Effect of the Angiotensin II Blocker 1-Sar-8-Ala-Angiotensin II on Renal Artery Clip Hypertension in the Rat

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ABSTRACT

Twenty-four conscious male Wistar rats, with hypertension induced by left renal artery clipping (two-kidney hypertension) were infused intravenously with 1-Sar-8-Ala-angiotensin II a competitive angiotensin II antagonist. The spectrum of responses was wide, ranging from a mild elevation in blood pressure to a marked fall in blood pressure, despite effective and specific angiotensin blockade in all cases. The change in blood pressure during 1-Sar-8-Ala-AII infusion activity showed a significant correlation with the level of plasma renin prevailing immediately before the infusion \( (r = -0.78, P < 0.01) \) but not with the prevailing blood urea level \( (r = 0.27, 0.1 > P > 0.05) \), the degree of hypertension \( (r = 0.42, 0.1 > P > 0.05) \), or the time since clipping \( (r = 0.02, P > 0.05) \). There was no significant correlation between the degree of hypertension and the plasma renin activity \( (r = 0.42, 0.1 > P > 0.05) \). In rats with blood pressure drops > 20 mm Hg in response to 1-Sar-8-Ala-AII, the final blood pressure level was still above the normotensive range. Excision of the clipped kidney reduced blood pressure to normal or to near normal within 24 hours in all of the rats tested. It is concluded that the degree of dependence of renal hypertension on the renin-angiotensin system is directly related to the increase in circulating angiotensin itself and not to an increase in sensitivity to angiotensin. Other factors appear to be involved in renal clip hypertension in addition to circulating renin and angiotensin, especially when the measured activity of plasma renin is normal.

The present study was undertaken to examine the nature, extent, and possible reasons for the variability in the response to 1-Sar-8-Ala-AII in two-kidney hypertension in the rat between 2 and 12 weeks after clipping, taking into account such factors as the time since clipping, the plasma renin levels, the severity of the hypertension, and the degree of impairment of renal function. Since acute surgery and anesthesia may have introduced some unwanted variability in previous studies (4), all of our experiments were performed in conscious rats studied 2 days after the implantation of arterial and venous catheters.

Methods

INDUCTION OF HYPERTENSION

Male white Wistar rats weighing 150-200 g were anesthetized with ether, and the left renal artery was constricted with a preset silver clip with an internal gap of 0.2 or 0.25 mm. After 1-2 weeks, blood pressure measurements were commenced using the micromanometric method of Friedman and Freed (6). Additional groups of rats were subjected to sham-clipping: the left renal artery was exposed, and a silver strip was placed in the perinephric fat.

Throughout the experiments, the rats were fed a regular pellet diet (Oxoid SG10) and given free access to tap water. No attempt was made to control sodium intake, since it has been shown that sodium status may be an important determinant of the renin levels achieved in this form of hypertension (6, 7); we wished to study hypertensive rats with spontaneously achieved variables.

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1-Sar-8-Ala-AII and Renal Artery Clip Hypertension

ADMINISTRATION OF 1-Sar-8-Ala-AII

Rats with established hypertension (systolic blood pressure over 200 mm Hg by tail measurements) were prepared for the experiment by inserting tapered polyethylene catheters into their right internal and external jugular veins and their right carotid artery under sodium pentobarbital anesthesia. The catheters were filled with heparinized saline, plugged, and implanted subcutaneously down the rats' backs. After a recovery period of 2 days, the catheters were recovered, and the arterial line was connected to a pressure transducer and a Devices two-channel recorder. Blood (0.5 ml) was then taken for the estimation of plasma renin activity.

After the blood pressure had stabilized, dose-response curves to 5-Ile-angiotensin II (infused at 0.05, 0.1, and 0.2 µg/kg min⁻¹ and l-norepinephrine (Levophed, Winthrop) (injected in doses of 10–160 ng) were obtained. A base-line blood pressure trace of at least 30 minutes was then recorded, and 1-Sar-8-Ala-AII was subsequently infused at 4 µg/min (a solution of 133 µg/ml in normal saline infused using a Meltec syringe pump at 0.03 ml/min). After 30 minutes of infusion, All blockade was confirmed by the loss of the pressor response to an All infusion at 0.2 µg/kg min⁻¹, and the norepinephrine dose-response curve was repeated. After an additional 10–30 minutes, the 1-Sar-8-Ala-AII infusion was stopped, but the blood pressure recording was continued until a steady level had been regained for 30 minutes.

At the end of such studies, 15 of the rats were anesthetized with a small dose of intravenously administered sodium pentobarbital, and the left (clipped) kidney was excised in 11 rats. In the remaining 4 rats, a sham-nephrectomy was performed (exposure and brief manipulation of the left kidney). The following day, direct blood pressure measurements were again made in a conscious state, and the four sham-nephrectomized rats were re-anesthetized and subjected to a left nephrectomy. Their blood pressures were measured directly again on the following day.

PLASMA RENIN ACTIVITY MEASUREMENTS

Blood (0.5 ml) was taken from the arterial catheter into a heparinized tube standing in ice. Following centrifugation at 2,050 g for 10 minutes at 4°C, 0.2 ml of plasma was added to 0.02 ml of 0.3M ethylenediaminetetraacetic acid (EDTA) and 0.01 ml of 0.2M 2,3-dimercapto-1-propanol (BAL) and incubated at 37°C for 4 hours. The incubate was diluted to 1 ml with immunoassay buffer, heated to 85°C for 10 minutes to stop the reaction, and then frozen.

For assay, the samples were thawed and centrifuged, and the supernatant fluid was assayed directly by the method of Boyd et al. (8) using 5-Ile-angiotensin I as the standard. Recovery of 60 ng of 5-Ile-Al added to the original plasma sample was 83.4 ± 6.1% (ss). Assay of replicate samples gave a within-assay coefficient of variation of 3%. In separate experiments, it was established in normal and hypertensive rats that bleeding of this amount of blood did not result in an increase in plasma renin activity in blood taken 1 hour later.

Statistical Analysis

Significance testing was based on double-tailed Student's t-testing or correlation coefficient calculations. Base-line blood pressures were calculated by taking the average of the mean blood pressure at 1-minute intervals over the 30 minutes immediately before the start of the 1-Sar-8-Ala-AII infusion. Mean blood pressure was calculated as the diastolic pressure plus one-third of the pulse pressure. The mean blood pressure change induced by the 1-Sar-8-Ala-AII infusion was taken as the difference between the base-line blood pressure and the average of the readings between 20 and 30 minutes after the start of the infusion.

Results

On the day of the experiment, directly measured mean blood pressure varied from 108 to 127 mm Hg in the 9 sham-clipped rats and from 131 to 227 mm Hg in the 24 clipped renal hypertensive rats. The two groups were of comparable weight at the time of the experiment; the sham-clipped rats weighed 313 ± 31 (s) g and the hypertensive rats weighed 335 ± 55.4 g. Plasma renin activities in the sham-clipped rats ranged from 1.1 to 5.5 ng Al/ml hour⁻¹ (mean 3.0 ± 1.58 [SD] ng Al/ml hour⁻¹) and in the hypertensive group (N = 22) from 4.2 to 85 ng Al/ml hour⁻¹ (mean 20 ± 18.2 ng Al/ml hour⁻¹) (Fig. 1).

Infusion of 1-Sar-8-Ala-AII at 4 µg/min resulted in complete blockade of the pressor effects of 5-Ile-AII infused at 0.2 µg/kg min⁻¹ in 19 rats (Fig. 2). In the remaining 5 rats, the maximum response was 10 mm Hg, a response indistinguishable from the slight spontaneous fluctuations in base-line blood pressure. The average mean blood pressure response to this dose of 5-Ile-AII prior to 1-Sar-8-Ala-

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Plasma renin activity in normal, two-kidney hypertensive (HT), and sham-clipped rats. Open circles in the hypertensive group represent rats whose mean blood pressures fell by more than 20 mm Hg during angiotensin II blockade with 1-Sar-8-Ala-AII.

FIGURE 1

Plasma renin activity in normal, two-kidney hypertensive (HT), and sham-clipped rats. Open circles in the hypertensive group represent rats whose mean blood pressures fell by more than 20 mm Hg during angiotensin II blockade with 1-Sar-8-Ala-AII.

1 Generously supplied by Dr. A. Castallion of the Norwich Pharmacal Corp.
All administration was 34 ± 2.1 (SEM) mm Hg. During 1-Sar-8-Ala-AII infusion it was 1.3 ± 1.4 mm Hg. Figure 2 also shows that the response to l-norepinephrine injections was unimpaired.

The blood pressure response to 1-Sar-8-Ala-AII varied widely from rat to rat. In 3 rats blood pressure fell by 5 mm Hg or less, and in 4 it actually rose slightly. In the other 17 rats, falls in blood pressure varied continuously up to a maximum of 65 mm Hg.

The maximum average fall in blood pressure for the whole hypertensive group was 18 ± 3.5 (SEM) mm Hg between 21 and 25 minutes of infusion. This decline was significant (P < 0.01), but, as seen in Figure 3, it was less than the fall observed 24 hours after the excision of the clipped kidney even in those rats showing greater declines (> 20 mm Hg) with 1-Sar-8-Ala-AII (P < 0.05). During 1-Sar-8-Ala-AII infusion, only four rats achieved blood pressures within the normal range observed in sham-operated rats.

In all of the rats subjected to left nephrectomy, blood pressure fell to within the sham-clipped range within 24 hours, regardless of the response previously seen to All blockade with 1-Sar-8-Ala-AII. This fall was not a result of anesthesia and surgery, since four rats subjected to sham-nephrectomy failed to show a fall in blood pressure at 24 hours but subsequently did return to normal blood pressure following excision of the clipped kidney (Fig. 3).

Various factors were analyzed to determine the basis for the variation in the individual responses to 1-Sar-8-Ala-AII (Table 1). There was no significant correlation with the blood urea level at the time of study (r = 0.27, P > 0.05), the time since clipping (15-80 days) (r = 0.02, P > 0.05), or the degree of hypertension indicated by average baseline blood pressure measured directly on the day of the experiment (r = 0.42, P > 0.05).

Plasma renin levels in the hypertensive group were significantly higher than those in the sham-clipped control group considered as a whole (P < 0.01) (Fig. 1). When the hypertensive rats were divided into those with marked falls in blood pressure (> 20 mm Hg) and those with lesser falls, the plasma renin activity in the former group was significantly higher (P < 0.01) (Fig. 1). Plasma renin activity in both groups was significantly higher than that in the sham-clipped control rats (P < 0.01 for the group with the lower blood pressure fall [13 ± 10 [SD] ng Al/ml hour⁻¹, N = 13] and P < 0.001 for the group with a fall > 20 mm Hg [34.4 ± 20.5 ng Al/ml hour⁻¹, N = 9]).

The fall in blood pressure during 1-Sar-8-Ala-AII infusion showed a highly significant correlation with the log of plasma renin activity (Fig. 4) (r = 0.78, P < 0.01). In addition, the regression line intercepted the zero point on the y-axis at a plasma renin activity of 5.7 ng Al/ml hour⁻¹, within the normal range but just above the upper limit of values obtained in the sham-clipped rats. This fact suggests that two-kidney hypertensive rats with a
There is a lack of correlation between the change in blood pressure (Δ BP) and the initial blood pressure (BP) (r = 0.42, P > 0.05), the blood urea level (r = 0.27, P > 0.05), and the days since clipping (r = 0.02, P > 0.05). The correlation between the blood pressure change and plasma renin activity (PRA) is shown in Figure 4.

Regression line between the log of plasma renin activity and the change in blood pressure (Δ BP) during 1-Sar-8-Ala-AII infusion, showing a highly significant correlation. The line intercepts the zero-change point on the y-axis at a plasma renin activity of 5.7 ng Al/ml hour⁻¹, almost exactly the mean normal value (5.6 ng/ml hour⁻¹).

In five rats, the 1-Sar-8-Ala-AII infusion rate was increased to 10 μg/min near the end of the infusion. None of these rats showed an additional fall in blood pressure or any fall at all if the 4-μg/min infusion rate had had no effect. These results indicate that 4 μg/min represented a maximal dose and suggest that differences in the blood pressure response were not due to differences in the effective infusion rates from rat to rat. This finding is in keeping with the observation of total blockade of the pressor effect of AII with 4 μg 1-Sar-8-Ala-AII/ min.

In the sham-clipped group (N = 9), the maximum blood pressure fall was 15 mm Hg, observed during two separate 1-Sar-8-Ala-AII infusions in a normal plasma renin activity would not show a fall in blood pressure with 1-Sar-8-Ala-AII infusion, as suggested in Figure 1.
Correlation between plasma renin activity and initial (baseline) mean blood pressure (B.P.). The correlation falls outside the 95% confidence limits.

The extent of involvement of the renin-angiotensin system in all types of renal hypertension is unsettled. Recent attention has focused on the two-kidney type (unilateral renal ischemia or constriction with the contralateral kidney left untouched). Various groups have presented data which suggest that this type of renal hypertension represents a model of renin- and angiotensin-dependent hypertension based on studies with All blockers or antibodies (3, 4) or with inhibitors of AI conversion (9, 10).

Such studies have not been unanimous. Eide (11) failed to relieve two-kidney hypertension with active All immunization in the rat, and Pals and co-workers (2), although they were able to reduce two-kidney hypertension in the early (up to 2 weeks after induction) phase using 1-Sar-8-Ala-AII, failed to find any significant effect in more chronically hypertensive rats (5-6 weeks from induction). Bing and Nielsen (4) found a variable effect using 1-Sar-8-Ala-AII in conscious and anesthetized two-kidney hypertensive rats 1 and 4 months after clipping.

It is true that plasma renin levels may be elevated in this form of hypertension (6, 12), but this phenomenon may be secondary to the hypertension because of sodium loss from the untouched kidney (6, 7). In many instances, however, plasma renin levels are within normal limits both in man and experimental animals (13, 14), and much work has been done on the physiology of renal hypertension and the pharmacology of renin and angiotensin to show how this hormone system might exert abnormal physiological effects in the presence of normal measured plasma renin levels. Various neural mechanisms of secondary hypertension following initial induction by renin and angiotensin have been suggested (15). The possibility that renin and angiotensin levels, although within the normal range, are “inappropriately” high for the prevailing body sodium status or the prevailing angiotensin receptor sensitivity (13, 16) has also been mentioned.

In the rats studied in the present series of experiments, the mean plasma renin activity of the hypertensive group was significantly elevated ($P < 0.01$) compared with that of the sham-clipped group. The hypertensive rats were readily divisible into those with blood pressure declines during 1-Sar-8-Ala-AII infusion of more than 20 mm Hg and those with lesser falls. Renin levels showed an almost clear-cut difference, with rats with the larger declines having significantly higher plasma renin levels than rats with the smaller declines ($P < 0.01$). For the hypertensive group as a whole, there was a strong correlation ($r = 0.78, P < 0.01$) between plasma renin activity and the blood pressure fall induced by 1-Sar-8-Ala-AII.

The susceptibility of the hypertension to angiotensin blockade suggests that renin and angiotensin contribute to the blood pressure elevation. However, our data show that this contribution is no simple relationship, since (1) the degree of hypertension did not correlate significantly with the plasma renin levels (Fig. 5) and (2) although 1-Sar-8-Ala-AII caused blood pressure falls in many rats, the levels reached were within the sham-clipped control range in only four rats and the mean levels reached during angiotensin blockade were significantly higher than the levels 24 hours after nephrectomy even in those rats with
major (> 20 mm Hg) 1-Sar-8-Ala-AII-induced declines (Fig. 3). Similar findings have been reported by Bing and Nielsen using 1-Sar-8-Ala-AII (4) and by Bumpus and his colleagues (17) using a slightly different angiotensin blocker.

The strong correlation between plasma renin activity and the fall in blood pressure with 1-Sar-8-Ala-AII suggests that in these rats the dependence of the hypertension on renin and angiotensin is closely related to the degree of elevation of plasma renin activity. Pals and Masucci (18) have reported a similar relationship between plasma renin activity and the depressor effect of 1-Sar-8-Ala-AII in conscious dogs with different types of hypertension, including chronic malignant one-kidney or acute two-kidney hypertension. If renin levels were for any reason inappropriately high in the normal-renin group, their hypertension should still have been susceptible to angiotensin blockade. In our series of experiments it was not. It may be inferred that in this normal-renin group, renin and angiotensin are not important factors in the maintenance of the hypertension.

Gavras et al. (16) have shown that sodium depletion can change the status of normal and one-kidney hypertensive rats, usually unresponsive to angiotensin blockade, so that blood pressure falls similar to those previously described in two-kidney hypertension occur (3). They have proposed that sodium depletion reveals a dependence of blood pressure on renin and angiotensin which exists even when 1-Sar-8-Ala-AII is ineffective. Our data are consistent with their experimental findings, since renin levels in salt-depleted rats are sure to be elevated, although these data were not presented by Gavras et al. (16). The rats would therefore fall into the group whose blood pressures we would expect to fall (Fig. 1). Rather than revealing an inherent dependence on renin and angiotensin, however, we think that the elevated renin levels represent an increased quantitative contribution to blood pressure maintenance regardless of the contemporary blood pressure. In this case, angiotensin blockade is predictably successful in reducing blood pressure. The distinction to be made, therefore, is not between one-kidney and two-kidney hypertension but between high-renin and low-renin hypertension.

If, in severe hypertension, sodium loss leads to elevated plasma renin levels (10), the hypertension will to some extent be dependent on renin and will be susceptible to angiotensin blockade, without renin and angiotensin being the initial causal factors in the elevation of blood pressure. The failure of 1-Sar-8-Ala-AII-responsive rats to achieve blood pressures within the normal range (assessed by the sham-clipped rats' blood pressure range and by blood pressures reached in the hypertensive group 24 hours after left nephrectomy) further suggests that, even when renin and angiotensin are implicated, they cannot account fully for the blood pressure elevation.

It might be postulated that these experiments have not covered the possibility that All exerts effects on a prolonged time course which are not altered by the infusion of an inhibitor for 2 hours or less. This situation would include the effects of aldosterone. It has been shown, however, that in hypertension induced in rabbits by All infusion over several months (102 days), cessation of infusion results in a fall to normal levels or below within as short a period as 2 minutes (19). Such a theory would also require that angiotensin induce these secondary mechanisms in inverse relationship to its plasma level, since they are apparently absent when renin levels are high and angiotensin blockade acts to reduce blood pressure.

Other factors which might have influenced the response to 1-Sar-8-Ala-AII were also studied. However, the severity of renal impairment (blood urea), the changing dependence on renin and angiotensin (time since clipping), and the initial blood pressure did not appear to influence the results of angiotensin blockade.

Bing and Nielsen (4) have postulated that a failure to reduce blood pressure with 1-Sar-8-Ala-AII may be related to an inherent pressor response to the drug which they assessed by the post-1-Sar-8-Ala-AII increase in blood pressure over control levels. In our experience, such an increase was uncommon and was not evident from the group mean postrecovery blood pressure. Neither was there any association between the presence or the absence of a preliminary rise and the absence or the presence, respectively, of a subsequent fall in blood pressure.

It is possible that this rebound increase in blood pressure is due to an increase in renin release from the kidneys induced by inhibition of angiotensin negative feedback by 1-Sar-8-Ala-AII (4, 20). Such an increase in renin may have partially opposed the depressor effect of 1-Sar-8-Ala-AII. It is unlikely that this phenomenon is the basis for the failure of many rats' blood pressures to fall in view of the highly effective blockade to a high dose of All infused during 1-Sar-8-Ala-AII administration, whether the analogue was associated with a blood pressure fall or not.
Our data suggest that renin and angiotensin contribute to two-kidney hypertension when plasma renin levels are elevated, although, even then, the evidence indicates that they are not the only factors involved. With normal circulating renin levels, there is no evidence of a significant role for renin or angiotensin on the basis of levels inappropriately high in relation to factors such as sodium status or increased receptor sensitivity. Other factors besides renin and angiotensin therefore appear to be involved in two-kidney hypertension. Their nature is unclear, but, since, without exception, blood pressure falls to normal within 24 hours of nephrectomy, our findings are strongly in favor of their originating in the kidney.

Addendum

Since the preparation of this paper, two relevant publications have appeared. Thurston and Swales (Circ Res 35:325-329, 1974) compared the effects of anti-angiotensin serum administration, 1-Sar-8-Ala-AII infusion, and nephrectomy on blood pressure in the two-kidney hypertensive rat. Since 1-Sar-8-Ala-AII and nephrectomy reduced blood pressure and the antiserum did not, they concluded that renin may be active outside the circulation in a site which is inaccessible to antiserum. Plasma renin values were not measured, and the rats were anesthetized during the blocking experiments. Another difference from the present experiments is that individual data were not given, even though individual variations may be of great importance, since one possible way of reconciling these data with our own is to suggest that increasing plasma renin concentration in our unanesthetized rats is associated with increased renin responsiveness, so that the ability of 1-Sar-8-Ala-AII to lower pressure still only correlates well with the plasma renin activity.

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