

Baggio *et al.* [72] isolated a phospholipid fraction from plasma and added it to a system of semipurified human renin and renin substrate. Inhibition of angiotensin II generation took place only when phospholipase A was added. Kotchen *et al.* [73] have reported the presence of an acetone-soluble renin-inhibitor factor in normal human plasma which is absent in uremic plasma. However, the exact role of these phospholipids (including that of phosphatidylethanolamine), or how they inhibit renin, is presently not known. Bunag and Walaszek [74] have postulated that phospholipid derivatives such as phosphorylated glyceryl acetals of olealdehyde and palmitaldehyde [75] caused a non-enzymatic inhibition of renin *in vitro* by formation of micelles on the renin molecule.

### *Angiotensin-converting enzyme and its inhibitors*

The enzyme, dipeptide carboxypeptidase ("peptidyl-dipeptide carboxyhydrolase", "peptidyl dipeptidase" or "angiotensin-converting enzyme") is widely distributed in the body and is present in circulating blood and in a number of other tissues; however, its highest concentration is found in lung and kidney. It has now been isolated in the pure form from a number of species and tissues and there are indications that it consists of a single glycopeptide chain with a molecular weight of approximately 140,000 and a molar equivalent of associated zinc. The difference in reported molecular weights from different species is thought to be due to isolation of artifacts of the enzyme based on methods used in its determination.

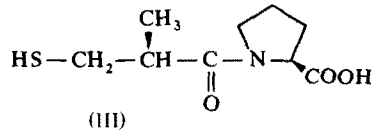
Angiotensin-converting enzyme is an exopeptidase. During pulmonary circulation this enzyme acts on the prohormone angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) to cleave the carboxyl-terminal dipeptide (His-Leu), thus generating angiotensin II which is delivered to the systemic circulation where it acts as the biologically active component of the renin–angiotensin system. This enzyme (synonymous with kininase II) also catalyzes sequential release of Phe-Arg and Ser-Pro from the carboxy-terminal of bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), thereby inactivating this powerful vasodilator. Thus, the resultant physiological action of this enzyme (i.e. generation of a vasopressor agent, angiotensin II, and inactivation of a powerful vasodilator, bradykinin) is considered to be vasopressor.

*In vitro* studies indicate that angiotensin-converting enzyme is not specific in its action on angiotensin I or bradykinin, but it cleaves dipeptide residues from the carboxyl-terminal of many peptides that possess a free carboxyl group at the carboxy-terminus. The pure enzyme cleaves successive dipeptide residues from the tetradecapeptide related to renin-substrate (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser) until angiotensin II is formed. However, this enzyme does not readily hydrolyze peptides containing a penultimate imino acid such as proline or a carboxy-terminal dicarboxylic acid such as glutamic acid. Substrates with a phenylalanine residue in the third position from the carboxy-terminus are bound much more tightly to the enzyme than the substrates having other residues in this position. The above aspects have been described in detail in recent reviews [76–80].

One of the ways to understand the mechanism of involvement of angiotensin II in the maintenance of hypertension, and also to develop antihypertensive agents, would be to block the generation of angiotensin II by inhibiting the action of angiotensin-converting enzyme. It is now known that angiotensin-converting enzyme is inhibited, particularly *in vitro*, by a variety of compounds of diverse chemical structure and chain-length such as angiotensin II, angiotensin III, bradykinin, angiotensin II antagonists (e.g. [$Sar^1$,$Ile^8$] angiotensin II), the end products of converting enzyme degradation of angiotensin I (viz. His-Leu) and bradykinin (viz. Phe-Arg) and the chelating agents [76–81]. In general,

the inhibitor peptides are bound to the enzyme in a manner analogous to the corresponding residues of substrate. Peptide inhibitors containing basic or aromatic residues have higher affinity for the converting enzyme than those containing acidic or branched amino acids. The inhibitory activity is greatly reduced when the peptides have an ester or amide linkage or a glutamic acid residue at the carboxy-terminus. On the other hand, the potency is increased when position 3 from the carboxyl end is substituted with a phenylalanine or tryptophan residue [76]. The most potent inhibitors are the peptides that have been isolated from the venoms of the South American pit viper *Bothrops jararaca* [82, 83]. The unique inhibitory effect of some of the venom peptides may be attributed to their high affinity for the converting enzyme. Most of these peptides contain *N*-terminal pyroglutamic acid and a Pro-Pro sequence at the carboxy-terminus. These substitutions are important for inhibition *in vivo* since they render the inhibitors insensitive to the action of exopeptidases, including converting enzyme. Contrarily, the penultimate prolyl residue is an unfavorable determinant with respect to inhibition of the "isolated" enzyme [76, 84]. Of the numerous venom peptides that were characterized and synthesized, the nonapeptide SQ 20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) and the pentapeptide $BPP_{5a}$ (Pyr-Lys-Trp-Ala-Pro) have been used most widely. SQ 20881 has been shown to decrease blood pressure in animal models and in hypertensive humans when plasma renin is high or normal [84, 85]. Recently, Niarchos *et al.* [86] have studied the hemodynamic effects of this inhibitor in normal subjects before and during sodium depletion. Their results suggest that blockade of angiotensin I conversion lowers blood pressure in normal subjects by inducing vasodilation and that these effects are enhanced by sodium depletion. Although SQ 20881 possesses great potential as a diagnostic and therapeutic agent, its clinical use is limited by the necessity of intravenous or other parenteral routes of administration.

In 1977, Ondetti *et al.* [87] synthesized carboxyalkanoyl and mercaptoalkanoyl derivatives of proline as specific and orally active inhibitors of converting enzyme. Of these 2-D-methyl-3-mercaptopropanoyl-L-proline (SQ 14225 or "captopril") (III) was found to be the most potent inhibitor.



(III)

This compound shares many of the common properties of the nonapeptide, SQ 20881. Potent and specific *in vitro* and *in vivo* inhibition of angiotensin I-converting enzyme was demonstrated by this compound in excised guinea pig ileum and in rats, cats, dogs and monkeys [88]. It markedly lowered the blood pressure of the renin-dependent aorta-ligated and two-kidney Goldblatt hypertensive rat, but failed to reduce blood pressure in the one-kidney Goldblatt hypertensive rat. In the two-kidney Goldblatt rat, SQ 14225 was about ten times as potent as SQ 20881 [89]. Oral doses of SQ 14225 moderately reduced the blood pressure of the Wistar–Kyoto spontaneously hypertensive rat but not that of the normotensive Wistar–Kyoto rat. Bilateral nephrectomy abolished the antihypertensive activity of SQ 14225 in the spontaneously hypertensive rat [89]. Administration of SQ 14225 in conscious normotensive rabbits indicated that it enhanced pressor response to angiotensin II while the vasoconstrictor effect of norepinephrine remained unchanged. It also had no effect on the vasodepressor response to acetylcholine, isoproterenol or prostaglandin $E_2$ [90]. Similarly, in normal male volunteers it inhibited the pressor response to intravenously administered angiotensin I [91]. After a dose of 20 mg, complete blockade was observed for more than 2 hr and partial inhibition for over 4 hr. There was no effect on pressor responses to angiotensin II.

Recently, a number of investigators [92–95] have studied the antihypertensive effect of SQ 14225 in hypertensive patients. These studies have indicated that this compound, administered alone or in combination with either a diuretic or sodium restriction in diet, has been effective in controlling moderate as well as severe and refractory hypertension.

In general, the higher the resting plasma renin activity the more profound the effect of SQ 14225 on mean arterial blood pressure. However, even patients with normal or low plasma renin activity have been found to respond to this compound. In most patients it caused a decrease in aldosterone production which was associated with retention of potassium and a rise in serum potassium concentration [93]. In contrast, in some patients, depending on the initial level of plasma renin activity, either no change or an increase in plasma aldosterone level was observed [95]. There were no significant changes in plasma cortisol and plasma norepinephrine levels. Although no severe side-effects have been observed, potentiation of a blood pressure lowering effect in volume depleted patients suggests that in such cases a careful monitoring of dosage should be carried out to assure a smooth and gradual lowering of blood pressure [95].

The data accumulated to date suggest that the antihypertensive response to converting enzyme inhibition with SQ 14225 is related to inhibition of angiotensin II generation. However, the lack of a strong correlation between basal levels of plasma renin activity and reduction in mean arterial pressure indicates that SQ 14225 might have effects other than decreasing circulating angiotensin II. Cody *et al.* [94] suggested that the pattern of reduction in peripheral resistance, along with unchanged cardiac index obtained with SQ 14225, is similar to that produced by vasodilators acting at both arteriolar and venular levels. This is plausible since inhibition of angiotensin-converting enzyme would lead to a decrease in vasoconstrictor agent (angiotensin II) and a concomitant increase in vasodilator agent (bradykinin). In addition to its direct vasodilator effect, bradykinin also releases prostaglandins which, in turn, could enhance the hypotensive effect of bradykinin [90]. Contrary to this hypothesis, there was no effect on urinary excretion of prostaglandin $E_2$ in renovascular hypertensive patients that were treated with SQ 14225, and which subsequently showed normal blood pressure [95]. Furthermore, Cody *et al.* [94] found unchanged cardiac output and heart rate in hypertensive patients responding to SQ 14225, which is in contrast with

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Angiotensin II: | Asp | Arg | Val | Tyr | Ile | His | Pro | Phe |
| [Sar$^1$, Ala$^8$] angiotensin II ("Saralasin") | Sar | Arg | Val | Tyr | Val | His | Pro | Ala |
| [Sar$^1$, Ile$^8$] angiotensin II: | Sar | Arg | Val | Tyr | Ile | His | Pro | Ile |
| [Sar$^1$, Thr$^8$] angiotensin II: | Sar | Arg | Val | Tyr | Ile | His | Pro | Thr |
| Angiotensin III: | | Arg | Val | Tyr | Ile | His | Pro | Phe |
| [Ala$^8$] angiotensin III: | | Arg | Val | Tyr | Ile | His | Pro | Ala |
| [Ile$^8$] angiotensin III: | | Arg | Val | Tyr | Ile | His | Pro | Ile |
| [Thr$^8$] angiotensin III: | | Arg | Val | Tyr | Ile | His | Pro | Thr |

Fig. 4. Antagonists of angiotensin II and III.

increased output and heart rate resulting from bradykinin infusion [96]. It has been reported that SQ 14225 has no central effects when given systemically, since its passage across the blood–brain barrier is severely restricted [97].

*Antagonists of the pressor and myotropic responses to angiotensin II*

In 1970, Khairallah *et al.* [98] observed that [8-alanine] angiotensin II blocked the myotropic response to angiotensin II on strips of guinea pig ileum. This observation generated great enthusiasm in both the chemical and the biological fields. It has long been felt that such antagonists could serve as useful tools for investigating the role of the renin–angiotensin system in renal hypertension. However, [8-alanine] angiotensin II proved to be a weak antagonist and, therefore, extensive studies on structure–activity relationship were carried out to develop potent antagonists of the pressor and myotropic action of angiotensin II. These develop ments have been described in a number of reviews [52–54, 99, 100].

Pals *et al.* [101, 102] reported the synthesis of [1-sarcosine, 8-alanine] angiotensin II and showed it to be more active *in vivo* than the 8-alanine analog. Khosla *et al.* [103] synthesized [1-sarcosine, 8-isoleucine] angiotensin II, an even more potent antagonist (Fig. 4), while Regoli and Park [104] synthesized [1-sarcosine, 8-leucine] angiotensin II. The potentiation of the *in vivo* potency by the substitution of sarcosine in the 1 position is possibly due to a decreased rate of enzymatic degradation as well as to an increased binding affinity [105]. Marshall *et al.* [106] reported that [4-phenylalanine, 8-tyrosine] angiotensin II, in high doses, blocked the myotropic effect of angiotensin II on isolated rat uterus and the pressor effect in anesthetized nephrectomized normotensive and hypertensive rats. Subsequent investigations by Khosla *et al.* [107–111] indicated that to produce potent antagonistic properties: (a) the aromatic ring in position 8 should be replaced with an aliphatic side chain branched at the $\beta$- or $\gamma$-position (Ile, Val, Leu, etc.), (b) position 1 should be replaced with unnatural amino acids such as sarcosine, dimethylglycine, or *N*-methylisoasparagine, (c) position 4 must be occupied by a tyrosine residue, and (d) all amino acids should have an L-configuration.

The two most widely used angiotensin II antagonists have been [Sar$^1$, Ala$^8$] angiotensin II [102] and [Sar$^1$, Ile$^8$] angiotensin II [103]. These antagonists reduce blood pressure in hypertensives with high plasma renin activity and, therefore, may be used as diagnostic agents to distinguish renin-mediated hypertensive patients. Another potential use of these antagonists is to induce and maintain a reduction in blood pressure in severely hypertensive patients. Above all, these antagonists provide useful tools to study the role of the renin–angiotensin system for the increase in blood pressure in human and experimental hypertension. These aspects form the subject of several reviews [52–54, 80, 100, 112–121].

It has been observed that these antagonists suffer from a number of limitations: (a) they cause an initial rise in blood pressure which is equal to 1–2% of angiotensin II [103]; subsequent work indicated that the pressor effect is partially due to a direct vasoconstrictor effect and the release of catecholamines [122, 123], (b) they are not tissue or organ specific for blocking the biological actions of angiotensin II, and demonstrate a variable agonist to antagonist ratio in isolated adrenal medulla, adrenal cortex, and possibly in other tissues [54, 122–125], (c) they cause significant increases in plasma renin activity [126], (d) they have short *in vivo* half-lives and (e) they are not orally active and must be given intravenously. The pharmacological half-life of [1-sarcosine, 8-alanine] angiotensin II has been reported to be less than 8 min in hypertensive patients [127].

We do not expect any problems if these antagonists are used only as diagnostic agents or for short-term treatment. However, for long-term administration the antagonists should be tissue specific with minimum, or no, agonist properties but with increased binding affinity to the receptor. Alternatively, the antagonistic potency of these peptides should be increased so that the peptides inhibit the angiotensin II induced responses at a low dose level. It was with this aim that Khosla *et al.* [110, 122, 128, 129] synthesized a number of analogs with modifications in position 8 and other positions of

the molecule; of these, [1-sarcosine, 8-threonine] angiotensin II appeared promising [110].

The antagonistic activity of [1-sarcosine, 8-threonine] angiotensin II was found to be comparable to that of [1-sarcosine, 8-isoleucine] angiotensin II with the additional advantage that the initial pressor activity of this analog was 50 per cent less than that of [1-sarcosine, 8-isoleucine] angiotensin II [110]. It was also observed that the antagonistic effect of [1-sarcosine, 8-threonine] angiotensin II lasted 2–3 hr after a 30-min infusion in rats at a dose level of 250 ng/kg/min [130].

Perfusion studies in isolated cat adrenals indicated that this analog did not induce the secretion of catecholamines [122]. A comparative activity in rabbit aortic strips, cat adrenal medulla and cat adrenal cortex indicated that this analog was a potent antagonist of the angiotensin II inducing responses in all the three tissues [131]. Comparative studies of the humoral and arterial pressure responses in sodium-depleted trained unanesthetized dogs indicated that, as compared to [1-sarcosine, 8-alanine] angiotensin II and [1-sarcosine, 8-isoleucine] angiotensin II, [1-sarcosine, 8-threonine] angiotensin II was the most potent antagonist in reducing arterial blood pressure [131]. Also, in marked contrast to [1-sarcosine, 8-alanine] angiotensin II and [1-sarcosine, 8-isoleucine] angiotensin II, the threonine analog had no agonist effect: (a) on vascular smooth muscle in doses with significant antagonistic activity, and (b) on the adrenal cortex even when given in very large doses, e.g. 10 μg/kg/min [131].

Masaki *et al.* [132] studied the effect of [1-sarcosine, 8-threonine] angiotensin II in a new model of two kidney, one clip hypertension* in conscious dogs. Sustained hypertension was produced by a two-step procedure involving complete occlusion of a renal artery 2 weeks after it was partially constricted. Intravenous infusion of [1-sarcosine, 8-threonine] angiotensin II caused arterial pressure to decrease during the acute but not the chronic phase of renal hypertension. In this latter phase, plasma renin activity had returned to control values. Thus, [1-sarcosine, 8-threonine] angiotensin II has been found to be a potent and long-lasting antagonist of the direct response of angiotensin II on blood vessels and of its indirect actions via the release of catecholamines from the sympathoadrenal system or aldosterone from adrenal cortex.

Munoz-Ramirez *et al.* [133] have reported that, under inactin anesthesia, intravenous infusion of [$Sar^1$, $Thr^8$] angiotensin II produced a hypotensive effect in young spontaneously hypertensive rats treated with furosemide and in mature spontaneously hypertensive rats fed a low-sodium diet. This antagonist also lowered blood pressure of young and mature spontaneously hypertensive rats receiving a normal diet. These data suggest that the renin–angiotensin system is involved in genetic hypertension.

In a continued effort to increase the potency and *in vivo* half-life of these peptides [1-sarcosine, 5-*O*-methylthreonine, 8-*O*-methylthreonine] angiotensin II was synthesized recently [128]. This analog has been found to be the most potent antagonist thus far discovered. Due to its high potency the effective dose could be lowered to minimize the initial agonist effects. It has also been observed that the introduction of α-methyl-amino acids and a sacrosylsarcosine or polysarcosyl linkage at the *N*-terminus increases the duration of action of these peptides [128]. Similarly, by using lipophilic vehicles (e.g. cottonseed or linseed oil) for subcutaneous injections, the antagonist is slowly released into plasma over a period of 6–8 hr [128]. These are some of the directions in which further work is being pursued.

*Elucidation of mechanism of experimental renal hypertension with angiotensin II antagonists*

Apart from their possible clinical use, the antagonists of the pressor action of angiotensin II have provided some insight into the mechanism of experimental renal hypertension. During the early phase, when the plasma levels of renin activity and angiotensin II are high, angiotensin II antagonists greatly reduce blood pressure [54, 102, 132, 134–138]. These results suggest that, in the early phase of experimental renal hypertension, acute vasoconstrictor effect of angiotensin II plays an important role. In contrast, in the chronic phase of experimental renal hypertension, when the plasma levels of renin activity and angiotensin II are almost normal, angiotensin II antagonists are less effective. These results suggest that in the chronic phase of renal hypertension rise in blood pressure is not due to a vasoconstrictor effect of angiotensin II alone. An additional mechanism, therefore, seems likely. One of the suggestions put forward is that perhaps slow developing pressor action of angiotensin II may be involved.

It has been observed that prolonged infusions of low doses of angiotensin II (3–5 ng/kg/min for 2–3 weeks) in dogs, rabbits, rats and man caused a gradual rise in blood pressure leading to a hypertensive state that appeared similar to chronic renal hypertension [139–143]. These results suggested that prolonged infusion of low doses of angiotensin II altered the ratio of angiotensin II concentration to that of increase in blood pressure. In other words, a given concentration of angiotensin II could maintain a higher level of blood pressure. It was based on this reasoning that Riegger *et al.* [144] thought that prolonged infusion of angiotensin II antagonists may also cause a decrease in blood pressure in chronic renal hypertension. These authors observed that prolonged infusion of [$Sar^1$, $Ala^8$] angiotensin II [10 μg/kg/min for 12 hr) indeed caused a marked fall in blood pressure in two kidney, one clip chronic renal hypertensive rats. This decrease in blood pressure was more marked than that due to brief infusion (1–2 hr) or to a single injection of 180 μg/kg of [$Sar^1$, $Ala^8$] angiotensin II.

The results suggest that perhaps a slowly developing pressor action of angiotensin II may be involved in

*Two kidney, one clip hypertension indicates that in the Goldblatt hypertension both kidneys are intact but only one kidney is clipped. Two kidney, two clip hypertension means that both kidneys are intact and both are clipped. One kidney, one clip hypertension implies that one kidney has been removed and the other clipped. Similar terminology follows for the Page and Grollman hypertensions. This nomenclature, proposed by Dr. I. H. Page, described explicitly the methods for producing hypertension and, therefore, is preferable to the currently confused terminology of "one-kidney hypertension" and "two-kidney hypertension".

chronic renal hypertension. However, the mechanism of this involvement is not clear. A change in the response to blood vessels and in the distribution of sodium and neurogenic activity are some of the factors that may be playing a role [144].

*Stimulation of aldosterone secretion and its inhibition*

It is now well recognized that, although a number of factors such as hemorrhage, changes in posture, sodium deficiency, plasma concentration of potassium and ACTH can stimulate the biosynthesis of aldosterone, angiotensin plays an important role in causing an increase in aldosterone secretion. Studies on the biosynthesis of aldosterone indicated that the zona glomerulosa contains the enzymes necessary for conversion of corticosterone to aldosterone, but little or no 17α-hydroxylase to produce 17-hydroxylated steroids. In contrast, the zona fasciculata and reticularis contains 17α-hydroxylase, but lack the enzymes for the conversion of corticosterone to aldosterone. It appears, therefore, that the mineralocorticoids, particularly aldosterone, are exclusively synthesized in the outer zone, while 17-hydroxylated steroids including glucocorticoids (e.g. deoxycortisol and cortisol) are synthesized in the inner zone of the adrenal cortex [15, 145].

Angiotensin II is known to stimulate the secretion of aldosterone. However, in 1971, Blair-West *et al.* [146] reported that a naturally occurring metabolite of angiotensin II, namely des-1-aspartic acid-angiotensin II or "angiotensin III" (Fig. 4), which has 30–50 per cent of the pressor activity of angiotensin II, is at least as effective as angiotensin II in stimulating the biosynthesis of aldosterone in sheep. These results were confirmed in a variety of animals as well as in humans and have led to the hypothesis that angiotensin II is being metabolized locally in the adrenal cortex into angiotensin III which then acts as the true stimulus for the biosynthesis of aldosterone (for detailed reviews see Refs. 53, 54, 112 and 145–149).

This concept led to the speculation that, if it is angiotensin III which is responsible for the adrenal cortical stimulation, then (in analogy to the antagonists of the pressor action of angiotensin II) angiotensin III analogs with aliphatic amino acids at the carboxyl-terminus would be potent antagonists for the secretion of aldosterone. Based on this reasoning, Khosla *et al.* synthesized analogs, such as [8-alanine] angiotensin III, [8-isoleucine] angiotensin III [109] and [8-threonine] angiotensin III [129] (Fig. 4). *In vitro* (adrenal cortical cell suspensions) and *in vivo* (ACTH suppressed, bilaterally nephrectomized dog) studies indicated that [8-isoleucine] angiotensin III is a potent antagonist of the angiotensin II- or angiotensin III-induced secretion of aldosterone [150, 151]. In contrast, the antagonists of the pressor action of angiotensin II, e.g. [1-sarcosine, 8-alanine] angiotensin II, failed to inhibit completely the angiotensin III-induced secretion of aldosterone in rats [152, 153]. Similarly, [8-isoleucine]- and other such angiotensin III analogs proved to be poor antagonists of the angiotensin II-induced elevation of blood pressure [151, 154].

For further insight, the nonapeptide, des-1-aspartic acid-angiotensin I, was synthesized to test this as a precursor of angiotensin III (Fig. 5). The results suggested that the nonapeptide is a substrate for converting enzyme (peptidyl-dipeptide carboxyhydrolase) from porcine lung and plasma and is indeed converted into angiotensin III [81]. Bravo *et al.* [147, 155] observed that infusion of this nonapeptide increased aldosterone secretion in dogs, while Campbell *et al.* [156] reported that it increased plasma concentration of aldosterone in rats. The converting enzyme inhibitor SQ 20881 attenuated both the plasma aldosterone and blood pressure responses to the nonapeptide. Recently, Freeman *et al.* [157] provided evidence that equipressor doses of both angiotensin III and its precursor nonapeptide influence renin secretion, renal function, and plasma aldosterone concentration in dogs in a qualitatively similar manner; SQ 20881 completely blocked the arterial pressure and renal blood flow responses to the nonapeptide but not the responses to angiotensin III. Ackerly *et al.* [158] observed that the perfused feline adrenal converts approximately 68 per cent of angiotensin I in the perfusate to the nonapeptide, des-1-aspartic acid-angiotensin I. Studies by Campbell and Pettinger [159] indicated the presence of large amounts of aminopeptidase A in the adrenal cortex.

Collectively, the above data suggest that the biological actions of the nonapeptide, des-1-aspartic acid-angiotensin I, depend on its conversion to angiotensin

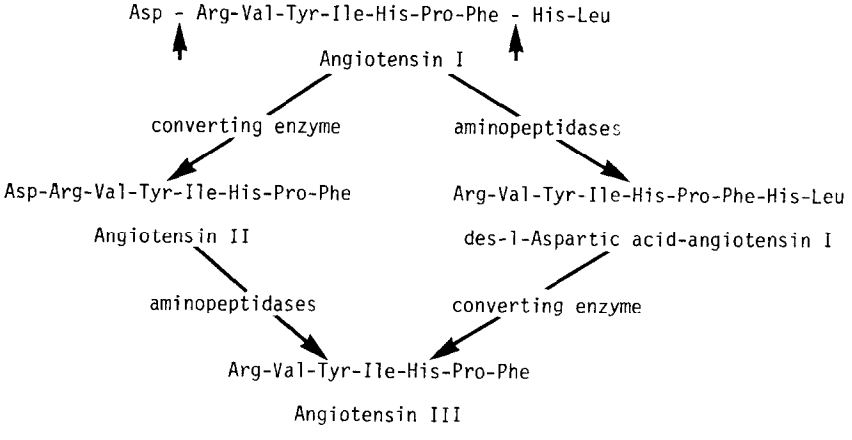



Fig. 5. Possible pathways for the formation of angiotensin III [81, 146].

III, and that endogenous generation of angiotensin III from the corresponding nonapeptide can occur via the action of converting enzyme, as originally postulated by Blair-West *et al.* [146]. Alternatively, angiotensin III could be generated from angiotensin II by the enzymatic cleavage of the *N*-terminal aspartyl residue by plasma and tissue aminopeptidases (Fig. 5). These concepts gained further support when Devynck *et al.* [160] demonstrated the presence of a specific binding site for angiotensin III in the rat adrenals.

So convincing were all these results that angiotensin III is recognized as a hormone in its own right. However, there are a number of points that do not fit into the hypothesis that angiotensin III is the stimulus for aldosterone secretion: (a) concentrations of angiotensin III in most species, including the plasma of dog and man are relatively low; only in rats does the concentration exceed that of angiotensin II [161]; (b) [1-sarcosine] angiotensin II, which is resistant to enzymatic degradation with aminopeptidases [105], stimulated aldosterone production to the same extent as angiotensin II or angiotensin III and in addition it had a longer duration of action [155], and (c) by using pure rat adrenal zona glomerulosa cells (with < 5% fasciculata cells), Khairallah *et al.* [49] showed that [5-isoleucine] angiotensin III and [5-valine] angiotensin III had only 7 and 16 per cent, respectively, of the steroidogenic activity of [5-valine] angiotensin II. Thus, with the present knowledge, we do not know whether it is angiotensin III alone or both angiotensin II and angiotensin III that are involved in the biosynthesis of aldosterone.

It can be reasoned that plasma levels may not be valid evidence if the formation and biologic action of angiotensin III is intracellular; there is some evidence to support this view [148, 162]. However, it is difficult to explain the results obtained in sodium-depleted conscious animals. Blair-West *et al.* [163] observed either no change or an agonistic action of [1-sarcosine, 8-isoleucine] angiotensin II on plasma aldosterone in conscious sodium-deficient sheep. Similarly, Stephens *et al.* [164] observed that administration of [8-isoleucine] angiotensin III to chronic, sodium-depleted conscious dogs failed to decrease plasma aldosterone; instead, apparently an agonistic response in aldosterone occurred. Clearly, further work is necessary to: (a) determine the role of angiotensin II and angiotensin III in stimulating the secretion of aldosterone in the normal and the sodium-depleted state, and (b) investigate the factors that give rise to discrepancies in stimulating aldosterone secretion with angiotensin II and angiotensin III in the *in vitro* and *in vivo* studies.

*Similar biological effects of angiotensin I, II and III*

It has been observed that, apart from adrenal cortex, a number of other tissues also respond to angiotensin III to produce biological effects similar to those of angiotensin II [165]. For example, angiotensin III exerts a positive inotropic action on the myocardium [166], it activates the enzyme tyrosine hydroxylase [167], and it affects renal blood flow and renal secretion [168]. It acts on peripheral arterioles to increase arterial pressure 30–50 per cent that of angiotensin II [169, 170], and it has been reported to be more potent than angiotensin II in increasing prostaglandin release from perfused rabbit mesentery [171, 172]. Similarly, it stimulates the secretion of catecholamines in adrenal medulla, although it possesses only 15 per cent of the secretory activity relative to an $ED_{50}$ molar concentration of angiotensin II [173]. When injected into the brain of cats, it exerts a pressor effect equal to that of angiotensin II [174]. Recently, Hutchinson *et al.* [175] have observed that the biologically active immunoreactive angiotensin present in normal canine cerebrospinal fluid is in the form of angiotensin III.

Due to rapid conversion of angiotensin I into angiotensin II, it is difficult to assess the biological importance of angiotensin I. However, investigations carried out by inhibiting the converting enzyme activity with peptides obtained from the venom of *B. jararaca* indicate that angiotensin I is equally potent to angiotensin II in stimulating the release of catecholamines from the adrenal medulla [176], and it acts on the central nervous system to raise systemic pressure and to induce thirst [177, 178]. In the zona fasciculata, it has been reported to stimulate steroid production [179], while in the kidneys it appears to modulate intrarenal blood flow.

It has been reported that [8-isoleucine] angiotensin I, [8-isoleucine] angiotensin II and [8-isoleucine] angiotensin III blocked angiotensin I-, II- or III-induced catecholamine secretion in retrograde perfused adrenal glands of cats [180]. [8-Isoleucine] angiotensin I and [8-isoleucine] angiotensin III were of particular interest since they are devoid of agonist activity. These results suggest that all three agonists and antagonists appear to interact with the same receptor site.

A likely explanation for similar biological effects of angiotensin I, II, and III (or their antagonists) could be that they possess a similar structural core that may "cross-bind" with each other's receptor sites. This hypothesis is supported by the finding that [1-sarcosine, 8-alanine] angiotensin II inhibits the steroidogenic effect of angiotensin I, II and III competitively in isolated fasciculata cells from bovine adrenal tissue [179]. On the contrary, it has been shown that adrenal cells from rats depleted of sodium were much more responsive to angiotensin III than cells from rats fed with a normal sodium diet [181]. Yet, change in dietary sodium did not affect the responsiveness to angiotensin II.

It appears that the involvement of the angiotensins in the regulation of blood pressure is a complex process that may interact with a number of other factors that can either cause hypertension (e.g. catecholamines, ACTH and adrenocortical steroids) or may show antihypertensive activity (e.g. prostaglandins and kallikrein-bradykinin).

*Brain renin-angiotensin*

In 1961, Bickerton and Buckley [182] demonstrated that angiotensin II can act directly on the central nervous system to increase arterial blood pressure. In 1966, Fitzsimons [183] reported that angiotensin II, infused intravenously, caused water-replete rats to drink. Further studies revealed that angiotensin II elicits a variety of other central actions including increased secretion of antidiuretic hormone (ADH or vasopressin), adrenocorticotropic hormone (ACTH) and acetylcholine. However, the doses of angiotensin II used to elicit increase in blood pressure or drinking reponse were very large and it is not clear if these effects of

angiotensin II represent its physiological actions. And if they do, then is angiotensin II synthesized in the brain or does the peripherally produced angiotensin II reach the central nervous system to elicit these responses? Some investigators believe that angiotensin II, being a polar compound, could not cross the blood–brain barrier.

Reid [184] showed that, when renin was administered centrally in rats, it increased water intake, raised blood pressure and increased the secretion of antidiuretic hormone, and that these effects were blocked by [1-sarcosine, 8-alanine] angiotensin II. This would imply an interaction between injected renin, cerebral renin substrate and converting enzyme to form angiotensin II. Existence of renin activity in brain has been demonstrated by the physiological effects produced by central administration of renin substrate. Further, renin substrate and immunoreactive angiotensin II have also been found in the cerebrospinal fluid of rats, sheep and man. These studies indicate that components required to generate angiotensins are present in the central nervous system. It has also been postulated that angiotensins produced within the central nervous system may affect structures in the midbrain and, possibly, areas of the third ventricle. (For detailed reviews on these aspects see refs. 184–187).

Contrary to the above hypothesis, there is evidence that angiotensin II synthesized peripherally can reach portions of the central nervous system, such as the area postrema and the subfornical organs, and may gain access to dipsogenic sites. This aspect has been reviewed recently [185]. Abdelaal *et al.* [188] observed that 24-hr dehydration of rats increased plasma concentration of angiotensin two and one-half times and that the individual increment in plasma angiotensin correlated positively with water intake. This is consistent with the findings of Summy-Long and Severs [189] and those of Hoffman and Phillips [190]. Malvin *et al.* [191] observed that cerebroventricular infusion of [1-sarcosine, 8-alanine] angiotensin II into rats for 75 min prior to their being allowed to drink significantly attenuated their water intake when they had been deprived of water for 30 hr. This indicated that [1-sarcosine, 8-alanine] angiotensin II blocked the endogenously generated angiotensin II. However, most of the rats receiving this antagonist finally began to drink water, suggesting other receptor mechanisms may also be involved.

The available data suggest that the role of brain angiotensin(s) in the regulation of blood pressure is perhaps related to its dipsogenic activity and/or its ability to stimulate the secretion of ADH, ACTH or acetylcholine. However, it is not clear whether these physiological actions are mediated by peripherally produced angiotensin(s) or by angiotensin(s) synthesized in the central nervous system.

*Participation of the nervous system in the regulation of blood pressure*

There is some evidence to suggest that serotonergic, central adrenergic and noradrenergic neurons may participate in the regulation of normal blood pressure and in the etiology of some experimental models of hypertension by their action on cardiac output, total peripheral resistance and blood volume and on the release of renin from the juxtaglomerular cells. Page and McCubbin [192] originally suggested that serotonin may have a role in regulating vascular tone, and since then considerable evidence has been put forward that suggests that serotonergic neurons are involved in the maintenance of vascular tone [193, 194]. They called serotonin an "amphibaric" substance because, when neural tone is low, it raises blood pressure powerfully, and when tone is high, it strongly depresses arterial pressure [192]. The mechanism by which serotonin plays this dual role is presently not clear.

Administration of the serotonin-synthesis inhibitor, *p*-chlorophenylalanine, or the α-methylserotonin precursor, α-methyl-5-hydroxytryptophan, lowers blood pressure in experimental hypertension [193, 195]. Similarly, the use of 5,6-dihydroxytryptamine, which destroys serotonergic nerve endings in brain and spinal cord, produces hypotension and bradycardia in unanesthetized rabbits [196].

The three neurotransmitters, dopamine, norepinephrine and epinephrine, collectively called catecholamines or sympathomimetic amines, are synthesized in nervous tissue or sites containing chromaffin tissue such as adrenal medulla. Their formation and release are mainly dependent on nerve impulses. The major synthetic pathway starts with an essential amino acid, L-tyrosine, which is taken up in the nerve ending and is hydroxylated by the action of tyrosine-hydroxylase to form dihydroxyphenylalanine (DOPA); the latter is then decarboxylated by the action of dopa-decarboxylase to form L-hydroxyphenylethylamine (dopamine). Dopamine is stored in the vesicles where dopamine-β-hydroxylase, which is exclusively localized in sympathetic nerve vesicles, converts dopamine into norepinephrine.

A major part of released norepinephrine is taken up into the nerve ending (re-uptake) where it is stored inside the granules in combination with ATP. Any free norepinephrine (or dopamine) in the sympathetic nerves is inactivated by oxidative deamination with monoamine oxidase (MAO) while circulating catecholamines are inactivated by *O*-methylation with catechol-*O*-methyltransferase (COMT).

The conversion of norepinephrine into epinephrine takes place exclusively in the adrenal medulla or possibly in some brain neurons (e.g. in the $A_1$ region of the brain). These sites contain the enzyme phenylethanolamine-*N*-methyltransferase (PNMT) that methylates norepinephrine to give epinephrine. The release of PNMT is dependent on cortisol synthesized in the adrenals [197].

The physiologic actions of epinephrine and norepinephrine are mediated through distinct receptors known as α- and β-adrenergic receptors (or adrenoceptors) [198, 199]. Both epinephrine and norepinephrine stimulate the α-adrenergic receptors in blood vessels to cause vasoconstriction of arterioles and venules. Similar contraction has been observed in uterine smooth muscle and stomach (sphincter). These effects are antagonized by α-adrenergic antagonists such as phentolamine, phenoxybenzamine, ergotamine and dihydroergocryptine.

There is some evidence that α-adrenergic effects may be associated with increased levels of cyclic 3′,5′-guanosine monophosphate (cyclic GMP) which may act as the second messenger. Most of the β-adrenergic

effects of catecholamines are associated with evaluation of intracellular levels of cyclic adenosine monophosphate (cyclic AMP) produced by stimulation of the membrane bound enzyme adenylate cyclase [200, 201].

Recently, dopamine has been found to mediate its physiological action through an adrenergic receptor called the dopaminergic receptor [202]. These receptors are found in certain brain regions and also in the renal vasculature where they apparently cause vasodilation. Epinephrine, norepinephrine and their antagonists are ineffective in blocking these effects. However, haloperidol has been found to be a selective antagonist of the dopaminergic receptors. Thus, neurotransmitters are in a dynamic state, being synthesized and metabolized, and yet they maintain a steady level in the nerves.

Blood pressure in hypertensive patients may be reduced by making adrenergic receptors unresponsive to norepinephrine by blocking norepinephrine biosynthesis, its release, re-uptake or storage, or by displacement of norepinephrine at the storage site. The last two decades have witnessed the synthesis of scores of drugs to block these junctions. However, the side effects often produced precluded most of these drugs from clinical use.

The effect of combination therapy also varied with the type of severity of hypertension, as also with the presence or absence of other diseases such as diabetes or asthma [203, 204]. For example, thiazides are contraindicated in hypertensive patients who are also suffering from diabetes or gout. Congestive heart failure should not be treated with propranolol or hydralizine (for detailed reviews see Refs. 205–210).

*Interrelations between various blood pressure regulatory systems and the mosaic theory of hypertension*

Thus, we have seen that the regulation of blood pressure is far from simple, as had been originally thought. There are a number of regulatory mechanisms that are interrelated into a dynamic equilibrium to ensure constant blood pressure and tissue perfusion. These, among other factors, include the adrenergic nervous system, the renin–angiotensin system and mineralocorticoids [119]. A derangement in any one of these regulatory systems could disturb the equilibrium of normotension, resulting in hypertension. Again, the renal hemodynamics and electrolyte transport systems may affect not only each other but they may also effect (or be affected by) blood pressure lowering mechanisms such as the kallikrein–kinin system, prostaglandins and the antidiuretic hormone. The observed blood pressure is the result of this equilibrium of various regulatory systems. And this is what the "mosaic theory of hypertension" is all about, and this is the reaon why Page [211] called hypertension a "multifactorial disease".

Since there are a number of regulatory factors that can raise blood pressure, so there are varieties of drugs that can reduce blood pressure. These drugs can be classified as vasodilators, diuretics, sympatholytic agents, angiotensin antagonists, converting enzyme inhibitors, renin inhibitors and so on. However, because of the multifactorial nature of this disease, none of these agents alone has proven effective in the long-term control of blood pressure of all hypertensives. But, when the right combination is found, they can be very effective. This is because we have barely started to understand this mosaic of regulatory mechanisms. Clearly, further work is necessary to understand it if we are to develop more effective measurement of the activity of each regulator.

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