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COMMENTARY

THE OTHER ANGIOTENSINS

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For more than three decades, the octapeptide, angiotensin II, has been considered as the mediator of renin-angiotensin responses. In 1971, two new findings raised the question of the importance of other angiotensins. Blair-West *et al.* [1] found that [des-Asp^1]-angiotensin II had aldosterone-stimulating activity similar to angiotensin II in sheep, and Peach [2] reported significant direct activity of angiotensin I on the adrenal medulla to increase catecholamine release.

Available data on the synthesis and metabolism of the angiotensins indicate that several pathways are involved (Fig. 1). The tetradecapeptide component of renin substrate is necessary for full activity. Renin splits the tetradecapeptide at the leucine—leucine bond to form the decapeptide, angiotensin I. The well-established conventional pathway for formation of angiotensin II is presented in the right side of the diagram. The initial product of aminopeptidase action on angiotensin II is [des-Asp^1]-angiotensin II (angiotensin III); this designation indicates that the heptapeptide fragment has biological activity. Further degradation of [des-Asp^1]-angiotensin II yields the hexapeptide, an inactive fragment. Endopeptidases and carboxypeptidases also degrade angiotensin II and angiotensin III with the formation of several inactive fragments.

An alternative pathway for the formation of angiotensin III is presented in the left side of Fig. 1. Plasma aminopeptidases compete with converting enzyme for the substrate angiotensin I; these aminopeptidases hydrolyze the decapeptide to the nonapeptide, [des-Asp^1]-angiotensin I. After the N-terminal aspartyl residue has been split off, aminopeptidases fail to attack the nonapeptide further. On the contrary, [des-Asp^1]-angiotensin I is an excellent substrate for converting enzyme which cleaves off the histidyl-leucine fragment to form angiotensin III [3].

Several approaches have been used to demonstrate the physiological or pathophysiological importance of the other angiotensins (angiotensin I, [des-Asp^1]-angiotensin I and angiotensin III). First, the qualitative and quantitative responses to these peptides have been compared to angiotensin II. Second, the characteristics of specific cellular receptors for the angiotensins including their relative affinity for angiotensins in comparison with angiotensin II have been studied. Third, angiotensin blockade has been employed extensively; several specific angiotensin analogs (both octapeptide and heptapeptide) have been used as well as converting enzyme inhibitors. Fourth, the plasma levels of the angiotensins have been measured. The first three approaches have been applied both *in vivo* and *in vitro*.

COMPARATIVE ACTIONS OF THE ANGIOTENSINS

The actions of angiotensin II and angiotensin III have been compared at several physiologically important receptor sites. The importance of the angiotensins in arterial pressure homeostasis was first revealed in one of the early studies with a specific competive antagonist of angiotensin II, [Sar^1, Ala^8]-angiotensin II; Johnson and Davis [4] found a striking decrease in arterial pressure with angiotensin II blockade in dogs with thoracic caval constriction and in sodium-depleted dogs. Angiotensin II appears to be twice as potent as the heptapeptide in its action on the peripheral arterioles to increase arterial pressure [5, 6].

In contrast, the comparative actions of angiotensin II and III on the adrenal cortex, the renal arterioles and the renal JG cells are very similar. Most of the studies on the adrenal cortex have dealt with the aldosterone response. Blair-West *et al.* [1] reported similar stimulatory actions for angiotensin II and III on aldosterone secretion in sheep. In adrenal cortical cell suspensions, Peach and Chiu [7] found angiotensin III to be more potent than angiotensin II in stimulating aldosterone biosynthesis. Campbell *et al.* [8] obtained almost identical dose–response curves for plasma aldosterone concentration during infusions of angiotensin II and III in conscious rats. To evaluate the qualitative as well as the quantitative responses to these two angiotensins (II and III), Lohmeier *et al.* [5] studied the steroid profile of adrenocortical secretion for aldosterone, corticosterone and cortisol in dogs. They found that both angiotensin II and III increased secretion of all three steroids and the quantitative responses were not significantly different. In this same study, the heptapeptide was only half as effective in increasing arterial pressure as angiotensin II, but the decline in adrenal plasma flow was essentially the same for the two peptides. In studies with [Sar^1, Ala^8]-angiotensin II, Johnson and Davis [4, 9] demonstrated a striking fall in cortisol secretion after angiotensin II blockade which provides strong evidence for the presence of functional angiotensin receptors in the zonae fasciculata and reticularis of the adrenal cortex. These results suggest a common pathway for the action of these two angiotensins on adrenocortical biosynthesis, and it seems likely that there are common angiotensin receptor sites in the zona glomerulosa for aldosterone and in the two inner zones of the adrenal cortex for cortisol.

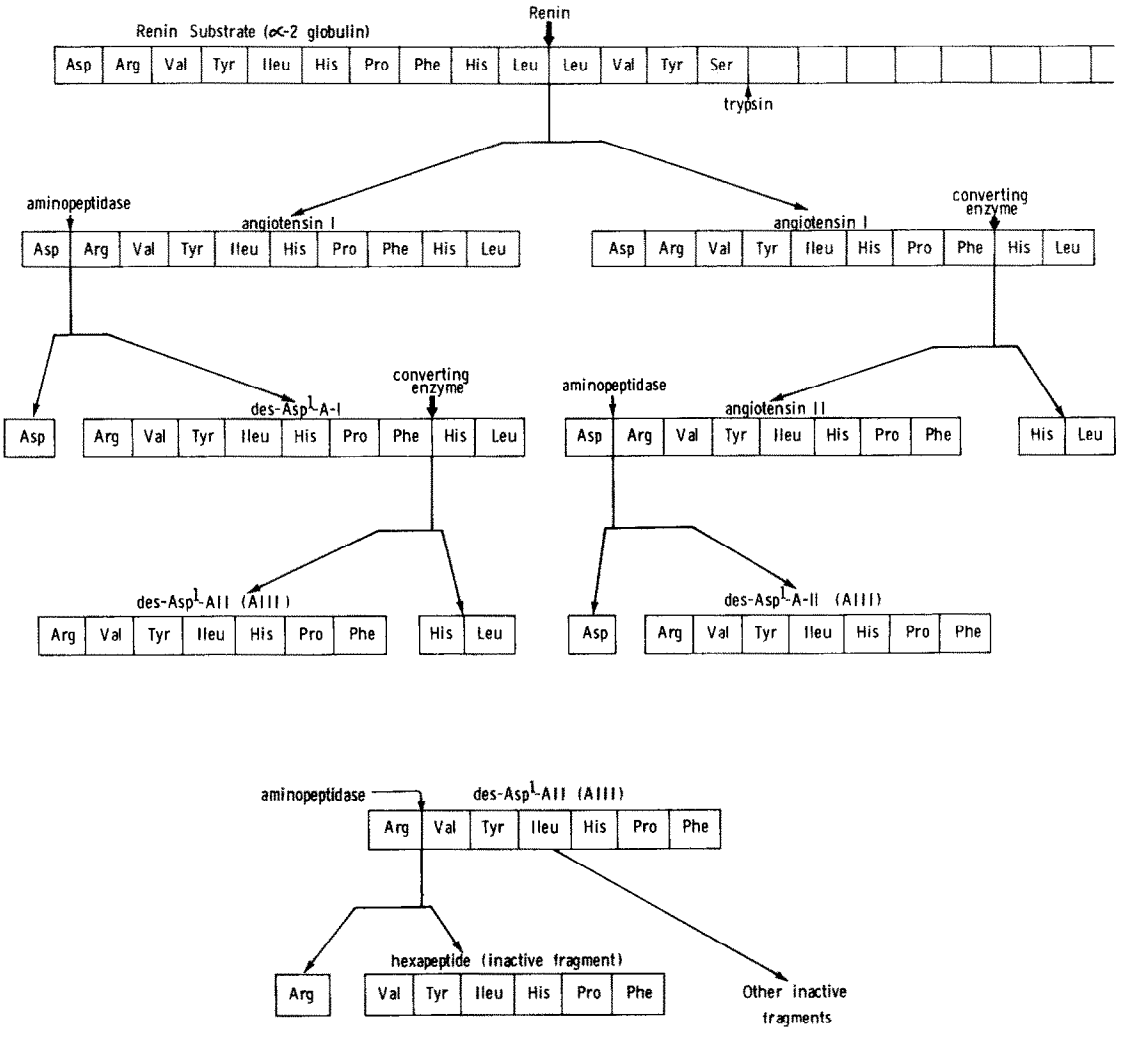

Fig. 1. Possible pathways for the formation of [des-Asp^1]-angiotensin II (angiotensin III). The abbreviations des-Asp^1-A-I and des-Asp^1-A-II are for [des-Asp^1]-angiotensin I and [des-Asp^1]-angiotensin II respectively.

Similar responses in the renal blood flow and in renin secretion have been observed with angiotensin II and III [10]. The importance of the angiotensins in the control of renal blood flow was demonstrated by angiotensin II blockdade in several experimental situations including sodium depletion, thoracic caval constriction and experimental high output heart failure [11, 12]. Freeman *et al.* [10] infused angiotensin II and III into the renal artery in normal dogs at rates which increased the renal blood levels by only 7 ng/100 ml. The decreases in renal blood flow and renin secretion were essentially the same for the two peptides. At this dose, neither peptide altered arterial pressure, glomerular filtration rate or the rate of renal sodium excretion. When similar studies were conducted in sodium-depleted dogs, renin secretion decreased about the same for the two peptides, but renal blood flow as well as arterial pressure, glomerular filtration rate and renal sodium excretion failed to change. Failure of renal blood flow to fall in sodium-depleted animals has been interpreted to reflect the occupancy of renal arteriolar receptor sites with endogenous angiotensin. Comparable dose–response curves for renal blood flow were also obtained after single intra-arterial injections of angiotensin II and III in normal and sodium-depleted dogs but, again, the sensitivity of the renal vasculature was less in the sodium-depleted animals. In a recent preliminary report, Taub *et al.* [13] have obtained similar threshold doses and dose–response curves for renal blood flow for angiotensin II and III during intrarenal arterial infusions; cross tachyphylaxis was also observed after infusion of each peptide. These findings on the kidney show that angiotensin II and III have similar actions on the renal vasculature and on renin secretion. The results provide support for the concept that angiotensin III mediates, at least in part, the renal response of the renin-angiotensin system.

Several other studies show that cellular receptor sites for the heptapeptide are present in other tissues. Blumberg *et al.* [14] reported that angiotensin III increased prostaglandin release from perfused rabbit

mesentery and was more potent that either angiotensin I or II. Both angiotensin II and III have been reported to exert a positive inotropic action on the myocardium [15], to activate the enzyme tyrosine hydrolyase [16] and to inhibit lung converting enzyme [17].

Other possible receptor sites for the angiotensins need to be studied. Both angiotensin II and III given intracranially in rats [18] and in goats [19] induced drinking, but angiotensin II was more potent. Angiotensin II given intravenously provoked thirst in the rat, and the response appeared to be mediated by the subfornical organ since its presence was found necessary for the response [20]. Similar studies with angiotensin III have not been reported.

A possible physiological role for both angiotensin I and its nonapeptide fragment has been considered. Peach [2] demonstrated that angiotensin I was equipotent to angiotensin II in inducing catecholamine release from the isolated perfused adrenal; conversion of angiotensin I to II was carefully and convincingly excluded. However, evidence is lacking to demonstrate a physiological role for angiotensin I in the control of adrenal medullary secretion. There is some evidence that angiotensin I contributes to the release of norepinephrine from the peripheral sympathetic neurons. Also, centrally administered angiotensin I has been reported to produce directly a pressor response and to stimulate drinking, but both the peripheral and central actions of angiotensin I need further study and clarification. Specific competitive antagonists for angiotensin I might help in resolving the question of the physiologic role of this peptide.

Another attractive proposal for the action of angiotensin I is in the control of intrarenal blood flow. The vasoconstrictor response to angiotensin I was not blocked by the nonapeptide converting enzyme inhibitor (SQ 20881) and a selective decrease in inner cortical and medullary flow has been reported [21]. It should be pointed out, however, that this converting enzyme inhibitor does not inhibit all the enzymes capable of converting angiotensin I to II [22]. It is well-known that angiotensin I blockade with SQ 20881 is seldom if ever complete.

The C-terminal nonapeptide is formed from angiotensin I by aminopeptidases (Fig. 1) and there is evidence [23, 24] to show that an alternative pathway for the formation of angiotensin III may exist. Indeed, recent preliminary reports [23, 24] indicate that [des-Asp^1]-angiotensin I has biological activity. Schmitz *et al.* [23] found that [des-Asp^1]-angiotensin I increased plasma aldosterone concentration and arterial pressure in conscious rats and pretreatment of the animals with a converting enzyme inhibitor significantly impaired the responses. The data suggest that the biological activity was dependent upon conversion of the nonapeptide to the heptapeptide by converting enzyme. Bravo *et al.* [24] have also reported that the nonapeptide had about half the steroidogenic potency of angiotensin II or III, but was devoid of pressor activity at the dose given. Also, in a cat adrenal glomerulosa cell preparation, [des-Asp^1]-angiotensin I has steroidogenic activity which was completely blocked by SQ 20881 [24]. Both of these reports lend support to the occurrence of an alternative pathway for the formation of angiotensin III *in vivo* and make it unnecessary to postulate a direct action of the nonapeptide.

CHARACTERISTICS OF CELLULAR RECEPTORS FOR THE ANGIOTENSINS

Evidence from studies both *in vitro* and *in vivo* has been purported to suggest a greater affinity of cellular receptors for angiotensin III than angiotensin II. Chiu and Peach [25], in studies of rabbit adrenal cell suspensions, found that a 25-fold higher dose of the competitive antagonist [Sar^1-ile^8]-angiotensin II was required to block angiotensin III induced-aldosterone biosynthesis compared with angiotensin II-induced aldosterone production. In the conscious rat, Campbell *et al.* [8] found that [Sar^1-ala^8]-angiotensin II was more effective in blocking the aldosterone-stimulating action of angiotensin II than the heptapeptide. Similarly, during measurement of the aldosterone secretion rate in the rat, Spielman *et al.* [6] found that a larger dose of [Sar^1-ala^8]-angiotensin II was required to block the response to angiotensin III than angiotensin II. Finally, studies by Sarstedt *et al.* [26] with a heptapeptide angiotensin antagonist, [des-Asp^1, Ile^8]-angiotensin II, showed that a lower dose than the effective dose of [Sar^1-ile^8]-angiotensin II was needed to achieve equal blockade of the aldosterone responses to both angiotensin II and III. Thus, the results, both *in vitro* and *in vivo*, can be interpreted to support the concept of greater receptor affinity for the heptapeptide than for angiotensin II. On the other hand, data obtained from binding studies have revealed that angiotensin II and III were approximately equally potent in displacing isotopically labeled angiotensin II from adrenal cortex or from various preparations of adrenal cortical tissue [27–29].

There are several findings which suggest that the cellular receptors for the angiotensins in the zona glomerulosa are functionally different from vascular smooth muscle receptors. First, angiotensin II has been found to be twice as potent as angiotensin III in increasing arterial pressure whereas the angiotensins are about equally potent in steroidogenic activity *in vivo*. Second, smaller doses of [Sar^1, Ala^8]-angiotensin II were required to block the pressor responses to both angiotensin II and III than were required to block the aldosterone-stimulating action of these peptides [6]. In studies *in vitro*, Williams *et al.* [30] found that the dose of the analogue, [Phe^4, Tyr^8]-angiotensin II, which blocked the action of angiotensin II in the isolated rabbit aortic strip preparation failed to block angiotensin II-induced aldosterone production in adrenal cortical cell suspensions from the rat. Third, Bravo *et al.* [31, 32] observed blockade of the pressor response to angiotensin II with an octapeptide antagonist, [Sar^1, Ile^8]-angiotensin II, while angiotensin II-induced aldosterone biosynthesis was not blocked by this antagonist. When, however, a heptapeptide antagonist, [des-Asp^1, Ile^8]-angiotensin II, was given, it failed to block the pressor response to angiotensin II but significantly decreased the aldosterone response to angiotensin II [32, 33].

These observations on the efficacy of antagonists to produce blockade of exogenous synthetic angiotensin II raise the question of their effectiveness against

endogenous angiotensin II in conditions with hyperangiotensinemia. This type of information is critical for more complete evaluation of receptor characteristics as well as for the determination of the relative importance of angiotensin II and III in homeostasis. The only data of this type have been provided by Sarstedt *et al.* [26]. They found that the octapeptide antagonist [Sar^1, Ile^8]-angiotensin II reduced arterial pressure in sodium-depleted rats but urinary aldosterone excretion was unchanged, while the opposite type of response occurred with a heptapeptide antagonist in rats depleted of sodium. Since the principal route for aldosterone excretion in the rat is fecal and only a small percentage of secreted aldosterone is excreted, this type of study needs to be repeated with measurements of either aldosterone secretion or the plasma aldosterone concentration.

EVIDENCE FOR A HOMEOSTATIC ROLE FOR ANGIOTENSIN III

Many of the findings cited above are consistent with the concept that angiotensin III mediates, at least in part, the renin-angiotensin response. First, angiotensin III was more potent than angiotensin II in stimulating aldosterone production *in vitro* and was equipotent *in vivo*. Similarly, the two peptides were equipotent in decreasing renal blood flow and renin secretion. It is clear from studies with angiotensin II blockade that both peptides exert a physiological action on the adrenal cortex and the kidney. Second, larger doses of the angiotensin II analogues which act as specific competitive antagonists were required for blocking the aldosterone-stimulating action of the heptapeptide than were needed for blockade of angiotensin II. Third, angiotensin III antagonists such as [des-Asp^1, Ile^8]-angiotensin II were more effective than octapeptide antagonists in blockade of aldosterone production produced by either angiotensin II or III. These data have been interpreted as evidence that the adrenal zona glomerulosa receptors have a greater affinity for angiotensin III than II and, thus, as favoring a physiological role for angiotensin III. Fourth, the observations of functionally different receptors with angiotensin II being selective for smooth muscle in the peripheral arterioles and with angiotensin III acting on the adrenal zona glomerulosa, the renal vasculature and the JG cells might be interpreted to support the concept of a physiological role for angiotensin III in the adrenal cortex and the kidney. Finally, it should be pointed out, however, that it is surprising that the potency of angiotensin III is not substantially greater than that of angiotensin II, if indeed angiotensin III plays an important role physiologically.

These considerations raise the question of the relative plasma levels of angiotensin II and III. Several reports have provided estimates which suggest that most of the circulating angiotensin is the octapeptide in normal man, dog and sheep. In the most complete study, Semple and Morten [34] reported that approximately 85–88 per cent of the plasma levels of the angiotensins was II and 12-15 per cent was III in normal man and dog; in contrast, the ratio of angiotensin II and III in normal rat plasma was 1:1. In normal animals, therefore, it seems unlikely that the circulating plasma level of angiotensin III contributes sugstantially to the action of the angiotensins at cellular receptor sites in the target tissues. It is possible, however, that angiotensin III is generated locally and there is substantial evidence for such a mechanism. Angiotensin III has been identified [35, 36] as a metabolite of [^{3}H]-angiotensin II in the effluent from both the coronary and renal circulations in the rat; this finding is consistent with the known actions of angiotensin III on both the myocardium and the kidney. Also, [^{14}C]-angiotensin II was degraded to angiotensin III in adrenal cortical cell suspensions [37] and angiotensin III has been found intracellularly in adrenal cortical cells exposed to labeled angiotensin II [22]. And, as with the myocardium and the kidney, the adrenal cortex is an important site of action of angiotensin III. Finally, it should be pointed out that no data are available yet on either the plasma level or the rates of local generation of angiotensin III in experimental or clinical states with increased activity of the renin-angiotensin system; either or both mechanisms for influencing the availability of angiotensin III at receptor sites might be altered. In this regard, the finding that the anoxic myocardium is more sensitive to angiotensin III than the normal myocardium emphasizes the need for data in pathophysiological conditions [15].

The possibility has also been suggested [22, 38] that angiotensin III acts as an intracellular hormone. It is conceivable that the action of angiotensin II is to bind to the cell membrane of the target cell with the subsequent formation of angiotensin III which induces the intracellular response. Indeed, there is evidence for aminopeptidases in various tissue preparations. This is an important area for investigation, and a final solution to the question of the physiologic and pathophysiologic role of angiotensin III must await the results of future research. A blocking agent specific for angiotensin III might be helpful in determining the role of the heptapeptide in homeostasis.

In summary, biological activity has been demonstrated for three angiotensins (angiotensin I, [des-Asp^1]-angiotensin I and angiotensin III) other than angiotensin II. Although a number of tissues respond to angiotensin I, evidence is lacking to demonstrate a physiologic role for this peptide. Available data indicate that [des-Asp^1]-angiotensin I is converted to angiotensin III which accounts for the observed responses. Angiotensin II and III exert actions in several areas including all three zones of the adrenal cortex, the renal arterioles and JG cells in the kidney and the peripheral arterioles. Angiotensin II appears to mediate the renin-angiotensin response in the peripheral arterioles, while potency studies *in vivo* have revealed that angiotensin II and III were equally effective in their actions on receptors in the adrenal cortex and the kidney. Considerable indirect evidence indicates that angiotensin III mediates, at least in part, the renin-angiotensin response in the adrenal cortex and kidney. This includes: (1) apparent greater receptor affinity of zona glomerulosa cells for the heptapeptide than the octapeptide, (2) heptapeptide analogues are better antagonists than octapeptide analogues in inhibiting aldosterone-stimulating activity induced by either angiotensin II or III, (3) equal potency of angiotensin II and III in their action on

the adrenal cortex and the kidney, and (4) local generation of angiotensin III in the renal circulation and in adrenal cortical cell suspensions. A more complete solution to this intriguing problem awaits additional study of the plasma levels of the angiotensins in different experimental and clinical situations and in the elucidation of the intracellular functions of the angiotensins.

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