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PHARMACOLOGIC INTERRUPTION OF THE RENIN-ANGIOTENSIN SYSTEM

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INTRODUCTION

In 1959, at a symposium on polypeptides that affect smooth muscle and blood vessels, Elliott recounted how, following elucidation of the structure of the decapeptide, angiotensin I, he set out to synthesize the agent and found that the synthetic product had only about 1% of the activity of angiotensin II on rat blood pressure despite close chemical resemblance (1). Elliott's key point in his talk was that the carboxyterminal amino acid, phenylalanine, contained almost equal mixtures of the D- and L-forms. Given the freedom of a symposium setting, he went on to make the daring and perceptive speculation that "the peptide containing D-phenylalanine was acting as a powerful inhibitor of hypertensin in vivo." He pointed out that proof required the unequivocal synthesis of a peptide containing pure D-phenylalanine, and expressed the hope that this would soon be achieved. Unfortunately, that synthesis was never reported and the importance of the carboxyterminal amino acid for the development of angiotensin antagonists was apparently forgotten for over a decade. Given the potential importance of pharmacologic interruption of the renin-angiotensin system in dissecting angiotensin's role in physiology and pathophysiology, the failure of the discussion that followed the presentations on angiotensin to respond to Elliott's novel suggestion was surprising, and perhaps discouraging.

In 1970 two groups, Khairallah, Bumpus, and their colleagues at Cleveland Clinic Foundation and Marshall, Vine & Needleman in St. Louis

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reported their rediscovery of the importance of the carboxyterminal amino acid for angiotensin's action and for the synthesis of angiotensin analogues with antagonist properties (2, 3). Khairallah et al demonstrated that the 8-alanine analogue of angiotensin II blocked angiotensin's action but not of serotonin and vasopressin on intestinal smooth muscle, but did nothing more to further characterize the blockade (2). In 1971 they reported competitive kinetics of the antagonism to angiotensin II induced by that analogue in a number of systems (4). Marshall et al (3) demonstrated that the 4-phenylalanine, 8-tyrosine (P_4T_8), analogue of angiotensin II showed competitive kinetics in vitro, blocked the action of angiotensin on blood pressure in vivo, and reversed renal hypertension acutely. Marshall et al were also aware of the intrinsic activity of P_4T_8, which, along with all of the antagonists defined to date, functions as a partial agonist, and went on to exploit that action to study the angiotensin receptor and the factors that went into the agonist responses (5). The first two agents developed, 4-phenylalanine,8-tyrosine angiotensin II and 8-alanine angiotensin II have been followed by a host of agents with increasing potency and, in some cases, a greater specificity.

At about the same time it was recognized that a series of peptides isolated from snake venom and initially called bradykinin potentiating factor (BPF) because of their enhancement of smooth muscle responses to bradykinin also blocked the conversion of angiotensin I to angiotensin II (6). Both the angiotensin analogues and converting enzyme inhibitors have found wide application in a variety of systems described below. Because of the differences in their structure, mechanism of action, and potential lack of specificity which leads to interpretative doubt, their parallel application has been a powerful tool for dissecting the system. Wherever they have induced an identical response, little doubt was left that angiotensin is a responsible mediator. Problems, of course, have arisen where the responses to the two classes of agent were disparate.

Why did these agents find such a rapid and wide use? Haber has pointed out the central role played by ablation of endocrine organs in the evidence which proves that a given biological response is mediated by a specific hormone (7). In the special case of the renin-angiotensin system, where the kidney is both the source of the hormone and a major responding organ, ablation has not been possible. The pharmacologic agents have, therefore, come to play a special role in this circumstance, as a replacement for ablation. However, as nonpharmacologists frequently forget, no agent has a single action and no agent provides data that leave us free of doubt. Both facts are self-evident to pharmacologists, but have often been forgotten by the rest of the scientific community in their eagerness to draw physiological conclusions from pharmacological data.

PHARMACOLOGIC PROPERTIES OF THE AGENTS

Levels of the System Where Antagonists Have Been Employed

The renin-angiotensin system offers a series of sites that have been exploited for more or less selective inhibition (Table 1). Renin release from the kidney is mediated, in part, by neural action, which is blocked by β-adrenergic blocking agents (8–10). More recently, evidence has accumulated that some agents that reduce renin release such as clonidine act via a presynaptic α receptor (9). Following release, renin reacts with a substrate synthesized by liver to form the decapeptide, angiotensin I, which is considerably less active on smooth muscle and on the adrenal than angiotensin II. Renin's action on substrate has been antagonized by nonspecific proteases such as pepstatin, by antibody to renin, and more specifically by structural analogues of the substrate on which renin acts (7, 11–13).

Angiotensin I is hydrolyzed by an enzyme in lung and peripheral tissues which cleaves the carboxyterminal dipeptide, his-leu, leaving the active octapeptide, angiotensin II. The same enzyme that activates angiotensin II (and is thus known as *converting enzyme*) is also responsible for the degradation of bradykinin and for hydrolysis of the β chain of insulin. In each case the enzyme has acquired a descriptive name which suggests a specificity that it does not enjoy (14). The same enzyme is variously described as converting enzyme or kininase II, depending on whether the investigator was primarily interested in angiotensin generation or bradykinin degradation. This enzyme is amenable to blockade by a number of agents. A series of peptides inhibit the degradation of bradykinin and are thus termed bradykinin-potentiating-factor (BPF). The most widely studied peptide in this series is a nonapeptide distributed as SQ 20881 (15, 16). Decapeptide analogues have also been shown to compete with angiotensin I for converting enzyme (12).

More recently, a novel nonpeptide blocker of dipeptidyl-peptide hydrolase has been synthesized (17, 18). Among the most exciting advances in the field, the agent was designed on the basis of a working model of the receptor site on the enzyme, based on the peptide structure-action relationships and on what is known of the structure of carboxypeptidase A. The advantages of a nonpeptide obviously include both activity when the agent is taken by mouth and relatively inexpensive synthesis. Until recently, work in this field has often been delayed by relative unavailability of the peptide analogues. Clearly, if our new understanding is to be exploited for long-term therapy, an agent that is effective when taken orally has enormous advantages.

Octapeptide analogues of angiotensin II act as antagonists by competing with angiotensin at its receptor site in vascular smooth muscle (11, 12),

cardiac muscle (20), the adrenal cortex (21–23), and the nervous system (24).

Until 1971 it was believed that aminopeptidase-mediated removal of the N-terminal aspartyl residue was the first step in degradation, because the heptapeptide was very inactive in some smooth muscle systems, especially uterine muscle (25). Evidence, reviewed below, is accumulating that the heptapeptide now known as angiotensin III may be the biologically active mediator in some systems, especially aldosterone secretion. Analogues of angiotensin III have shown preferential blockade of aldosterone release relative to their effects on blood pressure (Table 1 and see subsequent section on the adrenal). More circumstantial evidence suggests that the renal vascular receptor is also especially sensitive to angiotensin III (26).

Structure-Activity Relationships

Information is available on the relationship between structure of the pharmacologic agent and its biological effectiveness at each of the steps defined in Table 1. The recent reviews by Marshall (12), Regoli et al (11), and Bumpus (13) are available for detailed information on this subject. Emphasis is given below to agents with the widest use and the greatest specificity.

ANGIOTENSIN ANALOGUES As pointed out above, Elliott recognized the key position of the carboxyterminal amino acid, phenylalanine, in the action of angiotensin II and in endowing the molecule with antagonist properties (1). With the exception of a single agent, an analogue of angiotensin II in which the tyrosine residue in position 4 had been substituted by *p*-fluoro-1-phenylalanine (27), all of the angiotensin antagonists synthesized to date have been modified in position 8 (11–13). Marshall (12) pointed out that the lack of other examples of inhibitors that have a single substitution

Table 1 Pharmacologic interruption of the renin-angiotensin system

Mechanism	Product	Pharmacologic blockade
Renin release	renin	1. β-adrenergic blocking agents[a] 2. α-adrenergic agonists[a]
Renin's action on substrate	AI	1. substrate analogues 2. pepstatin
AI conversion to AII	AII	1. peptide inhibitors of peptidyl dipeptide hydrolase[a] 2. nonpeptide inhibitors of peptidyl dipeptide hydrolase[a]
AII and AIII action on receptor	response	1. peptide analogues of angiotensin II[a] 2. peptide analogues of angiotensin III

[a] Applied clinically.

at position 4 may reflect the unusually stringent requirements at this position for binding. A large number of alternate modifications at this level have not resulted in antagonists.

The general category of inhibitor has an aliphatic side chain substituted for the aromatic residue. The first agent synthesized in this class, 8-alanine angiotensin II, was effective but lacked potency and was thought to be inactive in vivo (4), although this has been denied (28). Increasing the hydrophobicity of the aliphatic side chain in the 8 position, by substituting valine, isoleucine, or leucine, for example, increases the binding of the inhibitor, and its effectiveness. The closer the resemblance of the aliphatic side chain to phenylalanine in size, the more potent both the antagonist and agonist properties. Of the 8-substitute analogues synthesized and tested, the most potent found to date has been the 8-isoleucine analogue, which is two orders of magnitude more active than the original 8-alanine analogue.

A second class of inhibitor has been distinguished in which the orientation of the aromatic side chain in position 8 has been perturbed, as in the case of the original *d*-phenylalanine substitution (1) and in *n*-methyl phenylalanine angiotensin II (29). The substitution of α-methyl-l-phenylalanine results in an even stronger conformational constraint and a more potent antagonist (30).

Myotropic, pressor, and hormonal effects of the analogues are all sensitive to spatial orientation at the carboxyterminus, as well as the length of the peptide backbone, and the relation of the carboxyl group to the aromatic side chain.

The N-terminal aspartyl residue has also been subject to considerable investigation. The original antagonists, 8-ala angiotensin II and 4-phe, 8-tyr angiotensin II were, respectively, inactive or relatively inactive in vivo. Because a major route of degradation involves aminopeptidase attack on the N-terminal amino acid, Pals et al synthesized 1-sarcosine, 8-alanine angiotensin II, and demonstrated a striking increase in the inhibitory effect, especially in vivo (19). While the initial goal of removal of the carb-amyl group of aspartic acid was to protect the molecule from degradation, this substitution in position 1 was also shown to have an additional influence. The resultant agent is considerably less polar, and thus potentially reaches the receptor site more easily. Perhaps more important is accumulating evidence that the binding affinity of the analogue for the receptor site is also enhanced by this substitution. Hall and co-workers synthesized an angiotensin II analogue modified only by replacing sarcosine in position 1 (31). This "super hormone" was almost an order of magnitude more active than angiotensin II in the rabbit aorta. Its agonist activity was blocked by angiotensin antagonists and showed cross-tachyphylaxis to angiotensin II on the

rat aorta, substantiating that it acted on the same receptor. 1-sar Angiotensin II produced very prolonged blockade that was not easily reversed by washing the tissue preparation.

The prolonged blockade induced by 1-sar–substituted analogues made it possible, by analogy with earlier studies with nonequilibrium antagonists such as the β-haloalkylamine, phenoxybenzamine, to assess the possibility that angiotensin may produce maximal concentrations by stimulating only a fraction of the receptors on the rabbit aorta. "Spare" receptors have been demonstrated in this preparation for catecholamines and serotonin (32). The 8-substituted short-acting agents induced parallel shifts without a reduction in the maximal response, characteristic of competitive antagonists (11). The 1-sar, 8-ileu analogue reduced the maximal response and flattened the slope of the angiotensin dose-response curve in a manner identical with that induced by phenoxybenzamine in high doses in the response to norepinephrine and serotonin (11). This experiment was interpreted to suggest that no "spare" receptors for angiotensin exists in the rabbit aorta. We have shown, however, that low concentrations of 1-sar, 8-ala angiotensin II, induce a parallel shift (21, 33); increasing concentrations also reduce the maximal response and flatten the slope. Spare receptors for angiotensin must exist in this system. Protection experiments in which the native hormone or 8-substituted analogues prevent the prolonged blockade induced by 1-sar substitute analogues indicate that all of the analogues act on the same receptor as the native hormone (11). The apparent noncompetitive antagonism induced by higher concentrations of the 1-sar–substituted agents truly reflects a nonequilibrium association with the receptor.

In an analogous approach, Paiva et al incorporated a nitrogen mustard group as an N-terminal residue, utilizing chloroambucil (34, 35). They demonstrated inhibition of the response of isolated smooth muscle to angiotensin II for hours after the inhibitor was removed but, as in the case of the other long-acting analogues, the agent possessed a great deal of agonistic activity.

Evidence that the heptapeptide, angiotensin III, might have important biological activity (25, 36) has also led to the synthesis of a series of heptapeptide analogues. These analogues have been reported to induce greater blockade in the adrenal cortex (22, 37, 38) and the renal blood supply (26), than on the vascular response that mediates the blood pressure rise following angiotensin administration. Because of the strong tradition in pharmacology of finding antagonists that discriminated between receptors which responded to an identical agonist—for example H_1 and H_2 receptors for histamine, α, β 1, and β 2 receptors for catecholamines and muscarinic, nicotinic, and skeletal muscle receptors for acetylcholine—it is tempting to speculate on the possibility that this approach will lead to analogues with

greater specificity in the renin-angiotensin system as well. While the evidence cited shows promising clues, as yet no agent with the specificity of the systems described above has been found.

One of the major difficulties in this field is that the structure-action studies have not yet defined true competitive antagonists, but rather partial agonists. All of the agents retain significant agonist properties, which have often been missed because the assay systems employed, such as the rabbit aorta and blood pressure in the rat, rabbit, or dog are relatively insensitive to angiotensin II. Mimran et al (39) in our laboratory observed that sar-1,ala-8 angiotensin II behaved as a competitive antagonist on rabbit and rat aorta and rat pressor assays, but clearly acted as a powerful agonist in more sensitive systems including the rat stomach and colon assay in vitro, and the renal blood supply of rabbits in which the renin-angiotensin system had been suppressed by a high sodium intake prior to study in vivo (39). In the latter systems, sensitivity to angiotensin II is approximately one order of magnitude greater. Because the concentration of the analogue producing threshold blockade was virtually identical in the various assays, it appeared that affinity of the receptors for 1-sar,8-ala angiotensin II was the same in all of the systems but that excitation-contraction coupling varied from tissue to tissue.

Needleman et al (5) exploited the partial agonist character of 4-phe,8-tyr angiotensin II to study factors responsible for the agonist response. They demonstrated that this agent can be converted from a pure antagonist to a pure agonist in rat uterine strips by increasing the pH of the incubation medium from pH 6.8 to pH 8.6. The same maneuver increased the sensitivity of the system to angiotensin II strikingly. Addition of magnesium at pH 8.6 reduced sensitivity to angiotensin II and reversed the action of P_4T_8 from agonist to antagonist. The effect of magnesium was possibly related to its ability to decrease the tissue calcium concentration. Multiple lines of evidence suggest that angiotensin acts in smooth muscle by increasing the level of intracellular calcium through inhibition of calcium uptake into sarcoplasmic reticulum (5). Marshall has suggested that the level of intracellular calcium prior to interaction of angiotensin II and, by inference of the analogues, may determine the agonist response by influencing the intracellular calcium level required to reach the threshold necessary for contraction (12). Clearly more information is required in this area before analogues free of intrinsic activity as agonists will be identified. In our experience with the rabbit renal blood supply as the assay system, all of the agents studied to date, including 8-ala, valine, tyrosine, leucine, isoleucine, glycine, and threonine (unpublished observations) display quantitatively important intrinsic activity which parallels approximately their activity as antagonists, suggesting that the substitutions primarily influence affinity.

CONVERTING ENZYME INHIBITORS Activity as a converting enzyme inhibitor appears to occur in two parts of the peptide molecules studied. Particularly important appears to be the pro-pro-sequence at the carboxyterminus, which interferes with the action of carboxypeptidase or dipeptidyl carboxypeptidase (15, 16, 40). The amino-terminal segment also has definite inhibitor activity that is sufficiently sensitive to degradation by proteolytic enzymes, including the converting enzyme itself, to limit its duration of action to a degree. The heptapeptide BPF 5a, for example, has an affinity 10 times greater than the nonapeptide for the converting enzyme receptor, but a striking increase in the rate of degradation of the pentapeptide was demonstrated in the absence of substrate, under conditions in which the longer inhibitors are completely stable. Because of its relative stability, the nonapeptide, distributed as SQ 20881, has been the most widely used. Recent reviews provide a more detailed description of the interesting structure-action relationships in this field (12, 16).

As pointed out by Marshall (12) and Wolfenden (41), the concept of transition state analogues uncovers an attractive site for inhibitor studies because an analogue which mimics the transition state that the substrate assumes during enzyme cleavage should have a binding affinity which is several orders of magnitude greater than that of the substrate itself. On theoretical grounds, therefore, compounds directed against the active sites of enzymes offer a potential gain of several orders of magnitude in antagonist-to-agonist ratios. No such theoretical basis for an increased affinity of a drug compared with a native hormone exists in considering binding of analogues to the receptor in effector systems directly. On this basis, and because of the potential utility of a nonpeptide antagonist reviewed above, Ondetti, Cushman, and their co-workers initiated a detailed analysis of the receptor site on the converting enzyme in an attempt to synthesize such an agent (17, 18), an attempt which was extraordinarily successful.

They devised a model of the receptor site on peptidyldipeptide hydrolase based on insights gained from the assessment of the binding of peptide inhibitors to the enzyme; information that had been gained on substrate specificity; and the assumption that the mechanism of action of the angiotensin-converting enzyme and its active site might be similar (since the angiotensin-converting enzyme is a zinc-containing exopeptidase with many properties similar to those of pancreatic carboxypeptidases). On the basis of these sources of information, (*a*) a positively charged locus was postulated to bind with a negatively charged C-terminal carboxyl group of the peptide substrate; (*b*) the bound zinc ion of angiotensin-converting enzyme, expected to play a role in peptide bond cleavage, was thought to be separated from the positively charged locus on the enzyme by the length of the dipeptide, rather than a single amino acid residue as in carboxypepti-

dase A; (*c*) a more distant site with affinity for the side chains of the two terminal amino acid residues of substrate was thought to be present; and (*d*) the converting enzyme was assumed to be bound, perhaps by hydrogen bonding, to the terminal peptide of the substrate. With these clues, extensive structure-activity studies were performed, with the ultimate finding that the D-3-mercapto-2-methylpropanoyl-1-proline (designated SQ 14225) was the most potent inhibitor of angiotensin-converting enzyme. The added 2-methyl group enhanced activity considerably, perhaps in part due to increased binding, but the investigators thought it more likely that the main contribution was the restriction in conformation, with the stereospecific substitution reducing the mobility of the acyl moiety.

The carboxyalkanoyl and mercaptoalkanoyl derivatives of proline inhibited the contractile responses of guinea pig ileum to angiotensin I and augmented responses to bradykinin in vitro. In vivo, when administered by mouth to rats, these agents inhibited the pressor effects of angiotensin I and augmented the vasodepressor effects of bradykinin. Finally, they lowered the blood pressure in models of renovascular hypertension. The activity of SQ 14225 was on a molar basis, equal to or better than that of the most active peptide in vivo, SQ 20881.

Metabolic Fate

The half-life of angiotensin II and of the heptapeptide, angiotensin III, is about 20 sec (42). Substitution of sarcosine in the 1 position renders the analogue relatively resistant to aminopeptidase (11–13, 28, 31). Pettinger et al demonstrated that this substitution in saralasin resulted in prolongation of the pharmacologic and biologic half-life to about 3 min in the rat and in man (43). Consistent with that observation, pharmacokinetic equilibrium on the influence of the agent on blood pressure is reached in 10–20 min (43). On the other hand, in the case of the renal blood supply when it was under the influence of endogenous angiotensin, pharmacokinetic equilibrium was not reached within 10 half-lives (44), suggesting that the receptors were not easily available to the agent. Given the accumulating evidence that angiotensin generated within the kidney operates on the vessels (45), and that the angiotensin reaches the receptor from the juxtaglomerular apparatus via the media in vessels too small to have vasa vasorum, it is possible that the prolonged period to reach equilibrium with renal vascular receptors reflects their relative unavailability, because of long diffusion distances from the blood stream.

The nonapeptide converting enzyme inhibitor SQ 20881 has a pharmacologic half-life of approximately 3 hr (46), considerably longer than the biologic half-life of this peptide is likely to be, suggesting a prolonged sojourn on the enzyme's receptor. Very little information is available on the

mechanism of its degradation. Not surprising for a molecule this size, it appears in high concentration in urine. The nonpeptide converting enzyme inhibitor SQ 14225 has a similar half-life. Again, the precise mechanism of its degradation is not known.

Toxicity

The toxicity of these agents has largely been related to their primary action. In animals and in patients with a volume deficit, often striking hypotension has been observed, a factor that may limit their utility in reversing the renal actions of angiotensin (26, 44, 47–50). For this reason, attempts to develop relatively selective agents for the renal vascular angiotensin receptor have been made, with some success (26). Because of its partial agonist properties, saralasin and 1-sar 8-ile angiotensin II have induced striking pressor responses in patients with low renin hypertension or primary aldosteronism (51, 52). To date no important damage from this action has occurred, but the observations suggest that caution should be employed in their use. Bolus administration induces a larger pressor response (53). The agonist properties of these agents are also probably responsible for the sharp reduction in renal blood flow that occurs when these agents are administered in high dose (54). Again, no untoward effect on the kidney has been documented from the relatively short periods of administration that have been employed, but in states in which renal vasoconstriction already exists this influence could potentiate the damage.

About 10% of patients receiving SQ 14225, generally in very high dosage, have suffered a reversible rash. This is likely to be dose related rather than idiosyncratic because in some patients reduction of the dosage have resulted in reversal of the rash (55). A transient rise in blood urea nitrogen and serum creatinine associated with normalization of blood pressure in a patient with previously uncontrollable hypertension occurred with SQ 14225 (55), but is also well known with other antihypertensive agents and probably reflects a nonspecific effect of blood pressure reduction, rather than a specific renal action of SQ 14225.

APPLICATION TO ASSESSMENT OF HYPERTENSION

In normal animals and man when the renin-angiotensin system is suppressed by recumbency and a liberal intake of sodium and potassium, neither angiotensin analogues nor converting enzyme inhibitors reduce arterial blood pressure (7, 56–58). Despite modest activation of the renin-angiotensin system induced by standing in subjects ingesting a liberal salt intake, a measurable vascular action of angiotensin does not occur, since

neither class of agent reduces blood pressure under that circumstance (56, 58). When the system is activated further by restriction of sodium intake, however, both classes of agent induce a reproducible reduction in arterial blood pressure despite recumbency (7, 54–61). In this setting the response is modest. In our study of 50 normal subjects in balance on a 10 meq sodium, for example, the average fall of diastolic pressure with saralasin was 5 mmHg with a standard deviation of 2.5. Thus, no more than a 10 mmHg fall in diastolic pressure is anticipated in normal man (59). Sitting or standing, however, potentiates the response to that antagonist (56, 58). Increasing degrees of negative sodium balance also result in an increasing role for angiotensin in maintaining arterial pressure. Indeed, aggressive sodium depletion will even make the "low-renin hypertensive" dependent on angiotensin for blood pressure maintenance (61). The relation between the state of sodium balance and angiotensin's contribution to normal pressure maintenance is probably best thought of as a continuum.

In states characterized by renin-angiotensin system activation but a reduced or normal arterial pressure such as hepatic cirrhosis, Bartter's syndrome, and congestive heart failure, both classes of agents induce a potentiated hypotensive response (49, 50). A similar hypotensive response to the antagonist occurs in animal models, such as glycerol-induced myohemoglobinuric acute renal failure (44) and partial occlusion of the thoracic inferior vena cava (26, 47). In all of these states activation of the renin-angiotensin system appears to play an important role in sustaining arterial blood pressure. The often cited paradox concerning the absence of hypertension despite activation of the renin-angiotensin system in these states has been resolved by the agents. In the absence of activation of the system, severe hypotension would be present.

The precise cardiovascular response to pharmacologic interruption of the renin-angiotensin system has not yet been defined in normal man. In the rabbit with a minor extracellular fluid volume deficit, pharmacologic interruption of the renin angiotensin system was associated with a well-sustained or even increased cardiac output (48, 62). The fall in blood pressure was due entirely to a fall in total peripheral resistance, consistent with earlier observations that angiotensin has minimal if any influence on veins (63). α-Adrenergic blockade induced greater hypotension despite an identical fall in total peripheral resistance, due to a fall in cardiac output (62). Direct assessment of venous tone demonstrated a striking venous dilatation induced by phenoxybenzamine, the α-adrenergic blocking agent; saralasin, however, had no demonstrable influence on veins, accounting for the well-sustained cardiac output (62).

Angiotensin's role in sustaining elevated blood pressure in various hypertensive models and in patients with a variety of diseases has, not surpris-

ingly, received more attention with these agents than any other area. Suggestions concerning angiotensin's contribution to the most common clinical problem, essential hypertension, have ranged from an uncommon role reflecting a response in no more than 5 to 10% of patients (59), to the daring suggestion that angiotensin may play an important role in as many as 70 to 85% of such patients (64). The differences largely depend on the agent employed, but the patient population selected and the circumstances under which the study was performed probably also contribute to this rather striking difference. Because this topic is so important and because it underlines effectively the controversies engendered when different classes of agent provide a different answer, it is considered in greater detail below.

In animal models of renovascular hypertension and their clinical counterpart, there is considerably more unanimity, but here also the specificity and sensitivity of the angiotensin antagonists have been debated (69). In normal man on a low salt diet a fall in diastolic pressure must exceed 10 mmHg to be significant. A potentiated response to saralasin occurs in about 10% of essential hypertensives when large series have been studied (51–53, 57, 59, 65–68). In patients with renovascular hypertension proved by 6 months surgical follow-up that revealed striking improvement or cure, over 80% responded to saralasin with a significant hypotensive response (51, 53, 59). Moreover, there was a close correlation between the magnitude of the hypotensive response and the plasma angiotensin II concentration in responders (59). Overall, there is considerably greater unanimity concerning angiotensin's role in the hypertension of early unilateral renal artery stenosis in animals [69; see reference (12) for 21 references], but with more prolonged hypertension even in this simple model there is debate concerning angiotensin's contribution (69). All of this debate, of course, is of immediate clinical relevance.

Essential hypertension remains a problem. When SQ 20881 is employed as the blocking agent, 70 to 85% of patients with essential hypertension show a significant response (64, 70). Conversely, responses to saralasin that exceed the normal occur in a much smaller fraction of patients with essential hypertension: Responses to which agent reflect more truly the contribution of angiotensin?

Saralasin's partial agonist activity (39, 48, 51), which has been suggested to account for the difference, may result in an underestimate of angiotensin's role in the maintenance of essential hypertension (64). It is equally reasonable to ask, of course, whether SQ 20881 overestimates the prevalence of angiotensin as a mediator (70).

Significant blockade of responses to angiotensin occurred at saralasin doses well below those required for intrinsic activity (54). A protocol in which saralasin doses were gradually increased from subthreshold for in-

trinsic activity (30–100 ng/kg/min) to the dose range likely to be effective was designed to identify the optimal dose for reducing blood pressure before intrinsic activity limits the response (59). This approach revealed a significant hypotensive response in only 5 of 62 (8%) patients with uncomplicated essential hypertension, and in only 7 of 32 (22%) patients with advanced essential hypertension likely to be complicated by nephrosclerosis. The capacity of saralasin to identify renovascular hypertension and the close correlation between the magnitude of the hypotensive response and the plasma angiotensin II concentration in responders suggest that saralasin, properly employed, does identify and provide a quantitative index of angiotensin-mediated hypertension.

The specificity and sensitivity of SQ 20881's action in reducing high blood pressure in essential hypertension is still open to debate. The enzyme is also responsible in part for degradation of bradykinin (6, 14–16). An increase in plasma bradykinin concentration following administration of the agent did not occur in the normal subjects (60) but has been documented in essential hypertension (70), an observation which does not prove that bradykinin contributes to the blood pressure fall but does indicate that caution is necessary before a final conclusion on the specificity of SQ 20881 can be drawn. Moreover, in patients with a particularly striking response to SQ 20881, both the control bradykinin concentration and the magnitude of the response were larger than in nonresponders (71). Finally, if after SQ 20881 administration one administered angiotensin II intravenously in a dose adjusted to return arterial blood pressure to a predrug level, the plasma angiotensin II concentration required to achieve that end was strikingly higher than it was prior to SQ 20881 administration (71). Some factor other than a fall in plasma angiotensin II concentration induced by SQ 20881 must have been involved in the response.

SQ 14225 has been employed to assess the response in only a small number of hypertensives (55), where it has shown striking preliminary success, even in patients previously resistant to conventional medical therapy.

APPLICATION TO THE KIDNEY

An intrarenal role of the renin-angiotensin system as a determinant of renal perfusion and renal function has been the subject of periodic speculation (45, 72–74), but definitive evidence for such a role in the overall contribution of angiotensin to renal perfusion and function in health and in disease has remained hypothetical. A compelling argument can be made for assigning a primal renal function to the system early in phylogeny (74), with other influences of angiotensin on blood pressure and on the adrenal gland arising

much later. The adrenal, for example, first appeared in amphibia (75). Renin, on the other hand, probably first appeared millions of years earlier in boney fishes (74). Little is known concerning blood pressure control in primitive organisms, but the lung fish as a representative example has an arterial pressure of about 15 mmHg which, at this level, appears to be effectively unregulated (76). In eurhyaline fish and eels, exposure to fresh water results in a renal adaptation which appears to be mediated by the renin-angiotensin system (74). The available data are compatible with the hypothesis that this system initially evolved as a control mechanism for the kidney, especially for the glomerular circulation, and that this role broadened with increasingly more ambitious ventures into more hostile environments.

The locus of angiotensin generation also provides insight into its action. Because the largest concentration of converting enzyme is found in the lung, it was believed until recently the conversion from angiotensin I to angiotensin II occurred only there (42). On this basis the function of angiotensin II was considered to be primarily systemic rather than intrarenal. Viewed in this context, the parallel phylogenetic appearance of lungs (where the bulk of conversion occurs) and the zona glomerulosa of the adrenal (the only unequivocally important normal systemic action of angiotensin II) in amphibia is unlikely to reflect pure chance.

Three observations suggest a critical, intrarenal focus for conversion. First, while the total amount of converting enzyme in the kidney is small, the available activity is sharply localized to the juxtaglomerular apparatus where renin is released (77, 78). Second, lymph draining the kidney contains far higher concentrations of angiotensin II than can be found in arterial or renal venous blood, and thus must have been generated locally (79). Third, specific competitive antagonists to converting enzyme, when infused into the renal artery, block the local action of angiotensin I but not of angiotensin II (80, 81). Angiotensin I, therefore, must require conversion to angiotensin II for its renal action and that conversion must occur locally. Taken in all, these observations demonstrate the capacity of the kidney to generate angiotensin II locally in a strategic location for a vascular or glomerular action, supporting phylogeny in suggesting a primary renal action. The remarkable sensitivity of the renal vasculature to angiotensin also supports this concept: A reproducible reduction of renal blood flow, glomerular filtration rate, and sodium excretion occurs with doses that are well below those required to induce a pressor response (54, 82).

Responses of the renal vasculature to pharmacologic interruption in many ways are analogous to the findings for arterial blood pressure. When the renin-angiotensin was suppressed by a high sodium intake in man and in animals (48, 54, 83), the administration of saralasin or a converting

enzyme inhibitor influenced renal blood flow very little or reduced it. The reduction of renal blood flow presumably reflects the intrinsic activity of saralasin. There appear to be important species differences in the renal vascular response to the agonist properties of saralasin. When the renin-angiotensin system was suppressed prior to study by a liberal sodium intake, this agent induced a striking, dose-related reduction of renal blood flow in man (54) and in the rabbit (39), a more modest but unequivocal response in the dog (83), but no apparent response in the rat (84).

When the renin-angiotensin system was activated and renal blood flow reduced by restriction of sodium intake in man (60) and in animals (47, 48, 83), both classes of inhibitor resulted in a dose-related increase in renal blood flow, the magnitude of which was consistent with the reversal of the effects of sodium restriction.

In animal models the renal vascular response to restriction of sodium intake (47, 48, 83), anesthesia and trauma (83), thoracic inferior vena caval obstruction (26, 47), experimental heart failure (85), renal hypertension (86), hemorrhagic shock (87) and impaired reflow after hemorrhage (88), and acute renal failure (44) have all been reversed in part or in toto by pharmacologic interruption of the renin-angiotensin system. In all of these models there is evidence for renin-angiotensin system activation, as indicated by increased plasma renin activity and by hyperplasia of the juxtaglomerular apparatus, associated with a striking abnormality of renal function.

Interpretation of the functional response of the kidney to pharmacologic interruption has been complicated by several factors. A blood pressure fall during administration of these agents occurs not only in some patients with secondary hypertension, but also in animals with activation of the renin-angiotensin system and a normal or reduced blood pressure (26, 44, 47, 48), and in patients with cirrhosis of the liver (49), Bartter's syndrome (50), and congestive heart failure (93; and personal observations in man). The resultant hypotension may well limit the renal vascular and functional response. When the agents have been infused into the renal artery, thus avoiding the hypotension, increased glomerular filtration rate has been noted in animals in which the system was activated by restriction of sodium intake (89, 90). We have found that adjustment of the dose of the angiotensin analogues to maximize renal blood flow and to minimize hypotension also results in an increase in glomerular filtration rate in dogs with partial occlusion of the thoracic inferior vena cava—a more profound stimulus. In some studies the use of a large dose and failure to define a dose-response relationship may well have obscured the influence on glomerular filtration rate, because of the intrinsic activity of the partial agonists and the complicating hypotension (91). Natriuresis has also been reported in response to the antagonists

in animals in which the primary stimulus involved the restriction of sodium intake and when the antagonists were properly employed (89, 90). In our studies on thoracic inferior vena caval occlusion, however, natriuresis did not follow the increase in renal perfusion and glomerular filtration rate. Presumably the increased stimulus recruited additional factors responsible for sodium retention by the kidney.

Several observations have suggested that the renal vascular receptor for angiotensin II differs from systemic vascular angiotensin receptors. First, the renal vasculature is especially sensitive (54, 82). Second, saralasin induces a dose-related reduction in renal blood flow that parallels the response to AII, but exerts only a limited pressor effect (54). Third, the 1-des asp analogue of angiotensin II (angiotensin III) constricts the renal vasculature as effectively as AII (26, 92) despite a limited pressor activity, suggesting that this agent does not influence other vascular beds in parallel. Fourth, a series of angiotensin analogues based on the octapeptide and heptapeptide was equally effective in blocking the effects of AII and AIII on the renal vasculature (26). Finally, angiotensin II and angiotensin III induced cross-tachyphylaxis in the renal vasculature (26). All lines of evidence suggest that AII and AIII act on a single receptor in the kidney, which differs at least functionally from other systemic vascular receptors.

The possibility that heptapeptide analogues will provide angiotensin antagonists with greater specificity for the renal vasculature was pursued in a model in which the renin-angiotensin system was activated—acute partial thoracic inferior vena caval occlusion (26). Saralasin in this model induced progressive, dose-related hypotension and a limited increase in renal blood flow, whereas the heptapeptide (1-des asp, 8-ile AII) analogue induced a consistent, larger dose-related renal blood flow increase with significantly less hypotension over a wide dose range. Unfortunately, 8-isoleucine substitution results in agents with substantial intrinsic activity, so a search for a more useful analogue continues. Taken in all, the evidence suggests that the renal vascular receptor differs sufficiently from systemic vascular angiotensin receptors that heptapeptide analogues of AII will be useful in exploring angiotensin's role in states characterized by disordered renal perfusion and function—and perhaps even therapy (93).

APPLICATION TO ASSESSING THE ADRENAL AND ALDOSTERONE RELEASE

The striking action of angiotensin II on aldosterone secretion has been known for almost two decades (94). What has remained controversial is the primacy of the renin-angiotensin system in mediating aldosterone's response to a volume challenge. One major objection was raised on the basis

of the failure of exogenously administered angiotensin II to produce an aldosterone response in normal man equivalent to that induced by restriction of sodium intake, despite the achievement of plasma angiotensin II concentrations of similar magnitude (95). This discrepancy was only apparent. As in the case of blood pressure and renal vascular control, restriction of sodium intake also modifies adrenal responsiveness to angiotensin II; responsiveness was enhanced during sodium restriction (96, 97), as opposed to the reduced response of vascular smooth muscle in that state (96).

Additional objections to this hypothesis have been raised on the basis of several lines of investigation (98, 99). Lowenstein et al documented a normal aldosterone response to restriction of sodium intake in rabbits despite effective immunization against angiotensin II (98). Because the size and polarity of antibody might limit its access to critical loci, they went on to assess the response to the infusion of saralasin in conscious rabbits rendered sodium deficient, and documented a failure of the agent administered in large doses to reduce plasma aldosterone levels (23). In contrast, saralasin effectively reduced plasma aldosterone levels in the anesthetized, sodium-depleted dog (100). Similarly, both saralasin and SQ 20881 clearly reduced plasma aldosterone in the anesthetized, sodium-depleted rat (101). Criticism, aimed at these studies because anesthesia has a profound effect on the renin-angiotensin system, led to a repeat of the study in conscious sodium-depleted dogs, with similar results (102). Perhaps the failure of saralasin to reduce plasma aldosterone in the rabbit (23) reflected the use of large doses of this partial agonist.

In man, Sancho et al reported that converting enzyme inhibition reduced plasma aldosterone levels in the recumbent individual on a low salt diet and, furthermore, blunted the aldosterone response to assumption of the upright position (56). We have confirmed and extended that observation (103). Both saralasin and SQ 20881 induced a dose-related reduction in plasma aldosterone levels in normal subjects in whom the renin angiotensin system was activated by restriction of sodium intake.

The adrenal response to saralasin resembles strikingly that of the renal blood supply (60). In normal subjects in whom the renin-angiotensin system has been suppressed by a liberal sodium intake, saralasin induces a dose-related increase in plasma aldosterone concentration (54, 103). When the renin-angiotensin system was activated by restriction of sodium intake, saralasin induced a biphasic response (103). An optimal dose of 0.1 μg/kg/min induced the maximal reduction in plasma aldosterone, and an increase in dosage blunted the aldosterone response. SQ 20881 also induced quantitatively similar changes in aldosterone secretion, but only in subjects in balance on a sodium-restricted intake. Neither agent, however, reduced aldosterone concentration to that measured in sodium-loaded sub-

jects. SQ 20881 induced an increase in plasma renin activity within minutes, presumably by interruption of the short feedback loop, which may well have limited its net effect on the adrenal. Similarly, the partial agonist action of saralasin may well have limited the adrenal response. The available data support the hypothesis that angiotensin mediates the adrenal's response to restriction of sodium intake in normal man, but do not prove that the entire response is mediated via that mechanism.

Interpretation of studies in this area have been complicated by the possibility that not angiotensin II, but rather the des-asp heptapeptide analogue is a mediator (22, 25, 36–38). After many years in which this agent was considered as a metabolic product of octapeptide degradation, Blair-West et al demonstrated that the heptapeptide was as effective as the octapeptide in inducing aldosterone secretion (25). Moreover, Blair-West et al raised the interesting possibility that the heptapeptide might be formed without the intervening formation of angiotensin II. Campbell et al confirmed their observation and suggested that the heptapeptide be labeled "angiotensin III" (36). Peach & Chiu demonstrated in an in vitro preparation of rabbit adrenal cortical tissue that angiotensin II and angiotensin III induced essentially identical aldosterone responses, and heptapeptide analogues were reported as being more effective than octapeptide analogues in blocking the effects of angiotensin (37). This observation was supported by studies in vivo, in the dog (22). Saltman et al, conversely, were unable to document a preferential action of heptapeptide analogues in an isolated canine glomerulosa cell preparation (104). The difference between the in vitro studies reported by Saltman (104) and by Peach & Chiu (37) are unlikely to be attributable to the species differences even though Saltman used a canine and Peach a rabbit adrenal, since Bravo et al, who also demonstrated a preferential action of the heptapeptide, did so in the intact dog (22).

The angiotensin analogues have also been useful in assessing the adrenal receptor. Thus, for example, we were able to demonstrate unequivocal evidence for a functional difference in the vascular and adrenal angiotensin receptor. Saralasin acted as a partial agonist or competitive antagonist in vascular muscle (105); 4-phe, 8-tyr angiotensin II, conversely, acted as a partial agonist in vascular smooth muscle but neither stimulated the adrenal in vitro nor blocked the effect of angiotensin (105). More recently, the nature of the change in response to angiotensin II with modifications of dietary sodium intake were explored. A threshold saralasin concentration reduced the response to angiotensin of aortas derived from rabbits on a high but not on a low salt intake. Similarly, a threshold saralasin concentration inhibited the aldosterone response to angiotensin in glomerulosa cells obtained from sodium-loaded, but not sodium-restricted rats (106). Because the competitive antagonist presumably shares affinity for the same receptor

on the basis of structural similarities, the results were interpreted to suggest that sodium restriction blunts the vasoactive effects of angiotensin by reducing the affinity of its receptor for angiotensin. In contrast, the enhanced adrenal response to angiotensin with sodium restriction could not be explained by an altered receptor-agonist interaction, but rather was due to some more distal process that was activated sufficiently to overcome the influence of reduced receptor affinity.

The partial agonist property of saralasin may well have utility in assessing the adrenal receptor in essential hypertension (107). SQ 20881, which is free of intrinsic activity on the adrenal, induced an identical reduction in a plasma aldosterone concentration in patients with essential hypertension who were in balance on a low salt intake as it did in normal subjects on the same diet (107). This observation suggests that angiotensin plays the same role in maintaining aldosterone release in essential hypertension as it does in the normal subjects. Saralasin, on the other hand, infused at a dose that reduced plasma aldosterone in normal man, induced a much smaller reduction in patients with essential hypertension, and in about one half actually stimulated the gland—a response seen with a high sodium intake in normal man. In patients with renovascular hypertension, saralasin induced a normal reduction in plasma aldosterone, so that the paradoxical response in essential hypertension could not be attributed to the hypertension per se. Because a potentiated adrenal response to angiotensin II had been documented in essential hypertension (108), the possibility that the agonist response to saralasin reflected an enhanced reactivity was also assessed. Patients in whom saralasin was an agonist, however, showed a blunted adrenal response to angiotensin II, ruling out that possibility. The best available interpretation at present is that the adrenal angiotensin receptor differs from the normal in patients with essential hypertension.

OTHER APPLICATIONS OF PHARMACOLOGIC INTERRUPTION OF THE RENIN-ANGIOTENSIN SYSTEM

Angiotensin analogues and converting enzyme inhibitors have also been employed to examine the receptors mediating the response to angiotensin in the central nervous system (109–111), in the myocardium (20, 112), in the peripheral nervous system (24, 113, 114), in the adrenal medulla (115, 116), and in physiological functions, especially thirst (117). As in the case of the systems reviewed in detail, the available evidence suggests that the angiotensin receptor in these systems differs, at least somewhat, from the receptor in vascular smooth muscle. The overall contribution of angiotensin to the control and function of these systems remains a matter of conjecture.

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Literature Cited

1. Elliott, D. F. 1959. In *Polypeptides, Which Affect Smooth Muscles and Blood Vessels, Proc. Symp. London,* ed. M. Schachter, pp. 99–105. New York: Pergamon
2. Khairallah, P. A., Toth, A., Bumpus, F. M. 1970. Analogs of angiotensin II. Mechanism of receptor interaction. *J. Med. Chem.* 13:181
3. Marshall, G. R., Vine, W., Needleman, P. 1970. A specific competitive inhibitor of angiotensin II. *Proc. Natl. Acad. Sci. USA* 67:484
4. Turker, R. K., Yamamoto, M., Khairallah, P. A., Bumpus, F. M. 1971. Competitive antagonism of 8-ala-angiotensin II to angiotensins I and II on isolated rabbit aorta and rat ascending colon. *Eur. J. Pharmacol.* 15:285–91
5. Needleman, P., Freer, R. J., Marshall, G. R. 1972. Angiotensin-receptor interaction: Influence of pH on angiotensin inhibition by Phe^4-Tyr^8-angiotensin II in rat uterine smooth muscle. *Arch. Int. Pharmacodyn. Ther.* 200:118
6. Ferreira, S. H., Bartelt, D. C., Greene, L. J. 1970. Isolation of bradykinin potentiating peptides from Bothrops jaracara venom. *Biochemistry* 9:2583
7. Haber, E. 1976. The role of renin in normal and pathological cardiovascular homeostasis. *Circulation* 54:849–61
8. Davis, J. O., Freeman, R. H. 1976. Mechanisms regulating renin release. *Physiol. Rev.* 56:1–56
9. Pettinger, W. A., Keeton, T. K., Campbell, W. B., Harper, D. C. 1976. Evidence for a renal α-adrenergic receptor inhibiting renin release in rats. *Circ. Res.* 38:1–10
10. Keeton, T. K., Pettinger, W. A., Campbell, W. B. 1976. The effects of altered sodium balance and adrenergic blockade on renin release induced in rats by angiotensin antagonism. *Circ. Res.* 38:1–10
11. Regoli, D., Park, W. K., Rioux, F. 1974. Pharmacology of angiotensin. *Pharmacol. Rev.* 26:69–123
12. Marshall, G. R. 1976. Structure-activity relations of antagonists of the renin-angiotensin system. *Fed. Proc.* 35:2494–2501
13. Bumpus, F. M. 1977. In *Hypertension: Mechanisms, Diagnosis and Management,* ed. J. O. Davis, J. H. Laragh, A. Selwyn, pp. 46–58. New York: HP Publ. Co. 254 pp.
14. Erdos, E. G. 1975. Angiotensin I converting enzyme. *Circ. Res.* 36:247–55
15. Ondetti, M. A., Williams, N. G., Sabo, E. F., Pluscec, J., Weaver, E. R., Kocy, O. 1971. Angiotensin-converting enzyme inhibitors from the venom of Bothrops jaracara. Isolation, elucidation of structure, and synthesis. *Biochemistry* 10:4033
16. Ondetti, M. A., Pluscec, J., Weaver, E. R., Williams, N., Sabo, E. F., Kocy, O. 1972. In *Chemistry and Biology of Peptides,* ed. J. Meienhofer, p. 525. Ann Arbor, Mich: Ann Arbor Sci. Publ.
17. Cushman, D. W., Cheung, H. S., Sabo, E. F., Ondetti, M. A. 1977. Design of potent competitive inhibitors of angiotensin-converting enzyme. Carboxyalkanoyl and mercaptoalkanoyl amino acids. *Biochemistry* 16:5484–91
18. Ondetti, M. A., Rubin, B., Cushman, D. W. 1977. Design of specific inhibitors of angiotensin-converting enzyme: New class of orally active antihypertensive agents. *Science* 196:441–44
19. Pals, D. T., Masucci, F. D., Denning, G. S. Jr., Sipos, F., Fessler, D. C. 1971. Role of the pressor action of angiotensin II in experimental hypertension. *Circ. Res.* 29:673

20. Rioux, F., Park, W. K., Regoli, D. 1975. Characterization of angiotensin receptors in rabbit isolated atria. *Can. J. Physiol. Pharmacol.* 54:229–37
21. Williams, G. H., McDonnell, L. M., Raux, M. C., Hollenberg, N. K. 1974. Evidence for different angiotensin II receptors in rat adrenal glomerulosa and rabbit vascular smooth muscle cells. Studies with competitive antagonists. *Circ. Res.* 34:384–90
22. Bravo, E. L., Khosla, M. C., Bumpus, F. M. 1974. Action of [1-des (aspartic acid), 8-isoleucine] angiotensin II upon the pressor and steroidogenic activity of angiotensin II. *J. Clin. Endocrinol. Metab.* 40:530–33
23. Steele, J. M. Jr., Lowenstein, J. 1974. Differential effects of an angiotensin II analogue on pressor and adrenal receptors in the rabbit. *Circ. Res.* 35:592–600
24. Khairallah, P. A. 1972. Action of angiotensin on adrenergic nerve endings: Inhibition of norepinephrine uptake. *Fed. Proc.* 31:1351–57
25. Blair-West, J. R., Coghlan, J. P., Denton, D. A., Funder, J. W., Scoggins, B. A., Wright, R. D. 1971. Effect of the heptapeptide (2-8) and hexapeptide (3-8) fragments of angiotensin II on aldosterone secretion. *J. Clin. Endocrinol.* 32:575–78
26. Taub, K. J., Caldicott, W. J. H., Hollenberg, N. K. 1977. Angiotensin antagonists with increased specificity for the renal vasculature. *J. Clin. Invest.* 59:528–35
27. Vine, W. H., Brueckner, D. A., Needleman, P., Marshall, G. R. 1973. Synthesis, biological activity, and ^{19}F nuclear magnetic resonance spectra of angiotensin II analogs containing fluorine. *Biochemistry* 12:1630
28. Pals, D. T., Masucci, F. D., Denning, G. S. Jr., Sipos, F., Fessler, D. C. 1971. Role of the pressor action of angiotensin II in experimental hypertension. *Circ. Res.* 29:673
29. Pena, C., Stewart, J. M., Goodfriend, T. C. 1974. A new class of angiotensin inhibitors: N-methylphenylalanine analogs. *Life Sci.* 14:1331
30. Turk, J., Needleman, P., Marshall, G. R. 1976. Analogs of angiotensin II with restricted conformational freedom including a new antagonist. *Mol. Pharmacol.* 12:217
31. Hall, M. M., Khosla, M. C., Khairallah, P. A., Bumpus, F. M. 1974. Angiotensin analogs: The influence of sarcosine substituted in position 1. *J. Pharmacol. Exp. Ther.* 188:222–28
32. Furchgott, R. F. 1967. The pharmacological differentiation of adrenergic receptors. *Ann. NY Acad. Sci.* 139:553–70
33. Strewler, G. J., Hinrichs, K. J., Guiod, L. R., Hollenberg, N. K. 1972. Sodium intake and vascular smooth muscle responsiveness to norepinephrine and angiotensin in the rabbit. *Circ. Res.* 31:758–66
34. Paiva, A. C. M., Nouailheatas, V. L. A., Miyamoto, M. E., Mendes, G. B., Paiva, T. B. 1973. New specific angiotensin antagonists. (8-valine)-, (8-isoleucine)- and chloroambucil-des-1-aspartic, 8-valine-angiotensin I. *J. Med. Chem.* 16:6
35. Paiva, T. B., Paiva, A. C. M., Freer, R. J., Stewart, J. M. 1972. Alkylating analogs of peptide hormones. 2. Synthesis and properties of p-[N,N-bis (2-chloroethyl) amino] phenylbutryl derivatives of angiotensin II. *J. Med. Chem.* 15:6
36. Campbell, W. B., Brooks, S. N., Pettinger, W. A. 1974. Angiotensin II and angiotensin III-induced aldosterone release in vivo in the rat. *Science* 184:994–96
37. Peach, M. J., Chiu, A. T. 1974. Stimulation and inhibition of aldosterone biosynthesis in vitro by angiotensin II and analogs. *Circ. Res.* (Suppl.) 34,35: I-7–I-13
38. Goodfriend, T. L., Peach, M. J. 1975. Angiotensin III: (Des-Aspartic Acid[1])-Angiotensin II. *Circ. Res.* (Suppl.) 36,37:I-38–I-48
39. Mimran, A., Hinrichs, K. J., Hollenberg, N. K. 1974. Characterization of smooth muscle receptors for angiotensin: Studies with an antagonist. *Am. J. Physiol.* 226:185–90
40. Cheung, H. S., Cushman, D. W. 1973. Inhibition of homogeneous angiotensin-converting enzyme of rabbit lung by synthetic venom peptides of Bothrops jaracara. *Biochim. Biophys. Acta* 293: 451
41. Wolfenden, R. 1972. Analog approaches to the structure of the transition state in enzyme reactions. *Acc. Chem. Res.* 5:10
42. Ng, K. K. F., Vane, J. R. 1967. Conversion of angiotensin I to angiotensin II. *Nature* 216:762–66
43. Pettinger, W. A., Keeton, K., Phil, M., Tanaka, K. 1975. Radioimmunoassay and pharmacokinetics of saralasin in the rat and hypertensive patients. *Clin. Pharmacol. Ther.* 17:146–58
44. Ishikawa, I., Hollenberg, N. K. 1976. Pharmacologic interruption of the re-

nin-angiotensin system in myohemoglobinuric acute renal failure. *Kidney Int.* 10:S183–90
45. Hollenberg, N. K. 1978. *Proc. VIIth Int. Congr. Nephrol.,* Montreal, 1978, pp. 677–85
46. Bianchi, A., Evans, D. B., Cobb, M., Peschka, M. T., Schaeffer, T. R., Laffan, R. J. 1973. Inhibition by SQ 20881 of vasopressor response to angiotensin I in conscious animals. *Eur. J. Pharmacol.* 23:90–96
47. Freeman, R. H., Davis, J. O., Vitale, S. J., Johnson, J. A. 1973. Intrarenal role of angiotensin II. *Circ. Res.* 32:692–98
48. Mimran, A., Guiod, L., Hollenberg, N. K. 1974. Angiotensin's role in the cardiovascular and renal response to salt restriction. *Kidney Int.* 5:348–55
49. Schroeder, E. T., Anderson, G. H., Goldman, S. H., Streeten, D. H. P. 1976. Effects of angiotensin II (AII) blockade with 1-Sar-8-Ala AII (Saralasin) in patients with cirrhosis and ascites. *Kidney Int.* 9:511–19
50. Sasaki, H., Okumura, H., Ikeda, H., Kawasaki, I., Fukiyama, K. 1976. Hypotensive response to angiotensin II analogue in Bartter's syndrome. *New Engl. J. Med.* 294:611–12
51. Anderson, G. H. Jr., Streeten, D. H. P., Dalakos, T. G. 1977. Pressor response to 1-Sar-8-Ala angiotensin II (saralasin) in hypertensive subjects. *Circ. Res.* 40:243–50
52. Ogihara, T., Yamamoto, T., Kumahara, Y. 1974. Clinical applications of synthetic angiotensin II analogue. *Jpn. Circ. J.* 38:997
53. Marks, L. S., Maxwell, M. H., Kaufman, J. J. 1977. Renin, sodium, and vasodepressor response to saralasin in renovascular and essential hypertension. *Ann. Int. Med.* 87:176
54. Hollenberg, N. K., Williams, G. H., Burger, B., Ishikawa, I., Adams, D. F. 1976. Blockade and stimulation of renal, adrenal, and vascular angiotensin II receptors with 1-sar, 8-ala angiotensin II in normal man. *J. Clin. Invest.* 57:39–46
55. Gavras, H., Brunner, H. R., Turini, G. A., Kershaw, G. R., Tifft, C. P., Cuttelod, S., Gavras, I., Vukovich, R. A., McKinstry, D. N. 1978. Antihypertensive effect of the oral angiotensin converting enzyme inhibitor SQ 14225 in man. *New Engl. J. Med.* 298:991–95
56. Sancho, J., Re, R., Burton, J., Barger, A. C., Haber, E. 1976. The role of the renin-angiotensin-aldosterone system in cardiovascular homeostasis in normal human subjects. *Circulation* 53:400–5
57. Macgregor, G. A., Dawes, P. M. 1976. Agonist and antagonist effects of Sar^1-ala^8-angiotensin II in salt-loaded and salt-depleted normal man. *Br. J. Clin. Pharmacol.* 3:483–87
58. Posternak, L., Brunner, H. R., Gavras, H., Brunner, D. B. 1977. Angiotensin II blockade in normal man: Interaction of renin and sodium in maintaining blood pressure. *Kidney Int.* 11:197–203
59. Hollenberg, N. K., Williams, G. H., Adams, D. F., Moore, T., Brown, C., Borucki, L. J., Leung, F., Bavli, S., Solomon, H. S., Passan, D., Dluhy, R. 1978. Response to saralasin and angiotensin's role in essential and renal hypertension. *Medicine.* In press
60. Hollenberg, N. K., Williams, G. H., Taub, K. J., Ishikawa, I., Brown, C., Adams, D. F. 1977. Renal vascular response to interruption of the renin-angiotensin system in normal man. *Kidney Int.* 12:285–93
61. Gavras, H., Ribeiro, A. B., Gavras, I., Brunner, H. R. 1976. Reciprocal relation between renin dependency and sodium dependency in essential hypertension. *New Engl. J. Med.* 295: 1278–83
62. Borucki, L. J., Levenson, D., Hollenberg, N. K. 1978. Cardiovascular responses to blockade of angiotensin and alpha-adrenergic vascular receptors in the hypovolemic rabbit. *Am. J. Physiol.* 235:F199–F202
63. Rose, J. C., Kot, P. A., Cohn, J. N., Freis, E. D., Eckert, G. E. 1962. Comparison of effects of angiotensin and norepinephrine on the pulmonary circulation, systemic arteries and veins, and systemic vascular capacity in the dog. *Circulation* 25:247–53
64. Case, D. B., Wallace, J. M., Keim, H. J., Weber, M. A., Sealey, J. E., Laragh, J. H. 1977. Possible role of renin in hypertension as suggested by renin-sodium profiling and inhibition of converting enzyme. *N. Engl. J. Med.* 296:641–46
65. Streeten, D. H. P., Anderson, G. H., Freiberg, J. M., Dalakos, T. G. 1975. Use of an angiotensin II antagonist (saralasin) in the recognition of "angiotensinogenic" hypertension. *N. Engl. J. Med.* 292:657
66. Wilson, H. M., Wilson, J. P., Slaton, P. E., Foster, J. H., Liddle, G. W., Hollifield, J. W. 1977. Saralasin infusion in the recognition of renovascular hypertension. *Ann. Int. Med.* 87:36–42

67. Beckerhoff, R., Vetter, W., Furrer, J., Tuma, J., Zaruba, K., Vetter, H., Siegenthaler, W. 1976. The effect of the angiotensin antagonist saralasin (1-sar-8-ala-angiotensin II) on blood pressure in secondary hypertension. *Schweiz. Med. Wochenschr.* 106:1738–41
68. Baer, L., Parra-Carrillo, J. Z., Radichevich, I., Williams, G. S. 1977. Detection of renovascular hypertension with angiotensin II blockade. *Ann. Int. Med.* 86:257–60
69. Davis, J. O. 1977. The pathogenesis of chronic renovascular hypertension. *Circ. Res.* 40:439–44
70. Williams, G. H., Hollenberg, N. K. 1977. Accentuated vascular and endocrine response to SQ 20881 in hypertension. *N. Engl. J. Med.* 297:184–88
71. Swartz, S. L., Williams, G. H., Hollenberg, N. K., Moore, T. J., Dluhy, R. G. 1978. Does SQ 20881 identify angiotensin mediated hypertension? *Circ. Res.* In press
72. Schmid, H. E. Jr. 1962. Renin, a physiologic regulator of renal hemodynamics. *Circ. Res.* 11:185–93
73. Brown, J. J., Chinn, R. H., Gavras, H., Leckie, B., Lever, A. F., McGregor, J., Morton, J., Robertson, J. I. S. 1972. In *Hypertension '72,* ed. J. Genest, E. Koiw, pp. 81–97. Berlin: Springer
74. Sokabe, H. 1974. Phylogeny of the renal effects of angiotensin. *Kidney Int.* 6:263–71
75. Bern, H. A. 1967. Hormones and endocrine glands of fishes. *Science* 158:455–62
76. Sawyer, W. H., Blair-West, J. R., Simpson, P. A., Sawyer, M. K. 1976. Renal responses of Australian lungfish to vasotocin, angiotensin II, and NaCl infusion. *Am. J. Physiol.* 231:593–602
77. Granger, P., Dahlheim, H., Thurau, K., 1972. Enzyme activities of the single juxtaglomerular apparatus in the rat kidney. *Kidney Int.* 1:78–88
78. Leckie, B., Gavras, H., McGregor, J., McElwee, G. 1972. The conversion of angiotensin I to angiotensin II by rabbit glomeruli. *J. Endocrinol.* 55:229–30
79. Bailie, M. D., Rector, F. C. Jr., Seldin, D. W. 1971. Angiotensin II in arterial and renal venous plasma and renal lymph in the dog. *J. Clin. Invest.* 50:119–26
80. DiSalvo, J., Fell, C. 1970. Effects of angiotensin on canine renal blood flow. *Proc. Soc. Exp. Biol. Med.* 133:1432
81. Hofbauer, K. G., Zschiedrich, H., Rauh, W., Gross, F. 1973. Conversion of angiotensin I into angiotensin II in the isolated perfused rat kidney. *Clin. Sci.* 44:447–56
82. Aurell, M. 1969. Renal response in man to plasma volume expansion and angiotensin. *Scand. J. Clin. Lab. Invest.* 24: Suppl. 112, pp. 1–59
83. Burger, B. M., Hopkins, T., Tulloch, A., Hollenberg, N. K. 1976. The role of angiotensin in the canine renal vascular response to barbiturate anesthesia. *Circ. Res.* 38:196–202
84. Ishikawa, I., Hollenberg, N. K. 1975. Blockade of the systemic and renal vascular actions of angiotensin II with the 1-sar, 8-ala analogue in the rat. *Life Sci.* 17:121–30
85. Freeman, R. H., Davis, J. O., Spielman, W. S., Lohmeier, T. E. 1975. High-output heart failure in the dog: Systemic and intrarenal role of angiotensin II. *Am. J. Physiol.* 229:474–78
86. Zimmerman, B. G. 1973. Involvement of angiotensin-mediated renal vasoconstriction in renal hypertension. *Life Sci.* 13:507–15
87. Lachance, J. G., Arnoux, E., Brunette, M. G., Carriere, S. 1974. Factors responsible for the outer cortical ischemia observed during hemorrhagic hypotension in dogs. *Circ. Shock* 1:131–44
88. Frega, N., Guerther, B., Dibona, D., Leaf, A. 1973. The role of angiotensin II in the impaired reflow following hemorrhagic hypotension. *Am. Soc. Nephrol.* 6:37
89. Kimbrough, H. M. Jr., Vaughan, E. D. Jr., Carey, R. M., Ayers, C. R. 1977. Effect of intrarenal AII blockade on renal function in conscious dogs. *Circ. Res.* 40:174–78
90. Hall, J. E., Guyton, A. C., Trippodo, N. C., Lohmeier, T. E., McCaa, R. E., Cowley, A. W. 1977. Intrarenal control of electrolyte excretion by angiotensin II. *Am. J. Physiol.* 232:F538–44
91. Slick, G. L., DiBona, G. F., Kaloyanides, G. J. 1975. Renal blockade of angiotensin II in acute and chronic sodium-retaining states. *J. Pharmacol. Exp. Ther.* 195:185–93
92. Freeman, R. H., Davis, J. O., Lohmeier, T. E. 1975. Des-1-Asp-Angiotensin II. Possible intrarenal role in homeostasis in the dog. *Circ. Res.* 37:30–34
93. Watkins, L. Jr., Burton, J. A., Haber, E., Cant, J. R., Smith, F. W., Barger, A. C. 1976. The renin-angiotensin-aldosterone system in congestive failure in conscious dogs. *J. Clin. Invest.* 57: 1606–17
94. Laragh, J. H., Angers, M., Kelly, W. G., Lieberman, S. 1960. Hypotensive

agents and pressor substances. The effect of epinephrine, norepinephrine, angiotensin II and other on the secretory rate of aldosterone in man. *J. Am. Med. Assoc.* 174:234–40

95. Boyd, G. W., Adamson, A. R., Arnold, M., James, V. H. T., Peart, W. S. 1972. The role of angiotensin II in the control of aldosterone in man. *Clin. Sci.* 42:91–104
96. Hollenberg, N. K., Chenitz, W. R., Adams, D. F., Williams, G. H. 1974. Reciprocal influence of salt intake on adrenal glomerulosa and renal vascular responses to angiotensin II in normal man. *J. Clin. Invest.* 54:34–42
97. Oelkers, W., Brown, J. J., Fraser, R., Lever, A. F., Morton, J. J., Robertson, J. I. S. 1974. Sensitization of the adrenal cortex to angiotensin II in sodium-deplete man. *Circ. Res.* 34:69–77
98. Lowenstein, J., Boyd, G. W., Rippon, A. E., James, V. H. T., Peart, W. S. 1972. See Ref. 73, pp. 481–89
99. Blair-West, J. R., Coghlan, J. P., Cran, E., Denton, D. A., Funder, J. W., Scoggins, B. A. 1973. Increased aldosterone secretion during sodium depletion with inhibition of renin release. *Am. J. Physiol.* 224:1409–14
100. Johnson, J. A., Davis, J. O. 1973. Effects of a specific competitive antagonist of angiotensin II on arterial pressure and adrenal steroid secretion in dogs. *Circ. Res.* 32: Suppl. I, I-159–I-168
101. Spielman, W. S., Davis, J. O. 1974. The renin-angiotensin system and aldosterone secretion during sodium depletion in the rat. *Circ. Res.* 35:615–24
102. Stephens, G. A., Davis, J. O., Freeman, R. H., Watkins, B. E., Khosla, M. C. 1977. The effects of angiotensin II blockade in conscious sodium-depleted dogs. *Endocrinology* 101:378–88
103. Williams, G. H., Hollenberg, N. K., Brown, C., Mersey, J. H. 1978. Adrenal responses to pharmacologic interruption of the renin-angiotensin system in sodium restricted normal man. *J. Endocrinol. Metab.* 47:725–31
104. Saltman, S., Fredlund, P., Catt, K. J. 1976. Actions of angiotensin II antagonists upon aldosterone production by isolated adrenal glomerulosa cells. *Endocrinology* 98:894–903
105. Williams, G. H., McDonnell, L. M., Raux, M. C., Hollenberg, N. K. 1974. Evidence for different angiotensin II receptors in rat adrenal glomerulosa and rabbit vascular smooth muscle cells. Studies with competitive antagonists. *Circ. Res.* 34:384–90
106. Williams, G. H., Hollenberg, N. K., Braley, L. M. 1976. Influence of sodium intake on vascular and adrenal angiotensin II receptors. *Endocrinology* 98:1343–50
107. Williams, G. H., Hollenberg, N. K., Moore, T. J., Swartz, S. L., Dluhy, R. G. 1978. The adrenal receptor for angiotensin II is altered in essential hypertension. *J. Clin. Invest.* In press
108. Kisch, E. S., Dluhy, R. G., Williams, G. H. 1976. Enhanced aldosterone response to angiotensin II in human hypertension. *Circ. Res.* 38:502–5
109. Sakai, K. K., Marks, B. H., George, J., Koestner, A. 1974. Specific angiotensin II receptors in organ-cultured canine supra-optic nucleus cells. *Life Sci.* 14:1337–44
110. Solomon, T. A., Cavero, I., Buckley, J. P. 1974. Inhibition of central pressor effects of angiotensin I and II. *J. Pharm. Sci.* 63:511–15
111. Sweet, C. S., Columbo, J. C., Gaul, S. L. 1976. Comparative antihypertensive effects of inhibitors of the renin-angiotensin system by central and peripheral administration in the malignant and spontaneously hypertensive rat. *Fed. Proc.* 35:397
112. Bonnardeaux, J. L., Regoli, D. 1973. Action of angiotensin and analogues on the heart. *Can. J. Physiol. Pharmacol.* 52:50–60
113. Sweet, C. S., Ferrario, C. M., Khosla, M. C., Bumpus, F. M. 1973. Antagonism of peripheral and central effects of angiotensin II by (1-sarcosine, 8-isoleucine) angiotensin II. *J. Pharmacol. Exp. Ther.* 185:35–41
114. Zimmerman, B. G. 1973. Blockade of adrenergic potentiating effect of angiotensin by 1-sar-8-ala angiotensin II. *J. Pharmacol. Exp. Ther.* 185:486–92
115. Peach, M. J., Ober, M. 1974. Inhibition of angiotensin-induced adrenal catecholamine release by 8-substituted analogs of angiotensin II. *J. Pharmacol. Exp. Ther.* 190:49–58
116. Sen, S., Smeby, R. R., Bumpus, F. M. 1974. Angiotensin antagonist and possible release of catecholamine (38453). *Proc. Soc. Exp. Biol. Med.* 147:847–49
117. Lehr, D., Goldman, H. W., Casner, P. 1973. Renin-angiotensin role in thirst: Paradoxical enhancement of drinking by angiotensin convertin enzyme inhibitor. *Science* 182:1031–34