Angiotensin II, Aldosterone and Arterial Pressure: A Quantitative Approach

Arthur C. Corcoran Memorial Lecture

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l AM deeply appreciative of the recognition accorded to me and to my colleagues by my being asked to deliver the A. C. Corcoran Memorial Lecture. I was, unfortunately, never privileged to meet the late Dr. Corcoran, although I was present at the meeting in Paris in 1965 when his death was announced. The manner in which this news was received, and the tributes paid to him at that time, left no doubt of the respect and affection he enjoyed. His scientific achievements stood then, and still stand, without need of an advocate.

Introduction

A proper understanding of the physiological and pathophysiological functions of a hormone system requires a strictly quantitative assessment. Not only has the concentration of the active component in blood to be measured in absolute terms, but the action of that component must be considered in relation to the shape and position of the dose-response curve in question, and to the part of that curve on which the endogenous concentration lies. Particular attention must be paid to these features when examining the effects of antagonists and inhibitors. In addition, consideration must be given to aspects of the hormone system that may not be reflected immediately in circulating blood, such as accumulation or local generation in various tissues.

The principal active component of the renin-angiotensin system in peripheral blood of man is the octapeptide angiotensin II. The present paper is largely an account of clinical and laboratory studies performed in this department since 1970, and is concerned with a quantitative evaluation, in terms of the above principles, of circulating plasma concentrations of angiotensin II in relation to various physiological functions and particularly to arterial blood pressure, water and electrolyte excretion, and plasma aldosterone concentration.

Methods

Plasma angiotensin II was estimated by radioimmunoassay; plasma aldosterone either by a double isotope derivative method, or by radioimmunoassay. Details of other techniques used are given in the various papers referred to in the text.

Advantages of Angiotensin II Assay

Although practicable methods have been available for the assay of angiotensin II in peripheral blood for over a decade, only a few laboratories have employed these on any scale. Indeed, some workers have argued against an investment in angiotensin II assays and in favor of renin methodology. In the present context, however, we consider measurement of angiotensin II concentration inherently superior for the following reasons.

Physiological Relevancy of Angiotensin II

In man, the octapeptide angiotensin II is the principal active component of the renin-angiotensin system in peripheral blood, the absolute values being generally only slightly lower in venous than in arterial plasma. Despite earlier reports to the contrary, it now appears that comparatively low concentrations of smaller peptide metabolites are present in venous plasma and thus measurements of venous plasma angiotensin II concentrations are more relevant than...
was previously supposed. However, during the intra-
venous administration of angiotensin II, arterial con-
centrations may considerably exceed those in venous plasma, an aspect which needs to be recognized in experimental studies.

Direct assays in patients undergoing cardiac catheterization have demonstrated higher concentra-
tions of angiotensin II in left ventricular plasma than in pulmonary arterial plasma drawn simultaneously, whereas angiotensin I levels were proportionately higher in pulmonary arterial blood. These findings are in accordance with a major formation of angiotensin II from angiotensin I in transit across the lungs.

Similar studies in patients with renal and essential hypertension have shown, across kidneys not secre-
ting renin, lower angiotensin II concentrations in renal venous than in arterial plasma, thus demonstrating net extraction of angiotensin II by the kidney. Chromatography of plasma from all these sites has confirmed the predominance of angiotensin II over smaller peptide metabolites.

In the dog, lower angiotensin II concentrations are found in hepatic venous than in arterial blood, but little is known of the proportion of the different peptide fragments in hepatic venous blood.

In the dog, as in man, peripheral concentrations of the heptapeptide angiotensin III (des-Asp\(^{1}\)-angiotensin II) are low compared to those of angiotensin II, and angiotensin III seems unlikely to be a major circulating hormone in these species. Only in the rat, of the animals studied so far, do the molar proportions of angiotensin III exceed those of angiotensin II in arterial plasma. Suggestions have been made that angiotensin III may be a mediator of the aldosterone-stimulant action of the renin-angiotensin system, perhaps being formed locally in the zona glomerulosa. However, following incubation of radioactively labeled angiotensin II with canine adrenal cells, Douglas et al. were able to recover the octapeptide unchanged. They conclude that such a conversion is not obligatory. These various hypotheses remain sub judice.

**Administration of Angiotensin II: Construction of Dose-Response Curves**

Angiotensin II is available as a pure synthetic pep-
tide and can therefore, with reasonable safety, be in-
flaged into both man and laboratory animals in a way not possible with renin preparations. Thus, dose-
response curves can be constructed of plasma angiotensin II concentrations against, for example, arterial pressure and plasma aldosterone concentra-
tions, a procedure which is crucial to a proper under-
standing of the actions of the renin-angiotensin system. It is emphasized that this requires the measurement of plasma angiotensin II concentra-
tions before and during infusion, because infusions alone, although providing often valuable inform-
ation, cannot show the position of the dose-
response curve. With due precaution, the effects also of prolonged administration of angiotensin II can be examined in man, as well as in experimental animals.

**Antagonists and Inhibitors**

Various synthetic analogues of angiotensin II are now available which, when infused, antagonize its ac-
tions. Clearly, it is more appropriate to interpret the effect of these antagonists in terms of dose-response curves and the prevailing level of angiotensin II, than in relation to less closely related components of the system, such as renin.

The need for angiotensin II assays is even more necessary when interpreting the effects of inhibitors of the enzyme responsible for the conversion of angiotensin I to angiotensin II. When these inhibitors are given, it is mandatory to demonstrate the quant-
itative effectiveness of the enzyme inhibition by a cor-
responding reduction in circulating plasma angioten-
sin II concentration. Simply to show diminution of the pressor effect of injected angiotensin I is not enough; apart from the crudeness of this test, any such diminu-
tion might be due to the masking effect of a vasodilator.

**Limitations of Renin Assays**

Renin assays, by contrast, have distinct limitations. A very wide diversity of techniques has been employed for renin assay, and these give very different quantitative information for the following reasons. Some methods ("plasma renin concentration" assays) have a constant substrate concentration in the incubation mixture, or the effect of variation in substrate concentra-
tion is corrected for in some way. Other methods ("plasma renin activity" assays) are influenced by differences in the concentration of both renin and sub-
strate in the sample. Certain methods activate inactive renin in the course of extraction; others probably do not. A wide range of buffers, of varied pH and os-
molality, has been employed by different workers in incubation mixtures; therefore the rate of angiotensin generation varies accordingly. The linearity of angiotensin generation during in vitro incubation is uncertain with some methods.

Despite these numerous sources of diversity, only rarely have attempts been made to calibrate the results of renin assay in terms of a standard renin; indeed, only in recent years has an acceptable International Human Renin Standard been recognized. In one collaborative study, in which 17 laboratories assayed the same seven human plasma samples, the returned results were quantitatively very different when expressed in terms of the rate of angiotensin generation in incubation mixtures, and even when calibrated and expressed in terms of a standard renin. Perhaps surprisingly, there was agreement on the ranking order of the renin content of the different samples.

Therefore, a great deal of information on the renin-
angiotensin system has been acquired in recent years, but much of this material is simply qualitative or, at best, semi-quantitative. Trends and correlations are indicated rather than absolute values, and such inform-
ation is of limited use, although usually adequate for clinical diagnosis.
Angiotensin II in Low-Renin States

Renin, rather than angiotensin II, assays are generally regarded as more suitable in circumstances where the renin-angiotensin system is suppressed. Although there is some substance in this view, it should be emphasized that in a group of normal subjects given fludrocortisone, a clear fall in peripheral plasma angiotensin II concentration was observed in every instance. Moreover, in a series of 37 untreated patients with primary aldosterone excess, from all of whom an adrenocortical adenoma was later removed, there was a significant inverse correlation between the high plasma aldosterone and the suppressed plasma angiotensin II concentrations (r = -0.30, p < 0.05), such as had earlier been reported for plasma renin versus aldosterone concentrations.

Advantages of Angiotensin II Assay

Briefly, the advantages of employing angiotensin II assay are that attention is addressed to the main hormonal component of the renin-angiotensin system; that dose-response curves can be constructed; that the actions of antagonists and inhibitors can be more critically assessed; and that direct quantitative comparison between the results obtained by different workers is possible.

Importance of Angiotensin II in Aldosterone Control

The concept that the renin-angiotensin system is a major influence on aldosterone secretion is based on the observation that aldosterone secretion is regulated by a renal hormone, and the demonstration that the administration of angiotensin II, at least in the short term, leads to an increase in the secretion, excretion and plasma concentration of aldosterone. In man, parallel changes are seen in renin (or angiotensin II) and aldosterone in a wide variety of clinical and physiological circumstances, albeit with some noteworthy exceptions, such as normal pregnancy, total fasting, ascent to high altitude, and in acute renal failure. We have reviewed the evidence elsewhere.

In man, elevation of angiotensin II seems capable of sustaining an increase in aldosterone secretion indefinitely. Thus, so far as we are aware, in every case of renin-secreting tumor in which measurements have been made, aldosterone excretion, secretion, or plasma concentration has been elevated. Moreover, the intravenous administration of angiotensin II to normal volunteers for up to 5 days sustained increased plasma concentrations and excretion rates of aldosterone throughout.

In the sheep, by contrast, it has been reported that continuous administration of angiotensin II leads to only a transient increase in aldosterone secretion. In the dog, the evidence is controversial, some workers finding sustained elevation, others not.

A key observation in man is that patients with renin deficiency, and hence low or absent plasma angiotensin II, show selective absence of aldosterone, even in the face of sodium depletion and marked hypokalemia. However, when such patients are infused with synthetic angiotensin II, plasma aldosterone can, at least in some cases, be restored to the normal range (fig. 1).

Angiotensin II: Aldosterone Dose-Response Curve in Sodium Depletion

Despite earlier reports to the contrary, we have consistently found that in man, simple dietary sodium restriction causes progressive elevation of plasma angiotensin II, in both peripheral venous and arterial plasma, detectable from as early as 24 hours from commencing the low salt diet.

Figure 2 shows data pooled from four separate studies in which normal subjects were given a diet containing 12 mEq of sodium or less daily for up to 5 days, while potassium intake remained constant and in the normal range. Mean plasma angiotensin II was significantly raised 24 hours after commencing the low sodium intake, and continued to increase thereafter. Plasma aldosterone was significantly elevated at 48 hours, and also continued to rise over 5 days. Moreover, the relationship of plasma aldosterone to angiotensin II steepened progressively during prolonged sodium deprivation, suggesting sensitization of the adrenocortical response to angiotensin II with sodium depletion.

When, in another experiment, the angiotensin II-aldosterone dose-response relationship was tested in
normal volunteers by giving incremental infusions of angiotensin II first in normal sodium balance and then after loss of an average of 150 mEq of sodium, the plasma aldosterone response to angiotensin II was seen to be both elevated and significantly steepened by sodium depletion. If the data from the infusion experiment are combined with those from the dietary sodium depletion studies (fig. 2), it can be clearly seen how progressive sodium depletion alters the angiotensin II:aldosterone relationship from the lower to the upper dose-response curve.

These observations, together with similar findings, resolved the questions raised by two reports in which it was noted that in sodium-depleted man, plasma aldosterone was disproportionately high for the concurrent plasma angiotensin II level, as judged from the relationship in normal sodium balance. In the absence of dose-response data, the authors had interpreted their results as not showing sensitization of the zona glomerulosa to angiotensin II in sodium depletion, and thus as evidence contrary to an important role of angiotensin II in the control of aldosterone secretion in these circumstances. However, it now seems clear that sodium depletion does enhance the adrenocortical response to angiotensin II.

This phenomenon was investigated more extensively in the dog. As in man, depletion of a mean of 2.4 mEq sodium/kg elevated and steepened the angiotensin II:aldosterone dose-response curve, but, conversely, sodium loading depressed and flattened the curve. Interestingly, severe sodium depletion (mean loss 6.3 mEq/kg), although further elevating the curve, caused individual aldosterone responses to become irregular and unpredictable, in marked contrast to the very consistent responses observed with more modest sodium depletion. This suggests that the upper plateau of a sigmoid dose-response curve may have been reached in very marked sodium depletion, and might explain the inability of Blair-West and colleagues to obtain an aldosterone response to administered angiotensin II in sodium-depleted sheep. Their animals were much more sodium-deficient, in both absolute terms and in relation to body weight, than even our most severely sodium-depleted dogs.
Mechanism of Shift in Angiotensin II-Aldosterone Curve

One possibility, which had been raised by the earlier studies of Ganong and his colleagues in the dog, was that lengthy exposure of the adrenocortical zona glomerulosa to high concentrations of angiotensin II might enhance the aldosterone-stimulant effect of additional increments of angiotensin II. This was further explored by Oelkers et al. Normal volunteers were fed a fixed diet containing known and constant quantities of sodium and potassium. Urine was collected and analyzed every 8 hours and sodium and potassium supplements were given to maintain constant sodium and potassium status. Three-day infusions of angiotensin II were made at doses calculated to mimic those occurring endogenously in sodium depletion. This procedure did lead to significant steepening of the angiotensin II:aldosterone dose-response curve, but not to the full extent observed in sodium depletion. In a subsequent series of experiments, in which volunteers on normal sodium intake were infused with angiotensin II, there was a sustained increase in plasma and urinary aldosterone, which further and significantly increased between Days 1 and 2 when gross sodium retention was prevented. Again, however, the steepening of the dose-response curve was less than that seen in sodium depletion. Both sets of experiments suggested that a trophic effect on the adrenal cortex of sustained high levels of angiotensin II might provide part of the explanation of the steeper dose-response curve, but an additional factor or factors was also necessary.

Further evidence consistent with this hypothetical trophic action of increased angiotensin II concentrations was obtained in patients with renal and/or malignant phase hypertension and elevated plasma angiotensin II concentrations. These subjects were found to have higher plasma levels of aldosterone for a given plasma angiotensin II concentration than normal subjects in normal sodium balance and briefly infused with angiotensin II. This exalted angiotensin II:aldosterone relationship was particularly well shown before operation in a patient with a renin-secreting tumor.

Hauger et al. have recently demonstrated that in the rat, administration of angiotensin II increases the number of angiotensin II receptors in the zona glomerulosa, and enhances the aldosterone response to angiotensin II. They regarded their findings as supporting the concept of a trophic action of angiotensin II on the adrenocortical zona glomerulosa.

If angiotensin II does have a trophic action of this kind, then the converse should also hold, in that subjects with chronically low levels of circulating angiotensin II should have depressed aldosterone responses to administered angiotensin II. Evidence of this was obtained in an elderly woman with isolated renin deficiency and analdosteronism. Although, when she was infused with angiotensin II, plasma aldosterone increased from undetectable values to the normal range, the response was subnormal in comparison with that in similarly infused normal subjects.

Anephric patients also have a depressed response of plasma aldosterone to administered angiotensin II. Such subjects are of special interest in this context because they permit an evaluation of the effects of sodium depletion on the aldosterone response to angiotensin II in the absence of changes in the endogenous plasma angiotensin II concentration, and hence may reveal adrenocortical sensitizing mechanisms that are independent of the renin-angiotensin system. In a series of six anephric patients studied by Deheneffe et al., sodium depletion led to highly significant enhancement of the response of plasma aldosterone to infused angiotensin II, although this response remained distinctly below that of normal subjects. In all the anephric patients, a ceiling of plasma aldosterone was identified, which could not be exceeded despite further marked increments of arterial plasma angiotensin II.

Thus at least two components of the steepened angiotensin II:aldosterone dose-response curve of sodium depletion have been demonstrated, one dependent on increased ambient levels of angiotensin II, and the other angiotensin-independent.

Further information was obtained by examining the relationship between plasma angiotensin II and other corticosteroids than aldosterone during angiotensin infusions in normal subjects before and after sodium depletion. Plasma concentrations of cortisol, corticosterone and deoxycorticosterone were not significantly affected by sodium deprivation or by infusion of angiotensin II. In normal sodium status, angiotensin II infusion caused an increase in plasma 18-hydroxycorticosterone, the dose-response curve having a slope very similar to that for aldosterone. Sodium depletion caused a much greater rise of basal plasma 18-hydroxycorticosterone than of aldosterone. As in earlier studies, sodium depletion steepened the aldosterone-response curve to infused angiotensin II.

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A final method by which the level of sodium intake may adjust the aldosterone response to angiotensin II is by an alteration of the number of octapeptide recep-
Angiotensin II and Renal Function

The direct effects of the renin-angiotensin system on the kidney are of considerable interest.\textsuperscript{90-94} There is little doubt that angiotensin II can be formed by the action of renin within the kidney, and we have suggested that the renin-angiotensin system may have evolved initially as an intrarenal control mechanism, and that only later in vertebrate phylogeny did angiotensin become a blood-borne hormone.\textsuperscript{91}

Injected angiotensin II causes marked changes in water and electrolyte excretion, although the precise intrarenal mechanism or mechanisms remain uncertain. Among the several possibilities are influences on the following: 1) tubular function; 2) glomerular filtration rate; 3) glomerulo-tubular balance; 4) autoregulation of renal blood flow and glomerular filtration; and 5) the distribution of blood flow and glomerular filtration within the kidney. These aspects are discussed in detail elsewhere.\textsuperscript{90, 91}

We have proposed that the reduction in urine flow that can be achieved in both pituitary-deficient and nephrogenic forms of diabetes insipidus by dietary sodium restriction, or the administration of benzothiadiazine drugs, may be largely mediated by increases in angiotensin II, either in the systemic circulation, or within the kidney, or both.\textsuperscript{99-101} Critics of this concept\textsuperscript{102-104} have based their arguments mainly on the patterns of renal function found in diuretic-induced anti-diuresis, which are said not to be such as would be expected from the action of angiotensin II.
However, in view of the many possible modes of action of angiotensin II on renal function, and the several ways in which these could be expressed, we are unconvinced of the validity of these counter-arguments, which are based largely on indirect evidence.

The data shown in figure 4 were obtained on different occasions in a patient with pituitary-deficient diabetes insipidus. Urine flow rate is plotted against peripheral plasma angiotensin II concentrations variously in the untreated patient, during dietary sodium restriction, during severe dehydration, following the administration of natriuretic agents and during the infusion of angiotensin II both in normal sodium balance and in sodium depletion. It can be seen that there is a highly significant inverse relationship between plasma angiotensin II concentration and urine flow in these different circumstances. Provided only that the long-term renal actions of angiotensin II are similar to those seen during briefer infusions, it appears to us difficult to avoid the conclusion that the high endogenous plasma angiotensin II concentrations achieved by natriuretic agents, or by dietary sodium restriction, could do other than contribute substantially to the observed reduction in urine flow.

**Angiotensin II-Blood Pressure Dose-Response Curves**

Various studies in different species have shown that the pressor effect of infused angiotensin II is diminished in sodium depletion. In many of the early experiments, what was meant by this was that the increments of arterial pressure obtained by a series of infusion rates of angiotensin II were less than those seen in normal sodium status, although the dose-response curves were parallel. When arterial plasma angiotensin II estimations became available, these aspects could be explored further. In the study by Oelkers et al., sodium depletion by a mean of 150 mEq sodium intake increased arterial plasma angiotensin II without consistently altering basal blood pressure. The pressor effects of administered angiotensin II were examined and could be expressed in different ways. In the original publication, the increment in mean pressure (ordinate) was plotted against the logarithm of the arterial plasma angiotensin II concentration (abscissa). Sodium depletion was then seen to shift the dose-response curves to the right, so that they were roughly parallel to the original sodium-replete curves, but blunted at the highest angiotensin II concentrations. If the absolute mean arterial pressure was plotted on the ordinate, the respective composite dose-response curves were parallel but that of sodium depletion was significantly depressed (fig. 5). The highest arterial plasma angiotensin II concentrations were similar in both the sodium-replete and depleted states, a point of some relevance in interpretation.

A further study was performed in a group of anephric patients before and after sodium depletion, endogenous plasma angiotensin II concentrations being the same in both circumstances. Unlike findings in normal subjects, sodium depletion caused a fall in arterial pressure. However, when angiotensin II was infused, the increments of blood pressure in relation to arterial plasma angiotensin II were closely similar in both states of sodium balance. Plotting absolute arterial pressure against angiotensin II revealed a similar but more marked parallel downward displacement of the curve for sodium-depleted anephric patients than was apparent for sodium-depleted normal subjects.

Davis and Thurston and Laragh have proposed that the decreased pressor response to angiotensin II in sodium depletion is due to prior occupation of available arteriolar smooth muscle receptor sites by endogenous angiotensin II, and that it is not necessary to invoke changes in the number or affinity of receptors. However, the studies in which arterial plasma angiotensin II was measured reemphasize that receptor occupancy is not a complete explanation of either the vaso relationship between angiotensin II and blood pressure or of the subsequent changes when angiotensin is infused. If this were the case, in sodium-depleted normal subjects basal blood pressure should be higher than, not equal to, what that in sodium-replete subjects; while in sodium-depleted anephric patients, arterial pressure would be unchanged. During angiotensin II infusion, normal subjects, both sodium-depleted and sodium-replete, should have similar blood pressures for similar arterial plasma angiotensin II concentrations, whereas in fact the sodium-depleted subjects have lower pressures.
The data suggest that sodium depletion tends to lower arterial pressure, partially by diminishing receptor availability, arteriolar smooth muscle responsiveness, and plasma volume. In normal subjects, but not in anephric patients, this tendency is countered by a rise in arterial plasma angiotensin II concentration.

Angiotensin II: Arterial Pressure Relationships in Renovascular Hypertension

The mechanism(s) of the hypertension that follows renal artery constriction has intrigued and eluded investigators from the time of the classic experiments of Goldblatt and his colleagues. There is general agreement that within hours of applying a renal artery clamp, peripheral blood levels of renin are raised. Subsequently, although arterial pressure remains elevated, renin levels are proportionately lower. Thus, the renin-angiotensin system might be responsible for the early hypertension following renal artery constriction, but other factors appear likely subsequently. A crucial question is whether these later mechanisms require the early rise of renin for their initiation, whether they represent slow effects of renin, or whether they are renin-independent.

A distinction must also be made between hypertension due to narrowing of one main renal artery, while the opposite kidney and renal artery remain intact (two-kidney hypertension) and that following narrowing of one renal artery with the opposite kidney removed (one-kidney hypertension). For example, Swales et al. have drawn attention to the sodium retention that occurs in one-kidney, but not in two-kidney, hypertension in the rat. Others have similarly emphasized the reciprocity of renin and sodium in the evolution of the hypertension.

Animal experiments are necessary to evaluate these various aspects, because it is rarely possible to follow the evolution of human renal hypertension, and there is consequently little or no information in man on the early stages. Caravaggi et al. investigated the quantitative relationship of arterial plasma angiotensin II concentrations to blood pressure in the initial stages of renal hypertension in the dog. Conscious animals both with one and two kidneys were studied. In the first part of the experiment, the dogs were given I.V. infusions of angiotensin II at various rates, and arterial plasma angiotensin II concentrations and blood pressure were recorded. Then, on a separate occasion, in the same conscious dogs, a renal artery clamp was progressively constricted and again the increases in arterial plasma angiotensin II and blood pressure were measured. When the data for the two parts of the experiment were compared, it was seen that they overlapped, and that the regression lines describing the relationship between plasma angiotensin II concentration and arterial pressure were almost identical. It was concluded that the immediate rise in blood pressure following renal artery constriction in the conscious dog, whether only one or both kidneys remained, could be explained solely by the acute pressor effect of the increase in circulating angiotensin II.

Within a few days of the onset of both one-kidney and two-kidney hypertension in the dog, plasma concentrations of renin and angiotensin II fall from the initially high values, although blood pressure remains elevated. The altered angiotensin II: blood pressure relationship is illustrated in figure 6, which

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**Figure 5.** Relationship in normal subjects between arterial plasma angiotensin II concentration and blood pressure before and during incremental I.V. infusions of angiotensin II in normal sodium balance (solid circles) and in sodium depletion (open circles). Calculated regression line for sodium depletion is significantly depressed (p < 0.001) but there is no significant difference in slope. Mean blood pressure (MBP) = diastolic plus 1/3 pulse pressure.
shows progressive changes in dose-response curves in two-kidney hypertension. The lowest curve is obtained in the normal conscious trained dog. One week after the application of a unilateral renal artery clip, both blood pressure and basal plasma angiotensin II rose, and when angiotensin II was infused, the dose-response curve was clearly elevated. Three weeks after applying the clip, blood pressure had risen further, while basal plasma angiotensin II was lower. Nine weeks after the start of the experiment, the dog was severely hypertensive, while basal plasma angiotensin II concentration was below the pre-clip value. The angiotensin II: blood pressure dose-response curve lies much higher than, but roughly parallel to, both the preoperative curve and the curves obtained 1 and 3 weeks after renal artery constriction.

Hutchinson et al. have likewise reported that in chronic experimental renal hypertension in the rat, the relationship between arterial pressure and plasma angiotensin II is higher than during acute infusions of angiotensin II into normal animals. These and similar findings have led several investigators to conclude either that factors other than the renin-angiotensin system are involved, or that the actions of angiotensin II are considerably modified in the later stages of renal hypertension.

In man, renal hypertension is usually first observed at a stage in the disease when any involvement of the renin-angiotensin system is also less clear than in the initial phase in the dog. Figure 7 shows, in a series of untreated hypertensive patients with renal artery stenosis, parenchymal renal disease, or in the malignant phase, the relationship between arterial pressure and the endogenous plasma concentration of angiotensin II. Overall, there is a significant relationship between plasma angiotensin II concentration and blood pressure. However, the regression line lies well above that correlating arterial pressure and plasma angiotensin II in normal subjects in normal sodium balance, who have been infused acutely with angiotensin II so as to give arterial plasma angiotensin II concentrations in the same range. In those patients with normal plasma concentrations of angiotensin II this is scarcely surprising; by definition, such subjects must show an abnormal angiotensin II: blood pressure relationship. Interest centers on those patients with elevated plasma angiotensin II concentration, since in at least some of these patients the renin-angiotensin system might be responsible for the high blood pressure. If angiotensin II is responsible for the elevated blood pressure in this group of patients, some factor or factors additional to the acute vasoconstrictor effect must be present. It might well be, of course, that the renin-angiotensin system was not responsible for the raised blood pressure in all. For this reason one patient with a renin-secreting tumor was of special interest, because blood pressure and plasma angiotensin II were restored to normal after tumor removal, and over-secretion of renin appeared to be the single ultimate cause of the hypertension. Before operation, with grossly elevated peripheral levels of plasma angiotensin II, such as were matched in the normal subjects only at the highest infusion rates, arterial pressure greatly exceeded that achieved during the acute infusion of angiotensin II in the normal volunteers. A similar disproportionate elevation of blood pressure in relation to plasma angiotensin II was seen before operation in another patient with renin-secreting tumor.

This aspect was explored in more detail in a woman with severe hypertension and unilateral renal arterial thrombosis. Untreated, while plasma angiotensin II was elevated, the angiotensin II: pressure relationship was similar to the enhanced relationship seen in other patients with renal disease and hypertension; and when angiotensin II was infused, the arterial pressure increased proportionately along this elevated regression line (fig. 8).

Nine days after the diseased kidney was removed, plasma angiotensin II had fallen into the normal range, and, while the blood pressure was also lower, the fall in both was along the elevated regression line of renal hypertension. When angiotensin II was infused, the increase in blood pressure and angiotensin II still followed this upper regression line.

Sixteen weeks after operation, arterial pressure had fallen further, while plasma angiotensin II remained within the normal range. Thus the relationship between arterial pressure and angiotensin II had moved from that of untreated renal hypertension toward, but not reaching, normal. Again, the still slightly abnormal correlation was confirmed when incremental infusions of angiotensin II were given.

These various observations in man, dog and the rat raised the possibility that prolonged elevation of
plasma angiotensin II might at least in part cause hypertension by mechanisms other than the acute vasoconstrictor action. Skulan et al. had previously demonstrated an altered pressor sensitivity to angiotensin II, of slow onset and offset, in rats with two-kidney hypertension. Work showing that prolonged low-dose infusion of angiotensin II had a progressive pressor action in rabbit and dog might also be relevant to this concept. It was therefore decided to study, in trained conscious dogs, the effect of prolonged administration of angiotensin II, examining particularly the relationship between blood pressure and arterial plasma angiotensin II concentration.

The dogs were unrestricted, carrying a portable infusion pump in a harness strapped to their backs. Angiotensin II was infused intravenously for 2 weeks, which were preceded and followed by 1-week periods during which 0.9% NaCl alone was given. Also, at weekly intervals incremental doses of angiotensin II were infused for 1 hour each so as to provide more detailed information on dose-response relationships.

It was found that arterial pressure, while showing an initial increment when the angiotensin II infusion was started, also rose slowly during continuous administration at constant dose (fig. 9). The weekly brief incremental infusions confirmed that the relationship between blood pressure and arterial plasma angiotensin II concentration was progressively elevated (but not steepened) during prolonged angiotensin II infusion (fig. 10). This advancing alteration in the angiotensin II: blood pressure dose-response curve was very similar to that seen in renal hypertension in the dog (fig. 6). Moreover, when the angiotensin II infusion was stopped, arterial pressure declined slowly to control values over 48 hours, still being significantly increased 24 hours after the angiotensin II administration had been discontinued (fig. 9).

There was, therefore, clear evidence of an effect (or effects) of angiotensin II, of slow onset and offset, enhancing the acute direct pressor action, and giving significantly higher absolute levels of arterial pressure for a given plasma angiotensin II concentration than was found during acute infusions of the peptide. Similar infusions of norepinephrine did not reproduce the upward resetting of the norepinephrine: blood pressure curve seen with angiotensin II.

These experiments demonstrated that increased plasma concentrations of angiotensin II were alone capable of inducing an enhanced blood pressure: angiotensin II relationship. On this evidence it seemed possible, although certainly not established, that an initially raised plasma angiotensin II level might both begin, and then maintain, hypertension due to renal artery stenosis. What could be the mechanisms of this effect? Six possible mechanisms are described below.
First, Folkow has emphasized the structural changes in arterial and arteriolar walls in hypertension, and has pointed out that an increased wall:lumen ratio can per se have a progressive pressor effect. Such structural alterations could be initiated and perpetuated by increased levels of angiotensin II.

Second, Cowley and DeClue found that part of the pressure increase seemed to result from a rise in cardiac output, possibly as a consequence of decreased vascular compliance.

Third, angiotensin II has a variety of central and peripheral sympathetic nervous actions that might well potentiate its initial pressor effect. These include an excitatory action on the area postrema; stimulation of the adrenal medulla and sympathetic ganglia; facilitation of sympathetic ganglionic transmission; potentiation of postganglionic neurotransmitter biosynthesis and release; and inhibition of neurotransmitter re-uptake.

Fourth, the prolonged infusion of angiotensin II at a low dose is accompanied by resetting of the baroreceptors.

Fifth, as discussed earlier, chronic exposure of the adrenal cortex to increased levels of angiotensin II potentiates the aldosterone-stimulant effect of angiotensin II. In renal hypertension in man there is evidence that the plasma aldosterone concentration is higher for a given plasma angiotensin II concentra-
In summary, it appears that circulating angiotensin II is entirely responsible, by acute vasoconstrictor effect, for the initial rise in pressure that follows renal artery constriction. Later, other mechanisms come into play. However, angiotensin II has undoubted pressor actions of slow onset, and these could, at least in part, be responsible for later phases of renal hypertension, both clinical and experimental; this remains unproved.

**Antagonists and Inhibitors: Physiological Considerations**

The various antagonists and inhibitors of the renin-angiotensin system permit very valuable complementary information to be acquired; adding to and confirming results obtained by direct measurement.

A distinction should be made between the prophylactic use of an antagonist, and its immediate effect when given during the course of a physiological phenomenon. Thus, interpretation is different if an antagonist is given before inducing renal hypertension or if it is given when pressure is already elevated. In the former circumstances, even if the renin-angiotensin system were completely inhibited, it might theoretically be possible for alternative mechanisms to operate, and lead to hypertension, even though these might not normally come into play in the absence of the inhibitor. Administration of the inhibitor during the course of the hypertension would more surely indicate, if blood pressure fell, that the renin-angiotensin system was involved.

A further contrast must be drawn between the results of brief and prolonged administration of antagonists. This is especially relevant to hypertension where, as discussed, long-term administration of angiotensin II can lead to quite different effects from those seen in the short term.

Each of the different types of angiotensin antagonist possesses certain theoretical disadvantages that limit interpretation of the results obtained.

Synthetic analogues and antagonists of angiotensin II, of which saralasin is the most widely studied, possess usually intrinsic agonist activity. While a clear inhibitory effect is seen when these substances are given in the presence of high concentrations of the more powerful agonist, angiotensin II, by contrast, when endogenous angiotensin II is low, the stimulant action of the administered analogue may appear, making interpretation difficult. This agonist effect is less with some analogues (such as Sar^Thr^angiotensin II) than with saralasin. Also, while the plasma concentration of angiotensin II usually rises when a competitive antagonist is given, part of the increase is only apparent, and is due to cross-reaction of the angiotensin II antibody with saralasin. However, plasma angiotensin II measurements made at this point have little relevance to physiological changes, because the peptide does not have ready access to receptors.

With inhibitors of converting enzyme, which prevent the formation of angiotensin II from angiotensin
I, rather different problems arise. Two such compounds have been employed fairly widely: the nonapeptide "teprotide" (SQ20,881), \textsuperscript{148, 149} administered intravenously, and the orally active D-2-methyl-3 mercaptopropionyl-L-proline "captopril" (SQ14,225). \textsuperscript{151}

It is mandatory to show that the dose of converting enzyme inhibitor employed in a given circumstance is sufficient to prevent angiotensin II formation, or, if it is not, the extent of the fall in plasma angiotensin II concentration achieved. \textsuperscript{152} As Morton and colleagues have pointed out, \textsuperscript{153} this presents problems of measurement. Most antibodies used in the assay of angiotensin II have some 2% cross-reaction with angiotensin I, and this is of negligible quantitative importance in most clinical and experimental circumstances. However, when converting enzyme inhibitors are administered, sufficiently high concentrations of angiotensin I build up in the circulation to cross-react with the angiotensin II assay, and to give falsely high values for plasma angiotensin II concentrations, unless angiotensin II is separated from angiotensin I before assay. With suitable precautions, however, it is possible to employ converting enzyme inhibitors clinically and experimentally, and to relate the fall in plasma angiotensin II concentration to the phenomena observed.

During the intravenous infusion of captopril at constant rate into conscious dogs, in the range 20–6000 μg/kg/hr, there was a dose-related fall in plasma angiotensin II, and a rise in angiotensin I, in peripheral blood. However, these changes were not progressive; despite the accompanying rise in plasma renin concentration, a new steady state was achieved within 30 minutes, and was then maintained during the 3 hours of captopril infusion (fig. 11). \textsuperscript{154}

Another difficulty of interpretation arises because the administration of converting enzyme inhibitors may also lead to the accumulation of the vasodilator bradykinin. The importance of this has not been agreed upon.

In man, teprotide was found by Haber et al. \textsuperscript{147} to give no measurable rise in plasma bradykinin. Similarly, Case et al. \textsuperscript{148} believed that at the doses they employed, bradykinin accumulation was probably not important, although no measurements were made. In the dog, Miller et al. \textsuperscript{149, 150} concluded that teprotide did not cause a rise in circulating bradykinin, but since, with the assay method employed, this was undetectable both before and after administration of the inhibitor, this view is questionable. In anesthetized normotensive and spontaneously hypertensive rats, Jaeger et al. \textsuperscript{145} found no evidence that teprotide reduced blood pressure other than by effects on the renin-angiotensin system, although this conclusion again was in the absence of bradykinin measurements.

By contrast, there have been several reports of

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig11}
\caption{Concentrations of angiotensin I and II (after chromatography and uncorrected for recovery) in arterial blood of conscious dogs before, and after 3 hours of I.V. infusion of captopril at 20, 200, 2000 and 6000 μg/kg/hour.}
\end{figure}
There is little doubt that further studies on converting enzyme inhibitors, in which a range of doses is given for various periods, and in which concurrent measurements are made of blood concentrations of bradykinin, angiotensin I and angiotensin II, will clarify several of these presently obscure or controversial aspects.

It should be emphasized that for the most ready interpretation, consideration should be given to the shape of the angiotensin II dose-response curve under study, and the position on this curve of the endogenous prevailing angiotensin II level. If the effects of inhibitors and antagonists are interpreted only in the light of the prevailing plasma concentration of angiotensin II (or of renin), there is at best a one-dimensional view. For example, sodium depletion, while steepening the aldosterone-response curve to angiotensin II, (fig. 3) moves the pressor dose-response curve in parallel downward (fig. 5). Understanding of these contrasted changes facilitates interpretation of the effects of saralasin when given in normal sodium balance and in sodium depletion.

Five normal volunteers were first given a 1-hour infusion of saralasin, 10 \( \mu g/kg/min \), in the recumbent position and after a 1-hour control infusion, when in normal sodium balance. No perceptible change in either arterial pressure or plasma aldosterone concentration occurred (fig. 12). In these circumstances, both the pressor and aldosterone-stimulating dose-response curves were shallow, and any antagonism to angiotensin II was presumably masked by the intrinsic agonist action of the administered saralasin.

In sodium depletion, with significantly higher basal levels of aldosterone and angiotensin II, the effects of saralasin were very different. A clear fall in plasma aldosterone was seen in every subject (fig. 12), the antagonist now being given at a steep part of the angiotensin II:aldosterone dose-response curve. By contrast, however, Stephens et al. did not see a consistent fall in plasma aldosterone when saralasin was given in sodium depletion, and considered the agonist effect as a possible explanation. By contrast, however, Stephens et al. did obtain a significant reduction in plasma aldosterone by administering saralasin to sodium-deficient dogs. Converting enzyme inhibitors seem consistently to lower plasma aldosterone in sodium-deplete man and the dog.

Antagonists and Inhibitors: Clinical Studies

When saralasin is infused for up to 1 hour in patients with hypertension of varied etiology, a significant inverse relationship is usually found between the

teprotide increasing bradykinin levels in man. Williams and Hollenberg found plasma bradykinin concentration was increased in patients with essential hypertension, but not in normal subjects. Mersey et al. reported that a 3-minute infusion of teprotide in normal sodium-deficient subjects caused a brief fall in plasma angiotensin II and a rise in bradykinin concentrations.

Potentiation of the effect of bradykinin has been reported with the administration of teprotide in the dog, and of captopril in the dog and the rabbit. A consistent rise in blood kinins was reported by McCaa et al. in sodium-deficient dogs given captopril. Both Bengis et al. and Muirhead et al. concluded that captopril probably caused changes in the kinin system in rats, although no assays were performed by either group.
plasma level of angiotensin II and renin before saralasin administration, and the fall in arterial pressure during saralasin. Those patients with high basal levels of angiotensin II show a large fall in pressure with saralasin. In those with low basal angiotensin II levels, the agonist effect of saralasin is seen, and a slight but distinct rise in pressure may occur. A similar, but less close, inverse relationship is evident between the change in plasma aldosterone on saralasin administration and the basal plasma angiotensin II concentration.

Such information is, however, in a single dimension; it would be more informative to relate these changes to dose-response curves, particularly if it were possible to consider the effective plasma angiotensin II concentration during saralasin administration. This cannot be done without marking certain assumptions. However, if we assume that when saralasin is given, the effective as opposed to the actual plasma concentration of angiotensin II is 10 pg/ml or less, we can, at least tentatively, make some interpretations in terms of dose-response relationships. Figure 13 shows the effect of infusing saralasin into a hypertensive patient with renal artery thrombosis when in normal sodium balance and when sodium depleted. First, when in normal sodium balance, basal plasma angiotensin II concentration is high, and with saralasin infusion, blood pressure falls, but not to normal levels; the fall is down the regression line previously obtained for renal and malignant hypertension. If the same patient is then sodium depleted, plasma angiotensin II concentration rises, while arterial pressure does not change appreciably. However, when saralasin is given under these conditions, a much greater fall in pressure is seen, dropping the values to the regression line for normal subjects. These findings do not lead to the conclusion that sodium retention, or a tendency thereto, is the cause of the upward shift of the regression of angiotensin II upon blood pressure in renal hypertension, but they are at least compatible with that interpretation. A further reason for caution in making interpretations of this kind is that the results will be in part distorted by the agonist, pressor action of saralasin. The increasing availability of converting enzyme inhibitors should, in the future, facilitate more detailed and accurate studies along these lines, because it will be possible to measure the actual plasma angiotensin II concentration during infusion of the inhibitor.

Antagonists and Inhibitors in Experimental Animal Studies

The use of inhibitors and antagonists in experimental hypertension has paralleled and amplified the clinical studies.

The short-term administration of octapeptide analogues of angiotensin II, and inhibitors of convert-
ing enzyme, in experimental renal hypertension has usually brought about a fall in arterial pressure in proportion to the prevailing level of peripheral plasma angiotensin II (or renin). Thus, the most marked reductions of blood pressure in the dog and rat have been seen when these antagonists are given in the early stages of renal artery clip hypertension, with greater falls usually occurring in the two-kidney rather than the one-kidney models, unless the latter animals have been salt-depleted.116-118, 120-129, 146, 160, 179-176, 193

Administration of teprotide before and for 4 days134, 135 and 6 days132 after constricting the artery to a sole remaining kidney in conscious dogs prevented a rise in pressure for the duration of infusion. Similar administration of saralasin attenuated, but did not prevent, a rise in pressure.124, 136 However, as saralasin at the doses given was itself pressor, this aspect is less easy to interpret.

The demonstration of a component of slow onset in hypertension due to angiotensin administration indicated the need to investigate the effects of prolonged administration of antagonists. Rieger et al.,178 examining the chronic phase of two-kidney hypertension in the rat, found that infusion of saralasin or of converting enzyme inhibitor for 12 hours, restored blood pressure gradually to normal, whereas briefer administration had no consistent effect. The depressor action of short infusions was significantly correlated with the preinfusion plasma renin concentration, but the prolonged effect was not. Similar infusions of antagonists into normal animals did not affect blood pressure, although a slight but distinct rise was observed when saralasin was given over a long-term period to rats with DOCA-salt hypertension.128

Possibly related observations were made by Sweet et al.,177 and by Mann et al.,178 who found, in rats with renal hypertension, that angiotensin antagonists were more effective in lowering arterial pressure when given into the cerebral ventricles than intravenously. Bravo and Tarazi179 have also produced evidence that part of the action of angiotensin antagonists is due to interference with neural control.

An alternative or additional possibility is that renin in blood vessel walls could have a local action not reflected by circulating angiotensin II concentrations.174, 180 Schalekamp181 has suggested that assays of plasma inactive renin could be important as perhaps indicating the quantity of renin being transported to vascular walls for local activation and hence angiotensin II generation.

Conclusions

The present paper is largely an account of the corporate philosophy of one research institute, if indeed it is not a contradiction in terms to speak of the composite view of a group of diverse and opinionated individuals. It is our belief that the evaluation of a hormone system requires the identification and assay of the relevant hormone, and a quantitative analysis in various physiological and pathophysiological circumstances. This has involved, in the present context, the development of assays, not only for angiotensin II, but also for renin, angiotensin I and angiotensin III; and the plotting of the dose-response curves of the several actions under study. To emphasize that dose-response curves are a necessary part of the critical interpretation of a hormonal effect is not especially profound; but as such a modus operandi is clearly unfashionable in connection with the renin-angiotensin system, the statement must be made. Examples have been given in this present account of misinterpretations due to failure to recognize that changes in slope, or lateral shifts in the position of the curve had taken place; or that the upper plateau of a sigmoid curve had already been reached.

By measuring angiotensin II rather than renin, attention is focused on the main active component of the system in the circulation. Moreover, the results of such assays can more readily be expressed in absolute terms, a prerequisite for quantitative assessment.

It is our view that a numerate approach of this kind is necessary in order to provide data capable of demolishing hypotheses or of sustaining theories; and that this is the essence of the scientific method. It is hoped that the present account represents some initial progress in that direction.

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Activation of a Prorenin-Like Substance in Human Plasma by Trypsin and by Urinary Kallikrein

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SUMMARY Inactive plasma renin (a prorenin-like substance) can be activated by trypsin. There are also endogenous neutral serine proteases in plasma that can activate inactive renin in vitro, but only after protease inhibitors are either destroyed by acidification (the alkaline phase of acid activation) or inactivated by cold (cryoactivation). In the present study we have shown that cold also facilitates trypsin activation. But even at -4°C, as much as 1 mg/ml of trypsin was required to overcome endogenous inhibitors and reproducibly activate inactive plasma renin during a 1-hour incubation at pH 7.4. After partial destruction of plasma protease inhibitors by acidification to pH 3.3, less trypsin was required for complete activation at pH 7.4: 200 μg/ml at 25°C and 100 μg/ml at -4°C. In contrast, 10 μg/ml of the renal enzyme urinary kallikrein completely activated inactive renin in previously acidified plasma at 25°C. Maximum activation of inactive plasma renin by trypsin or renal kallikrein was almost identical. Both enzymes caused activation in plasmas deficient in Hageman factor or Fletcher factor (prekallikrein), suggesting that their ability to activate inactive renin is not mediated by these neutral serine proteases of the intrinsic coagulation system.

Using maximum trypsin activation to define "total" renin, we found that among 22 normal subjects and hypertensive patients there was a direct relationship between the proportion of active renin in plasma (active/total) and the concurrent urinary kallikrein excretion (r = 0.46, p < 0.05). Normotensive white subjects had a higher proportion of active plasma renin than blacks, in whom urinary kallikrein is reported to be low. Altogether, these data suggest that there might be a link between prorenin and renal kallikrein in vivo. Further studies are required to evaluate this possibility and to determine whether prior hydrolysis of inactive renin, for example, by acidification, is required for renal kallikrein to activate inactive renin.

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Key Words • prorenin • trypsin • renal kallikrein • renin • cryoactivation • serine protease • trypsin inhibitors • urinary kallikrein
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