ANGIOTENSIN II IN RENAL HYPERTENSION/Caravaggi et al.


Blood Pressure and Plasma Angiotensin II Concentration after Renal Artery Constriction and Angiotensin Infusion in the Dog

[5-Isoleucine] angiotensin II and Its Breakdown Fragments in Dog Blood

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SUMMARY We measured arterial plasma angiotensin II concentration, renal blood flow, and arterial blood pressure in six conscious dogs during intravenous infusion of angiotensin II (5, 10, and 20 ng/kg per min). The same measurements were made on a different occasion in the same six animals, while they were conscious, before and during constriction of a main renal artery. Arterial blood pressure and plasma angiotensin II rose and renal blood flow decreased in both experiments. The similarity of regressions for plasma angiotensin II concentration and arterial blood pressure in the two experiments strongly suggests that the rise of circulating angiotensin II after renal artery constriction is sufficient to account for the hypertension by its direct pressor action. As discussed, a different mechanism seems likely to be involved in the later stages of renal hypertension. Angiotensin II is more likely to be in the 5-isoleucine form than in the 5-valine form in the dog. In contrast to the rat, plasma concentrations of the heptapeptide (angiotensin III), hexapeptide, and pentapeptide fragments of angiotensin II are low in the dog.

THE ROLE of renin in the pathogenesis of hypertension remains uncertain: after renal artery constriction in animals arterial pressure and plasma levels of renin and renin activity increase but thereafter hypertension persists while the renin level falls.1-4 Recent experiments with inhibitors of the renin-angiotensin system suggest its involvement in the early stages of renal hypertension.6-11 The purpose of the experiments described here was to determine whether the plasma concentrations of angiotensin II (the vasoactive component of the renin-angiotensin system) also rises in these circumstances and whether the levels reached are sufficient to account for the increase in arterial pressure in the early stages of renal hypertension. Scornik and Paladini12 used bioassay to show angiotensin blood levels to be high in early stages of renal hypertension, but normal in the later stages.

We measured arterial pressure and plasma concentrations of renin and angiotensin II in conscious dogs before and shortly after constriction of the main artery to one kidney; the results were compared with similar measurements in the same animals before and during pressor infusions of angiotensin II. The experiment was then repeated after removal of the contralateral kidney because of evidence (see Discussion) that hypertension of this type is less dependent on renin and angiotensin. To validate the angiotensin assay, we studied the immunoreactive breakdown fragments of angiotensin II in dog plasma and the reactivity of canine angiotensin II with the antiserum used.

Methods

TERMINOLOGY

Dogs with one renal artery constricted and the opposite kidney removed are described as one-kidney hypertensive dogs; dogs with unilateral renal artery constriction but with the opposite or "untouched" kidney in situ as two-kidney hypertensive dogs.

TECHNIQUES FOR EXPERIMENTS ON DOGS

Six male mongrel dogs were housed prior to and during the experiment in an air-conditioned room. After a period of training, they were anesthetized with pentobarbbitone (pento-
barbital) sodium (25-30 mg/kg, iv) and one kidney was exposed through a loin incision. An electromagnetic flow probe (IVM) was placed on the main renal artery to measure renal blood flow using a Statham sinusoidal flowmeter, model K-2000. A tourniquet (IVM) was placed on the artery 5 mm distal to the flow probe. In four dogs the left renal artery was used; in two, because of double left renal arteries, the opposite side was used. Flow probe cables and connecting tubing for the inflatable cuff were brought to the surface through the skin at the back of the neck.

After recovery from the operation the dogs received a standard diet calculated to maintain constant body weight. Sodium intake varied from 2 to 3 mEq/kg per 24 hours. Water was given ad lib. General health was checked each day by measuring food intake, body weight, and rectal temperature. In terms of these criteria no dog deteriorated during the study. From the 12th to the 15th postoperative day the dogs were trained to stand quietly with minimal restraint. Each dog was subjected to five experiments: both infusion of angiotensin and, on another occasion, constriction of the renal artery with contralateral kidney in situ; infusion of angiotensin and constriction of the renal artery after the contralateral kidney had been excised; and a control study with only infusion of saline and repeated blood sampling.

One hour before each experiment a polyethylene catheter (inner diameter, 0.86 mm) was passed, with the dogs under local anesthesia, through a surface branch of the femoral artery to measure arterial pressure (Statham transducer, model P23DB and Sanborn recorder, model 150). Infusions were given through a second polyethylene catheter in a saphenous vein. As indicated below, the sequence of experiments was varied for the different animals.

Experiment 1: Renal Artery Constriction in Two-Kidney Dogs. At 16 days after operation in dogs 3-6 and 10 days after operation in dogs 1 and 2 the renal artery was constricted by inflating the cuff, first for 1 hour at a pressure which reduced renal blood flow by approximately 20%; immediately after this for 30 minutes at a pressure which reduced flow by 60%; and finally, again for 30 minutes, at a pressure which reduced flow by 80%. Blood samples (20 ml) were taken from the arterial catheter for measurement of renin and angiotensin II at the times shown in Figure 1. Renal blood flow and arterial pressure were measured at intervals throughout the experiment (see Fig. 1).

Experiment 2: Angiotensin II Infusion in Two-Kidney Dogs. On the 16th postoperative day in dogs 1 and 2 and on day 23 in dogs 3-6 angiotensin II (Hypertensin, CIBA) was infused intravenously by a constant infusion pump (B. Braun, model 71032) at rates of 5, 10, and 20 ng/kg per min while the animals were standing quietly. The angiotensin was made up in 0.9% NaCl solution and the rate of infusion varied between 0.1 and 0.4 ml/min. Each period of infusion lasted for 30 minutes. Arterial blood samples (20 ml) for plasma angiotensin II determination (see below) were taken 10 minutes and 1 minute before the infusion began, at the 30th minute of the 10 ng/kg per min infusion, and at the 20th and 30th minutes of the 5 and 20 ng/kg per min infusions. Figure 2 illustrates the protocol.

Between days 27 and 28 the untouched kidney was removed under general anesthesia through a loin incision; 10 days later, after recovery, the third and fourth experiments were conducted.

Experiment 3: Renal Artery Constriction in One-Kidney Animals. On day 37 (in relation to the first operation) in dogs 3 and 6 and on day 44 in dogs 1 and 2 experiment 1 was repeated.

Experiment 4: Angiotensin II Infusion in One-Kidney Dogs. On day 37 in dogs 1 and 2 and on day 44 in dogs 3 and 6 experiment 2 was repeated.

Experiment 5: Control Study with Only Infusion of Saline and Repeated Blood Sampling. On day 52 measurements were made as in experiment 1 except that the renal artery was not constricted.

TECHNIQUES FOR DETERMINATION OF ANGIOTENSIN AND RENIN

Plasma angiotensin II concentration was measured in the Glasgow laboratory by the technique of Düsterdieck and McElwee. The experiments on dogs were performed in Milan, where blood samples for angiotensin II measurement were taken immediately into inhibitor solution containing ethylenediaminetetraacetic acid (EDTA) and o-phenanthroline to prevent further formation of angiotensin II by converting enzyme and destruction by angiotensinase. Samples were centrifuged at 5°C and plasma was stored frozen and transported by air to Glasgow in frozen form.
Results

Plasma concentrations of the octapeptide angiotensin II, the 2-8 heptapeptide and the 3-8 hexapeptide, together with the 4-8 pentapeptide fragments, were measured by the technique of Semple et al.\textsuperscript{15} Angiotensin II in dog plasma was further tested, as has been described,\textsuperscript{16,17} for its cross-reaction with two anti-angiotensin II sera; one (antiserum A) exhibited a 100% cross-reaction with both forms of angiotensin II (see Methods). Values obtained were therefore independent of whether the extracted peptide was in the 5-valine or the 5-isoleucine form. The remainder of the extract was assayed with antiserum B, which exhibited only a 10% cross-reaction with [5-isoleucine]angiotensin II.

Values for the percentage bound were plotted against the concentration of angiotensin II in each dilution as assessed with antiserum A. As shown in Figure 3, the curve obtained with the plasma extract was similar to that obtained with standard [5-isoleucine]angiotensin II but markedly different from the curve for the [5-valine]angiotensin II standard. The results of the experiment strongly suggest that in the dog angiotensin II does not have valine as its fifth amino acid. It also suggests but does not prove that isoleucine is the fifth amino acid. Because of these results antiserum A was used throughout the study for radioimmunoassay.

ANGIOTENSIN II AND ITS BREAKDOWN FRAGMENTS IN DOG PLASMA

The angiotensin assay technique used for human\textsuperscript{14} and rat\textsuperscript{18} plasma was capable of extracting and measuring separately octapeptide angiotensin II and its heptapeptide and hexa- plus pentapeptide fragments in the dog (Fig. 4). Six extracts of dog plasma were tested; regardless of whether endogenous angiotensin II was high or normal, or whether circulating levels had been raised by infusion of angiotensin II, the heptapeptide and the hexa- plus pentapeptide fragments together comprised less than 20% of the octapeptide fraction (Table 1). Routine chromatographic separation of the fragments prior to assay for angiotensin II therefore was not considered necessary.

Recovery of added angiotensin II (2 pmol/sample, $n=6$) varied from 79% to 87% (mean = 84%). Values for plasma angiotensin II as expressed are compensated for this loss. The coefficient of variation was 8% for 12 replicate mea-
IMMUNOREACTIVE MATERIAL (pmol) 

FIGURE 4 Eluate fractions (f) from paper chromatogram of dog plasma radioimmunoassayed for angiotensin II and its breakdown fragments. The position of standard markers of angiotensin II, heptapeptide, hexapeptide, and pentapeptide in adjacent lanes of the chromatogram is shown.

Measurements of angiotensin II made on different batches on 12 samples from the same plasma pool.

EXPERIMENT 1: RENAL ARTERY CONSTRICTION IN TWO-KIDNEY DOGS

Inflation of the renal artery cuff led within 10 minutes to a rise of arterial pressure in each dog (Fig. 1). Further inflation of the cuff at 60 and 90 minutes produced only a small additional increase. According to the protocol of the experiment, renal blood flow decreased sharply with each successive constriction; heart rate remained unchanged. Plasma concentrations of renin and angiotensin II rose 2- to 3-fold after the first constriction but did not change markedly thereafter (Fig. 1).

EXPERIMENT 2: ANGIOTENSIN INFUSION IN TWO-KIDNEY DOGS

Arterial pressure rose stepwise with increasing rates of angiotensin infusion (Fig. 2); renal blood flow fell and heart rate remained unchanged. Plasma angiotensin II concentrations increased; the magnitude of the increase was closely related to the rate of infusion (r = 0.83, P < 0.001).

EXPERIMENT 3: RENAL ARTERY CONSTRICTION IN ONE-KIDNEY DOGS

The effects of renal artery constriction on arterial pressure; renal blood flow, and heart rate were little affected by prior removal of the contralateral kidney, although levels of arterial pressure were slightly higher before and after constriction (Fig. 1). Probably because of removal of the untouched kidney, renal blood flow to the remaining kidney was higher initially but the proportionate reduction caused by constriction was similar—80% reduction in two-kidney dogs and an 82% reduction in one-kidney dogs.

EXPERIMENT 4: ANGIOTENSIN INFUSION IN ONE-KIDNEY DOGS

The effects of angiotensin infusion on arterial pressure, plasma angiotensin II concentration, renal blood flow, and heart rate also were little affected by excision of the untouched kidney (Fig. 2). As in experiment 3, blood pressure was slightly higher throughout.

EXPERIMENT 6: CONTROL STUDY

Plasma angiotensin II concentration did not change markedly while dogs stood quietly during infusion of saline but without constriction of the renal artery; values at the beginning of the experiment, at 1 hour, and at 2 hours were 42 ± 29 (SEM) pmol/liter, 40 ± 25, and 43 ± 24, respectively.

RELATION OF RENIN, ANGIOTENSIN II, AND BLOOD PRESSURE

Renin and angiotensin II concentrations correlated in the experiments in which both were measured for the same plasma sample (Fig. 5) and there was no difference in the relation between the two for one-kidney and two-kidney dogs (Fig. 5). Plasma renin concentration also was related to concurrent arterial pressure (Fig. 6); the regression for data from the infusion experiments lies slightly, but insignificantly, below that for the constriction experiments. There was a

TABLE 1 Angiotensin II and Its Three Immunoreactive Fragments in Dog Arterial Blood

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Angiotensin II</th>
<th>2-8 heptapeptide</th>
<th>3-8 hexapeptide and 4-8 pentapeptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal 7</td>
<td>9 (82%)</td>
<td>2 (18%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Normal 8</td>
<td>13 (87%)</td>
<td>2 (13%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Normal 9</td>
<td>12 (86%)</td>
<td>2 (14%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Normal 10</td>
<td>17 (78%)</td>
<td>4 (18%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Infused 11</td>
<td>420 (96%)</td>
<td>17 (4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Infused 12</td>
<td>480 (95%)</td>
<td>24 (5%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Results are expressed as picomoles of peptide per liter of plasma, uncorrected for recovery (values in Figure 2 are compensated for recovery). Dogs 11 and 12 were infused with [5-valine]angiotensin II for 60 minutes at a rate of 24 pmol/kg per min.

FIGURE 5 Relation between the plasma concentrations of renin and angiotensin II in plasma samples taken before and during renal artery constriction. Data were obtained from dogs with (•—•) and without (○—○) the contralateral kidney in situ.
small but insignificant difference between the regressions for one-kidney and two-kidney dogs (Fig. 6). Plasma angiotensin II concentration also correlated significantly with arterial pressure in each experiment (Fig. 7), and again the regression of data for the two-kidney dogs lay slightly but insignificantly below that for one-kidney dogs (Fig. 7).

Comparison of experiments which employed renal artery constriction and angiotensin infusion showed no significant difference in the regressions of arterial pressure on plasma angiotensin II concentration (Figs. 1 and 7), and there was considerable overlap of data from the two experiments. This suggests that arterial pressure rises in the two circumstances to approximately the same extent for a given increase of plasma angiotensin II concentration. This, in turn, suggests that the acute rise of arterial pressure after renal artery constriction can be partly or wholly accounted for by the rise in angiotensin II.

**Discussion**

Most antisera used for the radioimmunoassay of angiotensin II cross-react with its hepta-, hexa-, and pentapeptide breakdown fragments. Were these fragments to be extracted from plasma, radioimmunoassay would give falsely high results for angiotensin II. The heptapeptide (angiotensin III) is present in relatively large amount in rat plasma, and although it was originally thought that the hepta- and hexapeptide fragments predominated in human venous plasma, the finding has not been confirmed. For these reasons it was necessary to separate and measure angiotensin II and its fragments in dog plasma.

The relatively low concentration of angiotensin III is interesting because of the possibility that it may be more important than angiotensin II as a stimulus for release of aldosterone in the rat. It seems unlikely that this is the case in the dog since on a molar basis the two peptides are equipotent.

It has been noted previously that after renal artery constriction blood pressure rises more for a given increase in plasma renin concentration in one-kidney than in two-kidney dogs. The present experiments failed to show a significant difference either for renin (Fig. 6) or for angiotensin II concentration (Fig. 7). However, in contrast to the earlier work, we made a comparison of one- and two-kidney states in the same dogs and the interval between nephrectomy and renal artery constriction was shorter. Therefore, dogs with one-kidney and two-kidney hypertension do not differ markedly in their early stages.

Although it has been known for some time that renal artery constriction provokes release of renin, the mechanism involved is not clear. In the experiments described here the largest increase in renin and angiotensin levels occurred during the first period of renal artery constriction when blood flow was reduced by only 20%. A greater reduction in blood flow had little additional effect on levels of renin or angiotensin II or on blood pressure. There is other evidence to suggest that a reduction in blood flow, itself, probably is not the stimulus for renin secretion although the decrease in renal perfusion pressure might be. As in earlier studies on the dog, heart rate remained unchanged after renal artery constriction.

The main purpose of our experiments was to determine whether plasma angiotensin II concentration increased with renin to a level sufficient to account for the acute rise of blood pressure. Renin and angiotensin II increased together after renal artery constriction and, as in patients with renal hypertension, plasma levels of the two correlated well. Similar regressions were obtained for arterial pressure and plasma angiotensin II concentration in experiments which employed constriction and infusion (Figs. 1 and 7). This finding strongly suggests that the rise in arterial pressure after constriction can be partly or wholly accounted for by the increase in plasma angiotensin II concentration. Studies on conscious dogs infused with renin and converting enzyme inhibitor also suggest that this is so.

For reasons discussed by Bianchi et al. the role of
angiotensin II is more difficult to assess in animals which are anesthetized and subjected to surgery at the time of renal artery constriction. Nevertheless, most experiments suggest that the release of renin is increased under these conditions and that blood pressure rises as a result. *1-11, 26-28* On balance, therefore, it seems very likely that acute hypertension after renal artery constriction can be attributed to the vasoconstrictor effect of an increased plasma angiotensin II concentration.

Clearly a different mechanism is involved in the later stages when hypertension can persist or worsen while renin, renin activity, and angiotensin concentration in plasma are normal or only slightly increased. *11, 16, 23-25* It has been suggested that enhanced responsiveness to the pressor effect of angiotensin II, coupled with normal or only slightly increased plasma levels of the peptide, raise blood pressure at this time. *26* There now is support for the idea. *27, 29*

The mechanism for the enhanced response is not understood: abnormal retention of sodium and water in the arterial wall might be involved. *29* Arterial tissue sodium content is increased in experimental renal hypertension*29, 30* and sodium retention, with expansion of extracellular fluid volume and exchangeable sodium, sometimes occurs in animals with renal hypertension, particularly in the early stages. *3, 4, 41-42* The relation of exchangeable sodium and plasma volume to the renin-angiotensin system is often abnormal in patients with renal hypertension.*34-46* These points are discussed in more detail elsewhere. *38-47*

In conclusion we find that renin release, with a resulting increase in the level of circulating angiotensin II, probably is responsible by direct vasoconstrictor effect for the acute hypertension which follows renal artery constriction. Thereafter, hypertension persists and renin level falls. An abnormal relation between sodium and the renin-angiotensin system may be important in raising blood pressure during this chronic phase of hypertension.

References


The Role of Arterial Baroreceptors in Mediating the Cardiovascular Response to a Cardiac Glycoside in Conscious Dogs

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SUMMARY To determine the role of the arterial baroreceptor reflex in mediating the cardiovascular response to a cardiac glycoside, we examined the effects of ouabain (G-strophanthin), 17.5 μg/kg, iv, on direct and continuous measurements of left ventricular diameters, pressures, velocity of shortening, (dP/dt)/P, arterial pressure, cardiac output, and total peripheral resistance. These studies were conducted on healthy conscious dogs before and after total arterial baroreceptor denervation (TABD). Maximal pressor effects were observed in the first 3–5 minutes; mean arterial pressure increased by 11 ± 1 mm Hg in normal dogs compared to 33 ± 4 mm Hg in denervated dogs. In intact dogs at this time heart rate decreased by 18 ± 2 beats/min and cardiac output fell by 18 ± 3%; these values gradually returned toward control over 15–30 minutes. When heart rate was kept constant, cardiac output did not fall after injection of ouabain. In contrast, heart rate and cardiac output did not change significantly after ouabain in dogs with TABD. The maximal effects on the contractile state of the heart occurred between 15–30 minutes and were similar in both groups. Arterial baroreceptor reflexes appear to be responsible for the reduction in heart rate and cardiac output caused by administration of ouabain to the intact dog. They exert an important buffering action on the vasoressor effect but a less important action on the inotropic response.

CARDIAC glycosides increase cardiac output through a strong inotropic action on the failing heart. In contrast, cardiac glycosides, when administered to man or animals without heart failure, either reduce or do not change cardiac output. One of the most prevalent hypotheses offered to explain why digitalis exerts little effect on output of the nonfailing heart is that the arterial baroreceptors, stimulated either directly by the cardiac glycoside or by the rise in arterial pressure that occurs, attenuate the normally powerful inotropic action of the drug and thereby prevent cardiac output from rising. A corollary of this hypothesis, i.e., that the cardiac glycoside would cause a striking inotropic response sufficient to elevate stroke volume and cardiac output in the absence of arterial baroreceptors, was the subject of this investigation.

In order to accomplish this goal the effects of a subtoxic dose of ouabain were studied before and after recovery from denervation of arterial baroreceptors in conscious dogs which had been instrumented for direct measurements of stroke volume, cardiac output, left ventricular dimensions and pressures, (dP/dt)/P, and velocity of myocardial fiber shortening. It was considered important to conduct this study in conscious animals because general anesthesia, or se, depresses cardiac function, and cardiac glycosides exert a more potent action on the depressed myocardium.

Methods

Ten mongrel dogs were anesthetized with pentobarbital sodium (30 mg/kg, iv). Through a thoracotomy in the 4th left intercostal space an electromagnetic flow transducer (Zepeda Instruments, Seattle) was implanted around the ascending aorta and pacemaker electrodes were sutured to the left atrium. A catheter was implanted in the ascending aorta via the femoral artery. In another group of seven dogs under pentobarbital anesthesia and through a thoracotomy in the 5th left intercostal space, miniature pressure gauges (model P22, Konigsberg Instruments, Pasadena, Calif.) were implanted within the left ventricle through a stab wound in the apex. A Tygon catheter was implanted through

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