Comparison of Angiotensin II Antagonist and Antiserum Infusion with Nephrectomy in the Rat with Two-Kidney Goldblatt Hypertension

By Herbert Thurston and John D. Swales

ABSTRACT

Factors responsible for maintaining blood pressure were studied in rats with one renal artery constricted and the contralateral kidney intact. Rats with short-term (< 3 weeks) and chronic (> 4 months) hypertension were infused with angiotensin II antiserum until the pressor effect of exogenous angiotensin II was blocked. The blood pressure response to an infusion of an angiotensin antagonist (1-Sar-8-Ala-angiotensin II) was then recorded. Blood pressure was also measured following subsequent unilateral nephrectomy (ischemic kidney). Antiserum produced a small, sustained, nonsignificant fall in mean blood pressure, whereas the antagonist produced a major reduction. In rats with short-term hypertension, the antagonist reduced blood pressure to normal or near-normal levels. In rats with chronic hypertension, mean blood pressure fell but still remained above the upper limit of the normal range. Removal of the ischemic kidney produced a fall in blood pressure to a mean level close to that obtained after antagonist infusion. Mean cumulative sodium balance in the rats with short-term hypertension was slightly negative. On the basis of this and previously reported work, it is suggested that angiotensin II is generated at a vascular site which is inaccessible to antibody but readily accessible to antagonist. Moreover, since the effects of angiotensin II antagonist and nephrectomy do not differ significantly, it seems that the ischemic kidney sustains hypertension in this two-kidney model through activity of the renin-angiotensin system, although extrarenal factors assume greater importance when blood pressure remains elevated for longer periods.

KEY WORDS
sodium balance angiotensin II receptors vascular renin
mean blood pressure local angiotensin generation renal artery constriction

The role of the renin-angiotensin system in experimental hypertension is still uncertain despite the recent acquisition of powerful tools for investigating this question. Much of the uncertainty stems from the conflicting results that are obtained when different methods are used to suppress the renin-angiotensin system in experimental hypertension. Animals can be immunized against angiotensin II (1-11) or renin (12-15); alternatively, an inhibitor of angiotensin II formation (16-18) or an antagonist of its action (7, 19, 20) can be employed.

There is evidence that sodium retention occurs in rats, and perhaps in rabbits, with hypertension produced by renal artery constriction and contralateral nephrectomy (one-kidney Goldblatt model) (21, 22); such retention might sustain hypertension even when the renin-angiotensin system has been inactivated (7, 23). Even so, some workers who have used angiotensin II immunization have failed to lower the blood pressure of rats with hypertension produced by unilateral constriction of a renal artery with the contralateral kidney intact (two-kidney Goldblatt model) (1, 9, 11). We have recently put forward the explanation that renin generates angiotensin II in the peripheral blood vessels at a site which is inaccessible to the large antibody molecule but accessible to angiotensin II antagonist (24, 25). This possibility might explain some of the observed discrepancies. Other factors in addition to sodium retention could play a role in the resistance of experimental hypertension to inhibition of angiotensin II activity. Extrarenal changes such as alterations in the vascular tree, might perpetuate hypertension even after the relief of the ischemic stimulus (26). Also the kidneys might secrete or be responsible for the secretion of another pressor substance (27).

The present study was designed to decide between these possibilities. Angiotensin II antiserum and antagonist were infused into rats with two-kidney Goldblatt hypertension of short and long duration, and the action of each of these agents was compared with that of the other and with the effect of removal of the ischemic kidney.
Methods

White Wistar rats (150–250 g) of either sex were used, and hypertension was produced by the application of a silver clip (0.2 mm, i.d.) to the left renal artery. The rats were weighed and their blood pressures were measured by a photoelectric method (28) at least twice a week until cannulation was carried out.

**ANTISERUM**

Antiserum to 5-Val-angiotensin II amide (Hypertensin, CIBA) was prepared in a rabbit according to the method of Goodfriend et al. (29). When this antiserum was added to 5-Val-angiotensin II amide and titrated in the nephrectomized rat according to the method of Eide and Aars (5), 1 ml of antiserum neutralized the pressor action of 16,000 ng of angiotensin II. The antiserum was equally effective in blocking the blood pressure response of a nephrectomized rat to equipressor doses of rat angiotensin (prepared by incubating rat plasma) and 5-Val-angiotensin II amide. In an in vitro system, 0.1 ml of antiserum neutralized 0.1 ml of labeled 5-Val-angiotensin II amide. Binding of [125I]-labeled angiotensin I was less than 50% even with an antiserum dilution of less than 1:10.

**BALANCE PROCEDURE**

Rats to be subjected to short-term hypertension (indirect blood pressure > 120 mm Hg) were maintained in metabolic cages for a minimum of 5 days prior to renal artery constriction. Collections of urine and feces were made at 48-hour intervals. Weighed, powdered food mixed with deionized water was presented as a paste, and the uneaten residue was weighed. Free access to deionized water was also allowed.

Cumulative sodium balance was estimated by a previously described method (22).

**EXPERIMENTAL PROCEDURE**

Thirteen rats with short-term hypertension were studied 10–21 days after their left renal arteries had been clipped and approximately 7–10 days after they first became hypertensive. Nine rats with chronic hypertension were studied 10–21 days after their left renal arteries had been clipped and approximately 7–10 days after they first became hypertensive. Nine rats with chronic hypertension were studied 4–6 months after hypertension was established. Experimental procedures were otherwise identical. Rats were anesthetized with ether; the jugular vein and the carotid artery were then cannulated. Mean arterial blood pressure was measured using a Statham P23Gb transducer connected to a Grass polygraph recorder. After a steady base-line pressure had been established, 50 ng of 5-Val-angiotensin II amide dissolved in 0.1 ml of isotonic saline was injected as a bolus and the pressor response was measured. After restoration of the base-line blood pressure, 0.1 ml of antiserum was infused; 3–5 minutes later, the pressor response to 50 ng of angiotensin II was again recorded. Repeated doses of antiserum were infused until the response to exogenous angiotensin II was abolished. The stable blood pressure at this stage was noted, and a saline solution of angiotensin II antagonist (1-Sar-8-Ala-angiotensin II) was infused at the rate of 10 μg/min for 5 minutes. The stable blood pressure at the end of the infusion was noted, and blocking of the pressor response to 50 ng of angiotensin II was checked.

The cannulas were then withdrawn, and the rats were allowed to recover.

Two rats were infused with the antagonist before the antiserum injection, and one rat was infused with antiserum only. The specificity of the blood pressure response to antiserum was examined by infusing serial 0.1-ml doses into nine rats that had undergone bilateral nephrectomy 6–24 hours earlier.

**NEPHRECTOMY**

Indirect blood pressures were followed for 5–7 days. When blood pressure had stabilized at the preannulation level, the rat was again anesthetized and the left kidney was removed. After an additional 5–7 days had elapsed, the carotid artery was again cannulated and a direct blood pressure measurement was recorded.

**RESULTS**

Two of the rats with short-term hypertension and 2 of those with chronic hypertension died before a postnephrectomy cannulation could be performed. Thus, complete data on blood pressure after administration of antiserum and antagonist followed by unilateral nephrectomy were available for 17 rats. The 2 rats which received antagonist first were considered separately; therefore, complete data are reported for 15 rats (8 with short-term and 7 with chronic hypertension) which were observed after administration of antiserum and antagonist and following unilateral nephrectomy.

**SODIUM BALANCE**

Mean sodium balance in the 13 rats with short-term hypertension was \(-0.45 ± 0.25\) (SE) mEq. Balance in the 8 rats with short-term hypertension subjected to the complete protocol was \(-0.03 ± 0.30\) mEq. Of these rats, 4 showed a slightly negative balance and 4 showed a slightly positive balance.

**BLOOD PRESSURE RESPONSE TO ANTISERUM AND ANTAGONIST**

The initial mean direct blood pressure of the 13 rats with short-term hypertension was 142.1 ± 3.5 mm Hg. The 2 rats infused with antagonist before antiserum had been administered showed a fall in blood pressure to normal levels (100.0 and 101.5 mm Hg). No further sustained fall occurred on administration of antiserum, and, for this reason, it was felt that the results could not be combined with those from rats which were given antiserum first. The mean blood pressure of the remaining rats with short-term hypertension was 144.5 ± 5.1 mm Hg after administration of antiserum. This value is not significantly different from that before antiserum administration in a paired t-test (\(P > 0.05\)). After administration of antagonist, the mean blood pressure of these rats was 115.3 ±

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ANGIOTENSIN IN TWO-KIDNEY HYPERTENSION

3.3 mm Hg, a pressure significantly less than the initial blood pressure and the blood pressure after antiserum administration (P < 0.001). An identical pattern of results was obtained when data from the 8 rats with short-term hypertension which were subjected to the complete protocol were considered (Table 1). Of these rats, 4 showed a slightly higher blood pressure after administration of the antagonist and 4 showed a slightly lower pressure compared with the blood pressure after nephrectomy.

The nine rats with chronic hypertension had a base-line blood pressure of 151.7 ± 8.9 mm Hg. Pressure fell to 146.8 ± 7.4 mm Hg after antiserum administration and to 131.2 ± 6.9 after antagonist administration. The fall after antiserum had been given was not significant (P > 0.05), but the fall after administration of antagonist was (P < 0.005). The fall after antagonist administration was significantly less in the rats with chronic hypertension than it was in the rats with short-term hypertension (P < 0.05).

The nine bilaterally nephrectomized rats showed no sustained fall in blood pressure when antiserum was infused. There was, however, a transient fall in blood pressure (12.1 ± 2.7 mm Hg) lasting approximately 1 minute. This fall was observed even in rats that had been previously blocked with excess antiserum; it was therefore ignored in the present study.

BLOOD PRESSURE RESPONSE TO UNILATERAL NEPHRECTOMY

Blood pressure (measured indirectly) remained at a lower level for a day or two after the first cannulation procedure, but it had returned to the precannulation level by the time of nephrectomy. Following unilateral nephrectomy (ischemic kidney), indirect measurements yielded stable blood pressure values which were similar to the direct mean values subsequently obtained at the time of the second cannulation. Mean blood pressure measured directly by cannulation following nephrectomy was 111.8 ± 4.8 mm Hg for the 11 rats with short-term hypertension. This value was significantly less than the initial blood pressure and the blood pressure of these rats following antiserum administration (P < 0.01), but it was not significantly different from the blood pressure of these rats following antagonist administration (P > 0.05).

Blood pressure of the seven chronically hypertensive rats following nephrectomy was 131.7 ± 6.6 mm Hg. Although this value was less than the initial blood pressure and the blood pressure fol-

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<td><strong>Effect of Antiserum, Antagonist, and Removal of the Ischemic Kidney on the Mean Blood Pressure (mm Hg) of Rats with Short-Term and Chronic Hypertension</strong></td>
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For the rats with short-term hypertension, the blood pressure after either nephrectomy or administration of antagonist was significantly less than initial blood pressure or blood pressure after administration of antiserum (P < 0.005). For the chronically hypertensive rats, blood pressure after administration of antagonist was also significantly less than the initial blood pressure (P < 0.05), although blood pressure after nephrectomy was not (0.1 > P > 0.00). Although blood pressure fell in all seven rats after administration of antagonist, it fell in only six rats after nephrectomy. Also, the standard error after nephrectomy was higher. There was no significant difference between blood pressure after administration of antagonist and after nephrectomy (P > 0.06) for either group. All values are means ± SE, and the number of rats tested is given in parentheses.

Discussion

In the present study the blood pressure-lowering effect of the angiotensin II antagonist equaled that of removal of the ischemic kidney. In rats with short-term hypertension, each of these procedures restored blood pressure to values within or just above the normal range. The effect in rats with chronic hypertension was still present but less marked. These findings suggest that in the two-kidney Goldblatt model the kidney maintains hypertension through the mediation of the renin-angiotensin system. Extrarenal factors might assume greater importance in chronic hypertension; however, our results throw no light on the nature of these factors. In the rats with short-term hypertension, there was no significant correlation between the state of sodium balance and the fall in blood pressure produced by the inhibitor (r = -0.29, P > 0.05). Balance procedures are not feasible in long-term animal experiments but the studies of Tobian et al. (30) yield no evidence of elevated exchange-
able sodium levels. It is probable that sodium retention does not play a role in the maintenance of blood pressure in this model (21, 22). In one-kidney Goldblatt hypertension, on the other hand, the poor response to angiotensin antagonists can be enhanced by sodium depletion, and it is likely that sodium retention does play an important role (23).

Our results confirm the efficacy of angiotensin II antagonists in lowering blood pressure in the two-kidney model (7, 19, 20, 31); converting enzyme inhibitor (16) and renin preinhibitor (18) have also been shown to have a marked blood pressure-lowering effect. In most studies, blood pressure has nevertheless remained above the normal range. Our studies on the effect of nephrectomy suggest that this fact is related to the duration of hypertension rather than to other renal pressor mechanisms.

The poor blood pressure response to angiotensin II antisera remains to be explained. Although our antiserum was developed against synthetic 5-Val-angiotensin II amide, our in vivo studies indicated that the antiserum was equally effective in blocking the pressor effect of rat angiotensin II. Our results cannot therefore be due to low cross-reactivity. Observations on the efficacy of immunization have proved conflicting. In some hands, a substantial fall in blood pressure has been produced by the passive immunization of rats with two-kidney hypertension (3, 7, 8). This fall has not been observed by other workers (1, 9). Active immunization of this model has either alleviated or prevented hypertension in some studies (2) but not in others (10, 11). One source of confusion might be technical. The antiserum used in our experiments frequently caused a transient fall in blood pressure when it was injected as a rapid bolus. The fall was not due to binding of circulating angiotensin II. In the present study the pressor response to exogenous angiotensin II remained blocked for several hours after antiserum administration; moreover, injected antiserum produced a transient depressor effect of the same magnitude in both bilaterally nephrectomized rats and rats already blocked with antiserum. This transient blood pressure fall should, therefore, be neglected.

The poor response to antiserum cannot be due to extrarenal changes which perpetuate hypertension in the absence of renin-angiotensin activity. In the present study, rats which failed to respond to antiserum showed a substantial response to antagonist on the same occasion: control rats treated first with antagonist failed to show a further fall in response to antiserum. The dose of antiserum administered was far in excess of that required to neutralize endogenous angiotensin II, and additional doses of antiserum failed to exert any additional depressor action. There is no evidence that this antagonist has a depressor action in its own right. Indeed, our own unpublished studies on bilaterally nephrectomized rats have demonstrated a slight pressor effect, presumably due to a partial agonist action (19). It seems probable, therefore, that antiserum is ineffective because angiotensin II is generated at a site which is inaccessible to the large molecules of antibody but readily accessible to antagonist. It is well established that renin activity is present in the arterial wall (32), and increased levels have been demonstrated in the mesenteric artery wall of salt-depleted dogs (33). On the basis of studies of angiotensin II antiserum requirements (24), we have previously suggested that arterial wall renin activity is of physiological importance, i.e., that it at least partially determines the impaired pressor response of salt-depleted animals to angiotensin II, and that vascular renin levels are high in relation to sodium balance in rats with one-kidney Goldblatt hypertension. Studies on renin-induced hypertension, like the experiments described in the present paper, have shown a response to antagonist substantially greater than that to antiserum (25). Essentially similar conclusions have been reached by comparing the reductions in blood pressure produced by antagonist and antiserum in different groups of renin-infused rats (34).

The response to unilateral nephrectomy in our experiments points to a renal rather than a vascular origin for the renin which maintains elevated blood pressure levels. It would therefore be anticipated that antirenin would be more effective than antiangiotensin II antiserum, although the action might be delayed if peripheral vascular renin is inaccessible. The impurity of antirenin antibodies makes interpretation of such results difficult, and most observers have used a single kidney or a bilateral renal procedure to induce hypertension. A recent report, however, has described a somewhat delayed blood pressure-lowering action in rabbits with two-kidney hypertension (15).

The current work provides no evidence for a renal role in the maintenance of two-kidney hypertension other than through the secretion of renin, which is then bound at a peripheral vascular site where angiotensin II is generated. The inaccessibility of angiotensin II to antiserum might be relative rather than absolute, and it is possible that a longer period of antiserum infusion might have demonstrated a more profound effect on blood pressure.
ANGIOTENSIN IN TWO-KIDNEY HYPERTENSION

According to one group (8), angiotensin II antiserum takes up to 2 days to exert its maximal blood pressure—lowering action in the rat with two-kidney hypertension. This finding could indicate slow penetration. On the other hand, the failure of active immunization (10, 11) militates against such a hypothesis.

References
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HERBERT THURSTON and JOHN D. SWALES

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