Inhibition of Converting Enzyme of the Renin-Angiotensin System in Kidneys and Hindlegs of Dogs

By James W. Aiken and John R. Vane

ABSTRACT

The activity of angiotensin-converting enzyme in hindlegs and kidneys was compared in anesthetized dogs. Intra-arterial injections of angiotensin I or angiotensin II to one kidney or one hindleg caused dose-dependent decreases in blood flow in that vascular bed. An inhibitor of angiotensin-converting enzyme activity did not affect the vasoconstrictor activity of angiotensin II but substantially reduced that of angiotensin I. The reduction in vasoconstrictor activity of angiotensin I during enzyme inhibition was used to calculate conversion of angiotensin I to angiotensin II. There was 40% conversion in hindleg but only 2.1% in kidney. After intra-arterial injection of angiotensin I, bioassay of angiotensin II in the renal or iliac venous blood indicated that 60-90% of freshly formed angiotensin II was inactivated before leaving the kidney or hindleg. The results show that converting enzyme activity is much greater in hindlegs than in kidneys and that the endogenously formed angiotensin II following intra-arterial injection of angiotensin I is destroyed to the same extent as intra-arterially injected angiotensin II. Intra-arterial infusion of the enzyme inhibitor increased hindleg blood flow in half of the dogs; renal blood flow increased in only one dog. The inhibitor did not affect vascular responses produced by norepinephrine, histamine, vasopressin, or prostaglandin F₂α but the effects of bradykinin were potentiated.

KEY WORDS angiotensin I angiotensin II renal blood flow iliac blood flow converting enzyme inhibitor bradykinin Bothrops jararaca peptides
strips of renal arteries (8). Converting enzyme activity, including that extracted from kidney (9) and that responsible for the intramural generation of All in isolated strips of renal and other arteries (8) is inhibited by the pentapeptide, pyrrolidone carboxylic acid-Lys-Trp-Ala-Pro (10–13). We have, therefore, examined in vivo the vascular effects of AI by using the inhibitor of converting enzyme to compare in the kidney and hindleg of dogs the participation of local converting enzyme activity in the vascular responses produced by AI.

Methods

Nineteen dogs (14–30 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg, iv) and ventilated with a positive pressure pump via an endotracheal tube. A midline incision was made in the abdomen. Blood flow to the kidneys or hindlegs was measured by noncannulating electromagnetic flow probes placed around either the left and right renal arteries or the left and right external iliac arteries. The probes were connected to a two-channel flowmeter. Mean blood pressure was measured from a cannula in a carotid artery with a pressure transducer. All responses were displayed on multichannel rectilinear pen recorders.

Blood flow to the kidneys was studied in one group of dogs and to the hindlegs in another. Intra-arterial injections into one renal artery or one external iliac artery were made through nylon catheters with tip diameters of 1 mm. When renal blood flow was studied, a femoral artery was cannulated and the catheter was passed retrograde up the abdominal aorta. The tip of the catheter was bent and was directed by hand into the left renal artery. When external iliac blood flow was studied, an internal iliac artery was cannulated and the catheter was passed retrograde to the junction of the abdominal aorta and the two external iliac arteries. The bent tip of the catheter was then manipulated into one of the external iliac arteries. Thus substances injected through the intra-arterial catheter mixed with blood supplying one hindleg (or kidney) while blood flow in the contralateral hindleg (or kidney) was monitored as a control for responses mediated by reflexes or recirculation of intra-arterially injected drugs.

The internal volume of the arterial catheters was 0.35–0.45 ml. Drugs were injected into the catheter in less than 0.3 ml saline (0.9% NaCl w/v). This was then washed from the dead space of the catheter into the arterial blood with 0.5 ml saline. The inhibitor of converting enzyme was infused into the kidney or hindleg through the same catheter used for intra-arterial injections. During these infusions, drugs were washed in with saline containing the converting enzyme inhibitor. Intravenous injections were made through the cannula in a jugular vein. The dogs were given heparin (300 IU/kg, iv) at the beginning of the experiment.

In five experiments, the amount of All in the renal or iliac venous blood after injection of AI or All into the homolateral renal or iliac artery, was estimated by bioassay using the blood-bathed organ technique (14). These dogs were given heparin (1000 IU/kg, iv). Blood from the renal vein was sampled through a catheter inserted from a femoral vein up the vena cava. The bent tip of the catheter was directed by hand so that it lay in, but did not occlude, the left renal vein. Blood from the iliac vein was sampled through a catheter inserted into the femoral vein of the opposite leg and passed to the junction of the left and right common iliac veins. The bent tip of the catheter was then directed upstream so that it lay in, but did not occlude, the common iliac vein of the leg to which intra-arterial injections of AI and All were made. Blood was withdrawn continuously through the venous catheter at a rate of 10 ml/min with a roller pump and used to superfuse (15) a rat colon (16), a rat stomach strip (17), and chick rectum (18). Changes in lengths of the assay tissues were detected by transducers fitted with auxotonic levers. Blood which had superfused the isolated tissues collected in a small reservoir and flowed by gravity back into the dog through a cannula in the jugular vein.

The combination of assay tissues selected was suited for a specific assay of All in circulating blood. The rat colon is contracted by low concentrations of All (16, 19); the rat stomach strip is also contracted by All, but higher concentrations are needed (20). Both tissues are relatively insensitive to AI, and since the activity of AI is due mostly to intramural conversion to All (8), they can be rendered even less sensitive to AI by infusing the inhibitor of converting enzyme activity into the superfusion fluid. The chick rectum was included in the assay system because it is insensitive to AI or All, but is more sensitive to prostaglandin E₂ than the rat colon (21). Thus, any release of prostaglandin E₂ from the kidney (22) (which would add to the contraction of the rat colon and interfere with accurate assay of AI) would be detected by the chick rectum. Angiotensin II is the only known naturally occurring substance which contracts the rat colon and rat stomach strip but not the chick rectum. However, even if some as yet unidentified rat-colon-contracting substance (23) was released.
I. The AI used was L-Asp-$\beta$-amide-5-Val-angiotensin II. The AI used was 5-Ileu-angiotensin I. For every mg of AI there should be 0.77 $\mu$moles. On the basis of optical density measurements at 280 nm (reference, ethyl tyrosinate) the preparation contained 0.59 $\mu$moles/mg and by assay on the rat blood pressure (reference, AI from Skeggs), 0.46 $\mu$moles/mg. Thus the preparation contained, at best, 76% AI and by rat pressor assay, 60%. When we compared it with L-Asp-$\beta$-amide-5-Val-AII for pressor effect after intravenous injection in 12 dogs, the AI had $65 \pm 4\%$ (mean $\pm$ se) of the activity of the AII, after allowing for the difference in molecular weights.

The activity of AI was compared with that of AII by calculating the equipotent molar ratio. This is the ratio between the molar doses of AI and AII required to produce equal reductions in blood flow. Molar amounts of AI were calculated on the assumption that the AI preparation was only 65% pure. All calculations of percent conversion of AI to AII are based on molar comparisons. However individual doses and dose-response curves are expressed as weight of the AI (or AII) preparation, with no correction factor applied. Suitable precautions (24) were taken to prevent bacterial activation or inactivation of the peptides.

Other substances used were L-norepinephrine-D-bitartrate, 8-Arg-vasopressin, histamine acid phosphate, prostaglandin F$_2\alpha$ and bradykinin. Doses of norepinephrine and histamine are expressed as base.

The converting enzyme inhibitor was the synthetic pentapeptide pyrrolidone carboxylic acid-Lys-Trp-Ala-Pro. This pentapeptide is one of several peptides responsible for the pharmacological activities of "bradykinin potentiating factor" (BPF) extracted from Bothrops jararaca venom (25). It was first known as V-3-A (11, 26) but the name BPP$_{5a}$ was suggested for the synthetic substance (27). The sample of pentapeptide used in this work was synthesized at the Squibb Institute for Medical Research and called SQ 20,475. In view of the lack of agreement in naming the substance, we shall refer to it as "the pentapeptide." It was infused at a rate calculated to give a blood concentration of 1-3 $\mu$g/ml, previously shown (8) to produce near maximum inhibition of converting enzyme activity.

Results

Intra-arterial injections of AI or AII to the hindleg or kidney produced transient, dose-dependent decreases in the rate of blood flow. The response always began within 5 seconds and lasted from 1 to 6 minutes. Blood flow in the opposite hindleg or kidney was unaffected, except with high doses of AI and AII, which often caused a delayed increase or decrease in flow beginning 18 to 25 seconds after the injection. Infusion of the pentapeptide inhibitor of converting enzyme into either the renal or the iliac artery did not significantly affect responses produced by AII, but the effects of AI were always substantially reduced.

Figure 1 illustrates these effects in the iliac vascular bed. Injections of AII at 0.01, 0.03, and 0.1 $\mu$g and AI at 0.1 and 0.5 $\mu$g into the right external iliac artery caused dose-dependent decreases in flow without substantially affecting blood flow in the contralateral hindleg or the blood pressure. Higher doses of AI (1.5 $\mu$g) and AII (0.3 $\mu$g) injected intra-arterially caused even greater decreases in flow in the right iliac artery and, after a 25-second delay, a transient increase in blood flow on the left side and in blood pressure. Similar increases in blood pressure and blood flow were obtained when AI (0.5 $\mu$g) and AII (0.3 $\mu$g) were injected intravenously. During intra-arterial infusion of the pentapeptide at 250 $\mu$g/min, doses of AI (0.5 and 1.5 $\mu$g) which previously had caused large reductions in blood flow now produced only small decreases, whereas the effects of AII at 0.01, 0.03, and 0.1 $\mu$g were the same as, or slightly greater than, during the control period. Although local vasoconstriction in the hindleg produced by intra-arterially injected AI was antagonized during close intra-arterial infusion of the converting enzyme inhibitor, the effects of an intravenous injection of AI were unchanged. This suggested that the small amount of pentapeptide infused directly into the blood supply of the hindleg had little or no effect on converting enzyme activity in the lungs after being diluted by the total venous
At the dots, injections of AI or AII were made through a cannula into the right external iliac artery or (when designated i.v.) through a cannula in the jugular vein. Doses are shown in µg. At s saline was infused intra-arterially at 0.5 ml/min to the right iliac artery. Pentapeptide was infused where shown at a rate of 0.5 ml/min and a dose of 250 µg/min. At the break in the traces 20 minutes of the record is omitted.

Return. Twenty minutes after the pentapeptide infusion had been stopped, AI (0.5 µg) injected intra-arterially had regained most of its initial activity.

These results showed that the decreases in blood flow produced by intra-arterial injections of either AI or AII were caused by a local vasoconstriction and not by an action occurring after their recirculation. Furthermore, the selective antagonism of the effects of intra-arterially injected AI by the pentapeptide strongly suggested that AI caused reduction in blood flow in the iliac vascular bed predominantly through a rapid, local conversion to AII.

Similar results were obtained when renal blood flow was measured, except that much higher intra-arterial doses of AI had to be used to elicit a comparable degree of vasoconstriction. Also contrary to the results in the hindlegs, intravenous AI or AII usually produced a bilateral decrease in renal blood flow. Likewise, after large intra-arterial doses of AI or AII, there was a delayed decrease in flow in the contralateral kidney.

Both in renal and iliac vascular beds, the rate of decline in blood flow produced by AI was slower than that produced by AII. The latency from injection to the onset of the fall in flow was similar for both peptides (2-5 seconds), but the peak reduction in flow after AII was reached within 10 to 20 seconds, whereas that for an equiactive dose of AI took 3-6 seconds longer. Also, the duration of the response to equiactive doses of AI and AII was usually greater for the decapptide (in Fig. 1 compare AI at 0.5 µg to AII at 0.1 µg).

The effects of AI and AII were compared quantitatively in these two vascular beds by determining, in each experiment, the equipotent molar ratios of AI to AII before and during inhibition of converting enzyme. The peak vasoconstrictor effects of different submaximal intra-arterial injections of AI were each bracketed between smaller and larger effects of doses of AII. In this way, several determinations of relative activity of AI to AII were made in each experiment. The equipotent molar ratios were calculated and the means of these ratios are shown in Table 1. The changes in the equipotent molar ratios induced by inhibition of converting enzyme activity were used to estimate the apparent conversion of AI to AII. For example, in the hindleg a mean of 2.3 moles of AI were...
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TABLE 1

<table>
<thead>
<tr>
<th>Vascular bed</th>
<th>Control (Mean ± SE)</th>
<th>During pentapeptide (Mean ± SE)</th>
<th>Calc percent AI converted to All Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>27 ± 2.8</td>
<td>61 ± 12</td>
<td>2.1 (1.4–2.8)</td>
</tr>
<tr>
<td>Iliac</td>
<td>2.3 ± 0.2</td>
<td>28 ± 4.6</td>
<td>39.9 (31–51)</td>
</tr>
</tbody>
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equivalent to 1 mole of All (or 100 moles AI = 43.5 moles All). During inhibition of converting enzyme activity, 28 moles of AI were equivalent to 1 mole of All (or 100 moles AI = 3.6 moles All). Thus, for every 100 moles of AI, 39.9 (43.5–3.6) were converted to All; i.e., there was 39.9% conversion. In the kidney, there was only 2.1% conversion (100 moles of AI = 3.7 moles All before, and 1.6 moles during, converting enzyme inhibition). In one dog, flow was simultaneously measured in a renal and an iliac artery and catheters were inserted so that intra-arterial injections could be made to either vascular bed. Similar results were obtained.

Dose-response curves were plotted from the combined results of these experiments. The reductions in blood flow induced by AI or All were all expressed as percent decreases, taking rate of flow just before injection as 100%. The results are summarized in Figure 2. In kidney and hindleg, the dose-response curves for AI and All were parallel during the control period. During pentapeptide infusion, the activity of AI was decreased, but that of All was not; indeed, there may have been slight potentiation of the effects of the smaller doses of All.

Although the effect of inhibiting activity of converting enzyme on the activity of AI in the kidney was qualitatively similar to that in the hindleg, there were important quantitative differences. Whereas All had the same potency in hindleg and kidney (51% and 53% reduction in flow at 0.1 μg), AI was ten times
more potent in hindleg than in kidney (50% reduction in hindleg flow at 0.5 μg compared with 50% reduction in renal flow at 5 μg).

Thus, both from the molar ratios calculated from the individual experiments and from the dose-response curves, it is evident that AI is much more potent in the hindleg than in the kidney and that this difference in potency of AI in the two vascular beds is due to the much higher activity of converting enzyme in hindlegs than in kidneys.

VENOUS OUTPUT OF ANGIOTENSIN II-LIKE ACTIVITY

Ng and Vane (1) found that only 10–40% of the decapptide’s biological activity survived passage through kidneys or hindlegs. However, they could only roughly estimate whether the activity remaining in the venous blood was due to AI or a mixture of AI and All. The availability of the inhibitor of converting enzyme activity made it possible for us to clarify this point.

In four other dogs, All-like activity in the iliac vein, after intra-arterial injection of AI or All, was estimated by the blood-bathed organ technique. Pentapeptide (15 μg/min) was infused into the blood superfusing the assay tissues to give a concentration of 1.5 μg/ml. This prevented any conversion of AI to All either in the superfusing blood or in the assay tissues themselves. The amount of pentapeptide recirculating to the iliac vascular bed was insufficient to have any detectable effect on AI activity.

Injection of All into the iliac artery (0.1 or 0.3 μg) led to contractions of the rat colons bathed in the venous outflow. Assay of the All output confirmed previous results (1) and showed that 69 ± 6.3% of the lower dose and 62 ± 5% of the higher dose was inactivated in the hindleg. During pentapeptide infusion, this activity was substantially reduced; none was detectable after the lower dose (limit of assay: 16 ng of All) and 0.04 μg of All after the higher dose. These results showed that a substantial portion of the AI-like activity in the iliac vein after intra-arterial injection of AI was, indeed, due to All formed in the hindleg, and that All represented 13.5–15% conversion of the total AI dose. There was 60–70% disappearance of All in the hindleg, estimated by comparing the effects induced by the remaining AI on the blood pressure (after recirculation and conversion in the lungs) with intravenous AI injections. This was within the range (60–90%) found previously using infusions rather than injections (1). In none of these experiments was there evidence for release of prostaglandinlike activity.

In the renal vein of one dog All-like activity was measured in the same way. There was 75% inactivation of All injected into the renal artery. This degree of inactivation was unaffected by the pentapeptide. After injection of 2 μg AI, the activity emerging in the renal vein was equivalent to only 30 ng of All or 1.5% conversion of the injected dose. Infusion of pentapeptide reduced the activity which appeared in the renal vein after intra-arterial injection of AI, confirming that some of the activity was due to All formed in the kidney.

Further experiments on output of All-like activity in the renal vein were not attempted, for the amounts detectable were so small as to be near the limits of the assay procedure. Furthermore, contractions of the other assay tissues suggested that there was a release of prostaglandinlike material after AI injection.

OTHER EFFECTS OF THE PENTAPEPTIDE

Infusion of the pentapeptide at 250 μg/min into the renal artery (rate of blood flow 80–210 ml/min) had no direct effect on blood flow in 6 of the 7 dogs. In one dog there was a gradual increase in flow, amounting to 20% over a period of 25 minutes. In the iliac vascular bed, blood flow varied from 90 to 250 ml/min and in 5 of the 12 dogs the pentapeptide gradually increased flow by 13–24%. In the other 7 dogs there was either no
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change in flow or no increase greater than that produced by an intra-arterial infusion of saline at the same rate (0.5 ml/min). The experiment of Figure 1 was on one of the 5 dogs in which the pentapeptide gradually increased blood flow. During the first half of the trace the baseline flow in both the left and right iliac arteries followed the same pattern. However, during pentapeptide infusion, the left iliac flow gradually increased, whereas the right iliac flow maintained a constant baseline.

Pentapeptide in amounts (1-3 μg/ml) sufficient to antagonize substantially the vascular effects of AI did not affect the changes in iliac blood flow produced by intra-arterial injections of histamine, norepinephrine, vasopressin, prostaglandin F2α, or AII. Figure 3 shows some of these effects. Intra-arterial injections of histamine (0.01, 0.03, and 0.1 μg) produced dose-dependent increases in flow and AII (0.05 and 0.3 μg), AI (0.5 and 1.5 μg), and norepinephrine (0.1 and 0.3 μg) all produced dose-dependent decreases in iliac blood flow. During infusion of pentapeptide, only the effects of AI were reduced; all the other substances retained their activity.

Prostaglandin F2α, when injected intrarterially to the hindlegs (0.3-3 μg), produced a transient increase in flow which lasted to 5-10 seconds, followed by a prolonged decrease in flow lasting from 3 to 5 minutes. Both the initial vasodilatation and secondary vasoconstriction increased with increasing doses. The pentapeptide (1-3 μg/ml blood) did not change these effects of prostaglandin F2α. Similarly, decreases in blood flow produced by vasopressin (10 and 30 mU ia) were unaffected by the pentapeptide.

The pentapeptide potentiates several of the actions of bradykinin, including the contractile effect on isolated guinea pig ileum (26), the increased capillary permeability in rat skin and the decrease in the blood pressure of rats (27). We found that bradykinin (1-30 ng) injected into the iliac artery caused dose-dependent increases in flow. In the presence of the pentapeptide (1-4 μg/ml ia), bradykinin was five times more potent than before pentapeptide. Figure 4 shows that 2 ng bradykinin produced only a slight increase in flow but 5 and 20 ng produced larger ones. Thirty ng histamine produced an increase in flow approximately equivalent to 3 ng bradykinin. Neither the histamine nor the higher
doses of bradykinin altered the effects of a subsequent dose of 2 ng bradykinin. In the presence of pentapeptide (250 μg/min) the effect of 2 ng bradykinin was substantially potentiated (now 2 ng bradykinin was equivalent to a response produced by approximately 10 ng before pentapeptide), but the effect of 30 ng histamine was unchanged.

Discussion

Previous work from this laboratory has shown that in the pulmonary vascular bed there is high conversion of AI and no inactivation of AII. This has been extensively confirmed (8, 12, 23, 28-32) in several species. In other vascular beds, there is an overall inactivation of about 70% of AI or AII (1-3, 28). Because of the efficient inactivating mechanisms in peripheral vascular beds, it has been difficult to determine in vivo how much conversion of AI to AII takes place prior to destruction. However, the availability of an inhibitor of converting enzyme has made possible detailed analysis of local conversion of AI to AII (8).

Our present results show that AI injected into the arterial blood supplying either hindleg or kidney of the dog is partially converted to AII and that the AII generated induces local vasoconstriction. Converting enzyme in plasma has too low an activity to account for these results in the hindleg (1, 3, 29) so the enzyme must be fixed in the tissue. Isolated renal and femoral arteries have converting enzyme activity (8), so the enzyme may be located within the vessel wall. Conversion in the kidney, however, is so low that it may all be due to converting enzyme in plasma; in the dog, Oparil et al. (29) found 3% conversion in whole blood within 10 seconds. Conversion in the hindleg and kidney has been demonstrated by the reduction in local vasoconstrictor activity of AI when converting enzyme activity is inhibited and by the reduction during enzyme inhibition of the venous output of AII-like activity after intra-arterial injection of AI. Both methods indicate that conversion of AI to AII in the hindleg is 10 to 20 times greater than that in the kidney.

In our experiments, assay of AII was easier in hindleg venous blood, where it was present in higher concentrations, than in renal venous blood. Even though the immediate vasoconstrictor activity of AI in the hindlegs indicated up to 40% conversion (Table 1), the venous output of AII-like activity represented only 14% of the injected dose of AI. This agrees well with a 12% apparent conversion in venous blood from dog hindlegs found by Oparil et al. (29), although they believed this to be due to plasma conversion. Thus conversion of AI to AII probably takes place at a site which allows local vasoconstrictor activity, but thereafter there is about 70% inactivation of the newly formed AII before it reaches the iliac vein. This interpretation is supported by the fact that the same percent of injected AII is inactivated on passage through the hindlegs. A similar process may also occur in the kidney, where there is a qualitatively similar disparity: the immediate vasoconstrictor activity of AI represents 2.1% conversion, but less AII-like activity appeared in renal venous blood. The inactivation of newly formed AII in the kidney was probably greater than our experiments indicate, since the observed release of prostaglandinlike material from the kidney would have led to an overestimation of the amount of AII in the renal venous blood. Hence, there was probably even less than 1.5% of the intra-arterially injected AI in the form of AII in the renal venous blood. No prostaglandin release was seen in the experiments on hindlegs. Although the mechanism of inactivation of AI and AII in dog kidney and hindleg is unknown, inactivation of AII in rat kidney is due mostly to rapid metabolism and not to tissue sequestration (33).

Although an intrarenal role for the renin-angiotensin system is not excluded by our results, it seems unlikely that the system has a local role in the kidney for regulating blood flow. The very small proportion of blood-borne AI converted to AII in the dog kidney (2.1%) compared to the hindleg (40%), the intestine (25%) (34) and the lungs (50-100%) suggests that the kidneys may be exceptional for their low converting enzyme activity. Release of renin from the kidney could then
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The relative absence of converting enzyme in the kidney blood vessels would be a protective mechanism, and the renal vascular bed, which is highly sensitive to AII, would only receive AII formed by converting enzyme in the lungs, after the renin content of renal venous blood had been diluted with the rest of the cardiac output. Indeed, the kidney may have a dual mechanism for protecting itself from the vasoconstrictor effects of AII. First, the paucity of converting enzyme activity in the renal vasculature would minimize local generation of AII during renin release. Second, infusions of low concentrations of AII into dog kidney release a prostaglandin E2-like substance (22) which antagonizes the local vasoconstrictor action of AII (35).

The activity of AII cannot be completely abolished by the converting enzyme inhibitor (8), suggesting that AII has a small (1–4%, relative to AII) but significant inherent activity. Others have assumed that AII is completely inactive until converted to AII (4). Whichever view is correct, whenever AII has more than 5% (on a molar basis) of the activity of AII in any organ or isolated tissue it is likely to be due to local conversion to AII.

In dog kidney, even when the inherent activity is disregarded, the maximum possible conversion of AII to locally active AII described here and by Di Salvo et al. (36) and Ng and Vane (1) is very much lower than the 19% claimed by Franklin et al. (4). There are several possible explanations for the discrepancy between the 19% of Franklin et al. and the lower figures. First, implicit in their calculation of the relative potency of AII to AII is the assumption that the effects of a drug are linearly proportional to the dose. It is a cornerstone of bioassay that dose-response curves are sigmoid (37); a linear relationship exists between \( \log \) and the response. However, even if one plots the reductions in renal blood flow produced by AII and AII in Figure 1 of their published experiments (4) against log dose of the angiotensins, and assumes the dose response curves for AII and AII to be parallel (which they are in our experiments), then the percent conversion found by Franklin et al. in dog kidney is still relatively high (14–17%).

Another contribution to the discrepancy may be the difference in potency of the AII and AII of Franklin et al. compared to ours. From the data in their Figure 1 (4) it appears that their AII was more potent and AII less potent than ours. These differences in potency would be cumulative in any calculation of conversion based on relative potency of AII and AII. They did not state the relative activity of their AII and AII solution, either on isolated organs or after intravenous injection; so the size of this contribution to the discrepancy is difficult to assess. However, any differences in results due to contamination of AII or AII with impurities could be avoided by measuring and publishing the purity of the peptides (24) and by comparing their activity to that of international reference standards which are now available (38).

Our results on the kidney are also somewhat at variance with those of Oparil et al. (29). They showed that following the intra-arterial injection of AII, renal venous blood contained 7–9% of the injected dose in a form which reacted with antibodies for AII. However, it is possible that this does not represent 7–9% conversion, for the degree of reactivity of the AII antibodies with other substances in venous blood was not known, and this can sometimes be as high as 70% (39).

A further possibility is that converting enzyme activity varies substantially from laboratory to laboratory and from time to time with factors as yet uncontrolled. Many external factors can influence enzyme activity, including such chemicals as laboratory insecticides (40). A low salt diet reduces converting enzyme activity (5). Some uncontrolled factor may also be responsible for the discrepancies between the relatively high conversion in hindlegs found here and the lack of conversion in hindlegs found by Ng and Vane (1).

We have previously suggested that converting enzyme is located in vascular tissue (8), and fractionation studies on lung tissues (10,
41) are consistent with this view. A reninlike enzyme has been extracted from vascular tissue (42, 43). The contiguity of converting enzyme and renin in vascular tissue might allow intramural generation of All from renin substrate, thereby selectively contributing to the local control of vascular tone in those vessels sensitive to All.

The rise in hindleg blood flow, which was often associated with infusions of converting enzyme inhibitor, might also indicate a local role for the renin-angiotensin system, inhibition of local formation of All leading to vasodilatation. However, the inhibitor of converting enzyme activity also potentiates the actions of bradykinin, mainly by preventing its destruction (27). Thus the gradual vasodilatation might also have been due to potentiation of circulating or locally formed bradykinin.

Despite the presence of converting enzyme activity in some other vascular beds, that in the lung must play a predominant role in the conversion of blood-borne AI. The lack of inactivation of All in the lungs and high inactivation elsewhere, also suggests that lung conversion, leading to arterial concentrations of All, has a physiological function. Most estimates of lung conversion vary from 50-100%. Recently, Barrett and Sambhi (30) have shown that lung conversion depends on the concentration of AI, the lower the concentration the more the conversion. At the lowest dose which they used, conversion was 80% and the curve was quickly approaching 100%. Thus, when there is renin in the circulation, the pulmonary vein delivers predominantly All to the left ventricle. Whether converting enzyme in vascular beds such as the hindlegs or intestine has an important role in contributing to the total amount of All available for local receptors will depend on the rate at which renin generates AI in the arterial circulation between the left ventricle and the vascular bed concerned.

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References

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