Evidence for the Renal Conversion of Angiotensin I in the Dog

By W. G. Franklin, M. J. Peach, and J. P. Gilmore

ABSTRACT

Experiments were carried out on perfused canine kidneys in situ to determine if renal conversion of angiotensin I occurs. Various concentrations of decapeptide produced an immediate increase in renal vascular resistance when injected directly into the renal artery. Since the decapeptide is biologically inactive, the increased resistance was interpreted as indicating generation of angiotensin II. The extent of renal conversion of angiotensin I to angiotensin II was estimated to be approximately 19% in the normal dog. This percent conversion was reduced to about 10% in dogs maintained on high salt and DOCA for 7 to 10 days.

ADDITIONAL KEY WORDS converting enzyme angiotensin II

Although it is well documented that the kidney contains renin, there is still some question whether this organ also contains the enzyme which can convert angiotensin I, a decapeptide, to angiotensin II, an octapeptide. Ng and Vane (1) reported that intrarenal injection of angiotensin I in the dog was not associated with changes in renal resistance; however, after a single passage through the lung, renal resistance increased. From these experiments, Ng and Vane concluded that the kidney did not contain converting enzyme and that decapeptide conversion occurred primarily in passage through the pulmonary vasculature. Gocke and associates (2) reported that the concentration of angiotensin II is greater in the renal venous blood of patients with renal arterial stenosis than in arterial blood, suggesting that the kidney may indeed convert the decapeptide to the octapeptide. Oparil et al. (3) observed 20% conversion of angiotensin I across the kidney using 3H-leucine-10-angiotensin I. The findings of these investigators suggested to us a study to compare the influence of the decapeptide on renal resistance at normal blood flows with its effect on renal blood flow following a prolonged cardiovascular intervention, i.e., chronic deoxycorticosterone acetate (DOCA)-salt treatment. We thought that the hemodynamic state of the animal might influence the effect of angiotensin I on renal resistance. This would indicate physiologic or pathophysiologic modulation of converting enzyme activity and would resolve the apparent discrepancy between the findings of Ng and Vane and Gocke and associates.

Methods

Mongrel dogs of either sex anesthetized with pentobarbital sodium (30 mg/kg iv) were used in all the experiments. The trachea was intubated and the required blood vessels exposed. The left kidney was perfused using the technique described by Gilmore (4). Essentially, the lower abdominal aorta was approached retroperitoneally through a left flank incision and the lumbar arteries ligated. After administration of heparin (5 mg/kg iv), a modified Gregg coronary cannula perfused through a rotameter from a common carotid artery was inserted upstream into the aorta approximately 5 cm below the left renal artery. This cannula was then moved cranially and wedged into the left renal artery without interrupting blood flow. During this procedure the kidney was not disturbed; the position of the cannula was determined by palpation. The total length of the renal perfusion shunt from the carotid artery to the renal artery was approximately 110 cm. The internal volume of the perfusion shunt was 35 ml. In most instances, peptide injections were made into the perfusion...
system approximately 20 cm from the kidney.

Angiotensin I (1-asp-5-ileu-angiotensin I), 0.75 to 5.0 µg, or 1-asp-5-ileu-angiotensin II, 0.2 to 2.0 µg, or both were administered. In some experiments, the injection site was varied so that injections were made as far as 100 cm from the kidney. Renal arterial pressure was measured between the rotameter and carotid artery. Systemic blood pressure was monitored through a small peripheral artery.

Eight normal dogs and four dogs maintained on a high salt and DOCA regimen for 7 to 10 days were studied. The regimen consisted of 6 g of salt twice a day and 25 mg of DOCA once a day. The percent conversion of angiotensin I to angiotensin II was calculated as the percent change in renal blood flow per dose of angiotensin I divided by the percent change in blood flow per dose of angiotensin II multiplied by 1.25. The latter factor takes into account the fact that if a microgram of angiotensin I is completely converted to angiotensin II, it will yield only about 0.8 µg of octapeptide. In about half the animals, dose-response comparisons were obtained between the peptides, and percent conversion of angiotensin I was based on the dose-response curves.

The peptides (1-asp-5-ileu-angiotensin I and II) were supplied by Dr. F. M. Bumpus of the Research Division of the Cleveland Clinic and assayed by us periodically with the estrogen-treated rat uterine assay.

Results

The mean initial renal blood flow in control dogs was 2.53 ± 0.30 ml/min/g, in DOCA-salt animals, 2.56 ± 0.18. Mean systemic arterial pressure in animals under pentobarbital anesthesia was 128 ± 7.53 mm Hg for controls and 162.85 ± 9.73 for those on the DOCA-salt regimen (P < 0.01). Pressures in the carotid-renal shunt were 114.27 ± 10.9 mm Hg in controls and 147.72 ± 8.11 for DOCA-salt animals (P < 0.01).

All peptide concentrations were made up to a final volume of 1 to 2 ml with saline. Administration of 1 to 2 ml of physiologic saline produced a small injection artifact on renal blood flow which lasted about 15 seconds.

Eighty observations were made in normal dogs comparing the responses of renal blood flow to angiotensin I and II. An example of one such experiment is shown in Figure 1. It can be seen in the figure that doses of angiotensin I (0.75 or 1.50 µg) reduced renal blood flow by about 15 and 40 ml/min, respectively. With a control flow of about 110 ml/min, these represent flow decreases of 14 and 37%.

Angiotensin II in a dose of 0.50 µg...
TABLE 1
Percent Conversion of Angiotensin I to Angiotensin II

<table>
<thead>
<tr>
<th></th>
<th>Normal dogs</th>
<th>Salt and DOCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of dogs</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>No. of observations</td>
<td>80</td>
<td>32</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>18.97 ± 2.13</td>
<td>10 ± 1.32*</td>
</tr>
</tbody>
</table>

*Significantly different from control dogs.

reduced renal flow by 68 ml/min (a decrease of 63%). Net conversion of angiotensin I was calculated in the following way:

\[
\frac{0.14}{0.75} \times \frac{0.63}{0.50} = 0.16
\]

When this calculation was made using the percent change in renal blood flow with the high dose of angiotensin I compared to the dose of angiotensin II the conversion was 24.5%.

The mean calculated net conversion of angiotensin I to angiotensin II in normal dogs was 19% (Table 1). The conversion was independent of the decapeptide concentration. In dogs maintained on high salt and DOCA for 7 to 10 days, the mean conversion for 32 observations was 10%. This percent conversion of angiotensin I was significantly less than found in normal dogs.

The distance from the kidney of the site of injection of the peptides did not significantly alter the magnitude of the renal responses. For a given dose of angiotensin I the same quantitative change in renal blood flow was obtained whether the injection was made 20 cm or 100 cm from the kidney. Injecting the decapeptide 100 cm from the kidney lengthened the time from injection to peak renal blood flow response by 20 to 30 seconds, depending on the existing renal blood flow.

Discussion

These experiments clearly show that injections of angiotensin I directly into the renal arterial blood are associated with an increase in renal vascular resistance. This effect of the decapeptide could indicate that the decapeptide itself is vasoactive or that it is converted to the octapeptide, which is known to be vasoactive. With respect to the former possibility, in vitro experiments using isolated smooth muscle (5, 6) and renal flow in the saline-perfused kidney (7) have demonstrated the inactivity of angiotensin I. Conversion of the decapeptide to the active octapeptide could occur either between the site of injection and the kidney, within the kidney itself through plasma converting enzyme, or via a renal converting enzyme. With respect to this first possibility Ng and Vane (8) determined that "in one circulation time (15 to 20 seconds) there is little or no conversion of angiotensin I to angiotensin II in the blood." Oparil and associates (3) found that with incubation at 37° in heparinized plasma or whole blood the half time for conversion of angiotensin I to II was 15 and 3 minutes, respectively. These findings give direct support to the position that any conversion of the decapeptide observed in the present experiments was extrarenal, that is, did not occur between the site of injection and the kidney. Further support for this position are the experiments in which we found that if the distance of the decapeptide injection site from the kidney was increased up to fivefold (this increased the extrarenal contact between angiotensin I and plasma converting enzyme from about 5 seconds to 25 seconds), there was no significant increase in the calculated conversion of the decapeptide to the octapeptide.

The onset and duration of responses obtained with angiotensin I in the present study and the t1/2 of the decapeptide in whole blood (Oparil et al., 3) argue against significant intrarenal conversion due to plasma converting enzyme. The data indicate the existence of a renal angiotensin I converting enzyme.

In general, the results of these experiments are consistent with the suggestion of Cocke et al. (2) and report by Oparil et al. (3) but not consistent with the general findings of Ng and Vane (1) and Skeggs et al. (7). Of interest was the observation that with bioassay we found a 10% conversion of angiotensin I to angiotensin II, while Oparil et al. (3) found a 20% conversion using labeled angiotensin I. In
the experiments of Ng and Vane, angiotensin II or I were injected into the aorta immediately above the renal arteries. They reported that the injection of 1.33 μg of the decapeptide had essentially no effect on renal blood flow, whereas the intravenous injection of 2.0 μg of angiotensin I produced a substantial reduction in renal blood flow presumably due to recirculation of octapeptide that was formed in the lungs. Skeggs and associates (7) also found no influence of the decapeptide on renal resistance when it was injected into the renal artery. However, in these experiments the isolated rat kidney perfused with physiologic salt solution was employed. Under these conditions it is possible that there was depletion of the converting enzyme. In the present investigation, chronic DOCA and salt treatment appeared to modulate converting enzyme activity. This observation suggests that changes in renal converting enzyme might well influence the major in-vivo sites of the generation of angiotensin II and thus alter the in-vivo distribution of greatly elevated angiotensin II levels.

References
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