The Relation of Arterial Pressure and Plasma Angiotensin II Concentration

A Change Produced by Prolonged Infusion of Angiotensin II in the Conscious Dog

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SUMMARY Five unrestrained male beagle dogs were given a continuous intravenous infusion for 28 days. First, 0.9% NaCl solution was infused for 7 days, then angiotensin II at 3 ng/kg per min for 14 days, and finally 0.9% NaCl for 7 days. We found that the blood pressure rose gradually in each dog, reaching a peak toward the end of the 14-day infusion of angiotensin II. When angiotensin infusion was stopped, blood pressure fell gradually during 24 hours; the lowest pressure was not reached until 5 days later. To assess the relation between plasma angiotensin II concentration and arterial pressure, dose-response studies were done during the first saline infusion, after 7 and 14 days of angiotensin II infusion, and at the end of the second saline infusion. In these experiments, additional angiotensin II was infused intravenously at 3, 6, and 12 ng/kg per min, each rate for 1 hour. The increase of arterial pressure was then related to concurrent plasma angiotensin II concentration. In all dogs, prolonged infusion of angiotensin shifted the position of this curve upward. Thus, prolonged infusion of angiotensin raised the level of pressure maintained by a given plasma concentration of angiotensin II. Seven days after the angiotensin infusion, the curve had returned to the original position. Plasma aldosterone concentration also increased during all dose-response studies. The slope of the regression curve relating plasma concentrations of angiotensin II and aldosterone was steeper after, but not during, prolonged infusion of angiotensin II. Plasma potassium concentration did not change at any stage.

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THE INITIAL rise of blood pressure in renal hypertension is probably caused by vasoconstriction due to an increased plasma concentration of angiotensin II. Plasma levels of renin (Bianchi et al., 1972), the precursor enzyme, and angiotensin II (Caravaggi et al., 1976) are raised sufficiently. Inhibitors of the vasoconstrictor effect and of the enzyme producing angiotensin II restore normal blood pressure (Pals et al., 1971; Coleman and Guyton, 1975; Miller et al., 1975; Freeman et al., 1977; Masaki et al., 1977; Thurston and Swales, 1976). Chronic renal hypertension is less easy to explain. Blood pressure may be higher but plasma levels of renin and angiotensin II are not high enough to raise blood pressure by acute vasoconstriction alone (Bianchi et al., 1972; Hutchinson et al., 1975; Brown et al., 1976, 1977); inhibitors are less effective at this stage (Pals et al., 1971; Freeman et al., 1977; Masaki et al., 1977; Thurston and Swales, 1974; MacDonald et al., 1975). An additional mechanism therefore seems very likely. It is not agreed whether this mechanism is a slower-developing pressor action of angiotensin II (Brown et al., 1976, 1977) or something independent of angiotensin II (Thurston and Swales, 1974; MacDonald et al., 1975). Angiotensin II does have a slow pressor effect distinct from its acute vasoconstrictor action (Dickinson and Lawrence, 1963; McCubbin et al., 1965; Cowley and Declue, 1976; Cowley and McCaa, 1976). Two experiments suggest that the slow effect may be important in chronic renal hypertension: prolonged infusion of converting enzyme inhibitor can prevent the condition from developing (Miller et al., 1975), and, when hypertension has become established, prolonged infusion of converting enzyme inhibitor or saralasin can reduce blood pressure gradually (Riegger et al., 1977).

If a slow action of angiotensin is responsible for chronic renal hypertension, prolonged infusion of the peptide should gradually produce a hypertensive state in which arterial pressure is higher than can be explained by the acute vasoconstrictor action of angiotensin II. This was tested in the experiment to be described. Conscious dogs were infused with angiotensin II for 2 weeks, during which blood
pressure rose gradually. Before, during, and after the rise, the acute pressor effect of angiotensin II was tested by dose-response studies in which additional angiotensin II was infused briefly and the level of blood pressure was related to concurrent plasma angiotensin II concentration.

Methods

Male beagle dogs (11.4–17.0 kg) were used. One carotid artery had previously been exteriorized in a skin loop (Davey and Reinert, 1965). The dogs were anesthetized with thiopental sodium (25 mg/kg) followed by nitrous oxide, oxygen, and halothane; the jugular vein was catheterized with 0.88-mm Tygon tubing which passed subcutaneously to an opening over the thoracic spine. After the operation, the catheters were filled with heparinized 0.9% saline (200 U/ml) and plugged when not in use. Dogs were then trained to wear a special jacket covering the thorax and external part of the catheter, and to stand quietly on a table for dose-response studies and for measurement of blood pressure (see below). Throughout the experiment they lived singly in cages and were exercised twice daily. They consumed a fixed diet containing 47 mEq of sodium and 38 mEq of potassium every 24 hours.

Measurements during Prolonged Infusion

Constant rate infusions were given at 2.2 ml/24 hours into the jugular catheter with a portable pump (Parsons et al., 1977) contained in a pocket on the dog's jacket. For the first 7 days, 0.9% NaCl solution was infused; for the next 14 days, angiotensin II (Hypertensin, Ciba) was infused at 3 ng/kg per min; for the final 7 days, saline was infused as before. Figure 1 illustrates the form of the experiment and some of the measurements made. Five dogs were studied in this way; in one the experiment was repeated exactly after an interval of 4 weeks. Systolic blood pressure was measured daily or every 2nd day in a quiet room by an occlusive cuff applied to the carotid loop and connected to a special sphygmomanometer (Rose et al., 1964). Twenty measurements of pressure were made during 30 minutes on each occasion, and the mean of these was used to calculate change of pressure during prolonged infusion. Systolic pressure measured in this way correlated well with intra-arterial systolic pressure measured by transducer (Bell & Howel L221) and recorder (Devices, M2). The equation for 103 pairs of measurements made simultaneously in two anesthetized dogs was: Y (special sphygmomanometer) = 0.99X − 16.7 (r = 0.96, P < 0.001.) Body weight was measured at the time of blood pressure recording. In all other respects, the dogs' routine was unchanged.

Dose-Response Studies

These were done on the 7th, 14th, 21st, and 28th day of long-term infusion and were similar in form to an earlier experiment (Nicholls et al., 1978). Dogs stood on a table in the quiet room, and were lightly supported by a canvas cradle. The carotid arterial loop was cannulated with a 19-gauge needle connected in parallel with a mercury manometer and pressure transducer and recorder. Damped mean arterial pressure was recorded on both instruments, with frequent cross-calibration; catheters were filled with heparinized saline. A 0.25-mm i.d. polythene catheter was placed in a foreleg vein for intravenous infusion during the dose-response study, and the long-term infusion into the jugular vein was continued unchanged.

Dogs were allowed to become quiet before the study began. Then, as control, 0.9% NaCl solution was infused into the foreleg vein for 1 hour at 0.058 ml/min; after this, angiotensin II in 0.9% NaCl was infused at successive rates of 3, 6, and 12 ng/kg per min, each for 1 hour. Carotid blood samples were taken at the 45th and 60th minute of saline infusion, and at the 60th minute of each of the three rates of angiotensin infusion. Measurements of hematocrit (Hawksley), plasma angiotensin II (Caravaggi et al.,
1976), and plasma aldosterone (Fraser et al., 1973) were made on all samples. In addition, estimates of plasma concentrations of renin (see below) and electrolytes were made on the first and last samples.

Plasma renin concentration was assayed by a modification of the antibody-trapping technique (Poulson and Jorgensen, 1974). Separate 35-μl samples of dog plasma were incubated at 37°C for 2 and 5 hours each with 55 μl of a premixed solution containing 40 μl of nephrectomized ox renin substrate (at a concentration in the incubation mixture of 3360 nmol of angiotensin I/liter), 10 μl of 3 M Tris-HCl pH 6.9 buffer, and 5 μl of a 1/64 dilution of rabbit antiangiotensin I serum. This dilution gave optimal antibody trapping. Incubation was terminated by cooling tubes rapidly to 0°C and by adding 1.5 ml of 0.25 3 M Tris-HCl pH 7.4 buffer at 4°C. This procedure also altered the angiotensin-antibody equilibrium so that immunoassay could be performed. Plasma renin concentration was derived from the reaction velocity by external calibration using standard dog kidney renin prepared as previously (Brown et al., 1964). The relation between reaction velocity and renin concentration was

\[ Y(velocity) = 3.29X - 39.3. \]

The correlation coefficient for these data was \( r = 0.93 \), \( P < 0.001 \). Recovery of renin added to untreated and acidified plasma was 94% and 98%, respectively. Nineteen replicate estimates of plasma renin concentration had a coefficient of variation of 7.0%. The smallest amount of angiotensin I that can be measured is 30 pg. The Michaelis constant for the reaction of dog renin and renin substrate was 1155 ng of angiotensin I/ml. Units of renin are those defined by Brown et al. (1964).

Results

Changes during Prolonged Infusion of Angiotensin II

Infusion of angiotensin II raised the concentration of the peptide in the plasma of each dog, whereas plasma renin concentration fell (Fig. 1). Possibly because of this effect and the associated reduction of endogenous angiotensin II, the plasma concentration of total angiotensin II (the sum of endogenous and infused peptide) tended to fall during the prolonged infusion (Fig. 1). Mean arterial pressure did not rise during the 1st hour of infusion at 3 ng/kg per min, nor did systolic pressure change significantly during the subsequent 2 days of infusion at this dose. Thereafter, systolic pressure rose gradually in each dog, reaching a peak toward the end of the 14-day angiotensin infusion. Mean arterial pressure had also risen significantly by the 7th day, and this increase was sustained (Fig. 1). On reversion to saline infusion, mean and systolic pressure fell in each dog. The rate of fall of systolic pressure was greater than the rate of rise during angiotensin infusion, and although the largest fall occurred during the 1st day, blood pressure after 24 hours was still significantly higher than control (rank sum test, \( P < 0.05 \)). The lowest systolic pressure was not reached until the 6th or 7th day (Fig. 1).

Plasma aldosterone concentration increased within 1 hour, but a significant increase was not maintained for the whole period of angiotensin infusion, even though mean values were higher than control (Fig. 1). Body weight did not change significantly either during the control period or, subsequently, during angiotensin infusion, although every dog lost weight on returning to saline for the final control period (mean loss = 0.7 kg, paired t-test = 3.8, \( P < 0.02 \)). Hematocrit was slightly but insignificantly lower at the end of the experiment (35.9%) than at the outset (39.4%, paired t-test = 2.12, \( P < 0.05 \)), but there were no systematic changes during prolonged infusion or during dose-response studies. Plasma sodium and potassium concentrations measured in two dogs showed no trends or significant changes.

Dose-Response Studies

Brief infusion of angiotensin II during the dose-response studies raised the plasma concentration of angiotensin II. The extent of the increase was related to the rate of infusion but was not different in the second and third studies, in which long-term infusion of angiotensin II was being given as well (Fig. 2). This finding suggests that the clearance rate of angiotensin II from plasma was unaffected by long-term infusion of the peptide. Plasma renin concentration was also reduced by acute infusion of angiotensin II, but this reduction was apparent only in the first and fourth studies, in which basal renin values were higher. (Figure 1 shows data for the
first study; for the fourth, paired t-test = 2.75, \( P < 0.05 \).) During prolonged angiotensin infusion, basal renin values were lower (Fig. 1), and infusion of additional angiotensin did not suppress renin further.

In each dog, mean arterial pressure rose acutely during each dose-response study. Curves relating plasma angiotensin II concentration and arterial pressure before, during, and after prolonged infusion of angiotensin II are shown in Figure 3. Prolonged infusion displaced the curve upward, and after infusion the curve returned to its control position. Thus, prolonged infusion of angiotensin II had two effects: it raised arterial pressure gradually (Fig. 1), and it altered the relation of arterial pressure and angiotensin II, so that a given plasma concentration of angiotensin II maintained a higher level of pressure (Fig. 3). These changes were apparent in all experiments during the 2nd week of angiotensin infusion, and in five of six experiments during the 1st week of infusion. The changes varied in extent in individual dogs. The one dog in which the experiment was repeated showed the change on both occasions.

Plasma aldosterone concentration also was increased acutely by angiotensin infusion during each study in each dog. Figure 4 shows mean values for the four studies. In addition, the relation of plasma angiotensin II concentration and aldosterone was altered by prolonged infusion; in three of six experiments, the curve was distinctly lower in the second and third studies than in the first. Figure 5 shows the two experiments in which this result was most marked; the regression for all experiments was not significantly reduced, however (Fig. 4). On reversion to saline for the fourth study, the curve shifted significantly upward in intercept (\( P < 0.001 \)) and in slope (\( F = 4.55, P < 0.05 \)).

Discussion
Infusion of angiotensin at a rate which initially was without marked effect raised blood pressure gradually in each dog. The phenomenon has been noted previously in rabbits (Dickinson and Law-
renin-angiotensin mechanism often fails to restore raised: acute administration of inhibitors of the vasoconstrictor effect, is the chief explanation for slowly developing pressor effect of angiotensin II, sin II (McDonald et al., 1975), others that it is a certain. Some hold that it is unrelated to angiotensin II, and that this, with a contribution from the direct vasoconstrictor effect, is the chief explanation for the elevation of blood pressure (Brown et al., 1976, 1977). Two objections to this latter idea have been raised: acute administration of inhibitors of the renin-angiotensin mechanism often fails to restore normal blood pressure, and plasma levels of renin and angiotensin II are too low to have produced the usually marked elevation of blood pressure. Failure of inhibitors to reverse the hypertension is not necessarily a reason for rejecting the above theory, since in most experiments the inhibitors are given by single injection or by infusion for 2 hours at most. As shown here, blood pressure takes more than 24 hours to return to normal when infusions producing the slow rise of pressure are stopped. Infusion of inhibitor for less than 2 hours may therefore be insufficient. In support of this argument, infusion of saralasin, a competitive inhibitor of angiotensin II, or of a converting enzyme inhibitor for 11 hours, restores normal blood pressure gradually in rats with chronic two-kidney hypertension (Riegger et al., 1977). Injection of larger doses of the inhibitors, and infusion for up to 2 hours in the same rats had a less marked effect. Prevention of one-kidney hypertension in the dog by prolonged infusion of converting enzyme inhibitor (Miller et al., 1975) is also consistent with this argument. The second objection is that plasma levels of angiotensin II are too low to have produced severe hypertension. They are certainly too low for angiotensin II to have produced such hypertension solely by its acute vasoconstrictor effect, since blood pressure is higher for a given level of angiotensin II in patients with chronic renal hypertension than it is in normal subjects in whom pressure is raised acutely by infusion of angiotensin II (Brown et al., 1976, 1977). Again, this does not exclude a role for the slow-developing pressor effect of angiotensin II: our experiment shows that prolonged infusion of the peptide can increase the level of arterial pressure maintained by a given plasma concentration of angiotensin II. Thus, angiotensin II can alter its own dose-response curve as assessed by acute infusion of angiotensin II. The direction of this effect is the same as that seen in chronic renal hypertension. The finding is compatible with, but does not establish, a role for the slow pressor effect in chronic renal hypertension. However, other agents, such as aldosterone, could alter the curve in a similar way (Brown et al., 1977). Further evidence suggesting a role for the slow effect of angiotensin in chronic renal hypertension (in particular, data from patients with renin-secreting tumor) has been reviewed elsewhere (Brown et al., 1976, 1977).

Mechanism of the Slow Pressor Effect

Our experiment demonstrates an upward shift in the dose-response curve but does not explain it. Mechanisms that may be involved have been discussed (Brown et al., 1977). An action of angiotensin II on the nervous system is particularly likely (Ferrario et al., 1972). In our experiments, a persistent increase of aldosterone did not seem important (Fig. 1); work by Cowley et al. (Cowley and Declue, 1976; Cowley and McCaa, 1976) also suggests that aldosterone is not involved. Three other mechanisms contributing to the slow rise of pressure were identified in their study: a change in baroreceptor response, direct vasoconstriction by angiotensin II, and increased cardiac output. Their experiment was similar to our own, except that the higher rate of angiotensin infusion in their study probably produced greater direct vasoconstriction. Sodium balance was not measured in our experiment. Absence of weight gain suggests that there had not been marked retention of sodium and water, but this is not certain, as angiotensin II may also suppress appetite and food intake (McFarland and Rolls, 1972). However, all dogs lost weight when angiotensin was stopped. In other experiments, long-term infusion of angiotensin did produce sodium retention (Oelkers et al., 1978; Ames et al., 1965; Uruquhart et al., 1963). Also, the increased rate of rise of pressure when dietary sodium is increased (Cowley and McCaa, 1976) suggests that sodium is important. It is of interest, then, that increased dietary sodium intake also produces an upward shift in the angiotensin-arterial pressure curve in anephric subjects (Deheneffe et al., 1976).

Interpretation of Dose-Response Data

Measurement of plasma angiotensin II concentration was a necessary part of our experiment. Relating arterial pressure to the rate of angiotensin infusion would have been inadequate; the rise of blood pressure produced by exogenous angiotensin II is influenced by the plasma concentration of endogenous peptide (Chinn and Dusterdieck, 1972). If not increased, the pressor response to exogenous peptide cannot be properly assessed. Nor can conclusions be drawn on the mechanism of the displaced curve. The gradual rise of pressure
is produced partly by increased cardiac output and partly by increased vascular resistance (Cowley and Declue, 1976); separate measurements of these were not made here. Moreover, for proper interpretation of a change of resistance, measurements should be made over a wide range extending to maximal vasodilation (Folkow, 1971). This was not done either.

Response of Aldosterone

Infusion of angiotensin II produced acute stimulation of aldosterone release, a well-documented response (Freeman et al., 1977; Cowley and McCaa, 1976; Nicholls et al., 1978). It was distinct when angiotensin was infused at 3 ng/kg per min, a dose that produced little immediate effect on blood pressure. Infusion at 6 ng/kg per min increased aldosterone levels and blood pressure. The threshold for the aldosterone effect is therefore similar to, or slightly lower than, that for blood pressure. However, continued infusion of angiotensin II did not maintain the increase of aldosterone after the initial peak either in our experiment or in that of Cowley and McCaa (1976). The slope of the line relating angiotensin II and aldosterone decreased in three of six experiments, although the decrease for the group was not marked or significant. Sodium retention has this effect in other circumstances (Nicholls et al., 1978) and is a possible explanation for the decline.

When angiotensin infusion was stopped, the slope of the line relating angiotensin and aldosterone shifted upward and steepened. There are two possible explanations for this finding: sodium loss (cf. Nicholls et al., 1978) or persistence of a trophic effect of angiotensin on the adrenal gland (Oelkers et al., 1975). Favoring the second explanation but not excluding the first, Cowley and McCaa (1976) noted a secondary rise of aldosterone during the latter stages of a prolonged but constant infusion of angiotensin. Also, in man, when sodium retention is prevented during infusion of angiotensin, aldosterone does not fall significantly, and the slope relating angiotensin and aldosterone steepens (Oelkers et al., 1975). Similarly, in chronic renal hypertension, including patients with renin-secreting tumor, the curve is steeper than normal (Beeverers et al., 1975). These observations raise the possibility that prolonged exposure of the adrenal cortex to an increased plasma concentration of angiotensin II enhances the response of aldosterone tested acutely. Other stimuli to aldosterone might be involved in these changes. Infusion of angiotensin II at 23 ng/kg per min in the dog reduces plasma potassium concentration (Cowley and McCaa, 1976). This would tend to reduce aldosterone secretion. However, at 5 ng/kg per min in the study of Cowley and McCaa, and at 3 ng/kg per min in our study, plasma potassium did not change. ACTH is another potential influence. Angiotensin II infusion suppresses ACTH acutely (Semple et al., in press), and ACTH can stimulate aldosterone in some circumstances (Kem et al., 1975). Absence of a change of plasma cortisol during angiotensin infusion, as in the study of Cowley and McCaa (1976), does not necessarily mean that ACTH is unchanged, because cortisol and ACTH are not closely related during angiotensin infusion (Semple et al., in press).

Whatever the explanation for the changes of aldosterone, it could have produced the gradual rise of pressure only if the response of pressure to aldosterone were delayed, or if it increased as the plasma level of aldosterone decreased. These points were not tested.

We conclude that prolonged infusion of angiotensin II raises blood pressure slowly and produces an abnormality like that present in renal hypertension—a level of blood pressure higher than can be explained by the acute vasoconstrictor action of angiotensin II. Although this removes a potential objection to a role for angiotensin II in the pathogenesis of chronic renal hypertension, it does not establish that angiotensin II has such a role.

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