Scientific Contributions

Renoprotection by ACE Inhibition or Aldosterone Blockade
Is Blood Pressure–Dependent

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Abstract—Renin-angiotensin-aldosterone system blockade has been shown to protect against renal damage in salt-supplemented, stroke-prone spontaneously hypertensive rats (SHRsp). Based on intermittent tail-cuff blood pressure (BP) measurements, it has been claimed that such protection is BP-independent and mediated by a blockade of the direct tissue-damaging effects of angiotensin and/or aldosterone. BP radiotelemetry was performed for 8 weeks in ≈10-week-old male SHRsp who received a standard diet and either tap water (n = 10) or 1% NaCl to drink. Saline-drinking SHRsp were either left untreated (n = 12), received enalapril (50 mg/L) in drinking fluid (n = 9), or had subcutaneous implantation of time-release 200-mg pellets of aldactone (n = 10). The average systolic BP (mean±SEM) during the final 3 weeks was significantly higher (P < 0.05) in untreated saline-drinking (215±6 mm Hg) SHRsp but not aldactone-treated (198±4 mm Hg) or enalapril-treated treated SHRsp (173±1 mm Hg), as compared with tap water–drinking SHRsp (197±3 mm Hg). Histological renal damage scores at 8 weeks paralleled the BP in all groups, with an excellent correlation (r = 0.8, P < 0.001, n = 41). Moreover, a renal damage score of >5 was only observed in SHRsp whose average systolic BP during the final 3 weeks exceeded 200 mm Hg, indicating a threshold relation with BP. These data show that protection by renin-angiotensin-aldosterone system blockade in this model is BP-dependent and mediated by preventing the severe increases in BP seen in untreated salt-supplemented SHRsp and further underscore the limitations of interpretations based on conventional tail-cuff BP measurements. (Hypertension. 2003;41:201-206.)

Key Words: hypertension, renal • rats, stroke-prone SHR • nephrosclerosis • autoregulation

The stroke-prone spontaneously hypertensive rat (SHRsp) is a widely used model to investigate hypertensive target organ damage because of an enhanced susceptibility to develop stroke and renal damage as compared with its progenitor SHR strain.1–9 Both hypertension and target organ damage are markedly accelerated by salt supplementation.2–5,8 Nevertheless, the pathogenesis of such target organ damage is widely believed to be at least in part blood pressure (BP)-independent and mediated by the direct tissue-damaging effects of the renin-angiotensin-aldosterone system (RAAS).5,10–17 This is based on the fact that ACE inhibitors or angiotensin receptor blockers and more recently aldosterone receptor antagonists have been shown to markedly reduce the severity of renal damage and/or the incidence of stroke without significantly reducing BP. However, BP in such studies has only been measured intermittently with the tail-cuff methodology. Given the spontaneous, rapid, and often large BP fluctuations that are characteristically observed in conscious hypertensive rats, such methodology is inherently inadequate for an accurate assessment of the BP load, the quantification of the antihypertensive effects of pharmacological interventions, or the precise contribution of hypertension to renal damage, as has been clearly demonstrated in this and/or other models of renal damage.6–8,18–25 The present studies were therefore undertaken to examine the effects of RAAS blockade on continuous radiotelemetrically measured BP and the contribution of such BP effects to the observed renoprotection in salt-supplemented SHRsp rats.

Methods

Animals and Animal Care

The SHRsp were obtained from a colony transferred to Hines, Ill, from the colony maintained at the University of Michigan in Ann Arbor since 1981. Only male rats were used, and all were cared for in accordance with the Principles of the Guide for the Care and Use of Laboratory Animals (Department of Health, Education, and Welfare). They were housed in a constant-temperature room with a 12-hour light and 12-hour dark cycle as described below, and they had free access to a standard (1.05% NaCl) rodent chow (Purina) and drinking fluid (tap water or 1% NaCl).

Experimental Protocols

When the rats were ≈10 weeks old, they were anesthetized (sodium pentobarbital, 30 mg/kg body wt IP) and instrumented for radiotelemetric BP monitoring as previously described.5,18–20,24,25 The rats were housed individually in polycarbonate cages placed on radio
receivers. The LabPro System was used for data acquisition (Data Sciences International). Systolic BP was recorded every 10 minutes throughout the course of 1 week, with each value representing the average of BP readings during 50 to 60 heart beats in a 10-second interval (the rat heart rate is 300 to 400 beats/min). During the first 7 to 10 days, all rats received a standard diet and tap water, and the systolic BP during the last 3 days of this period was considered as the baseline BP. At the end of this period, the rats were either continued on tap water or were given 1% NaCl to drink. The saline-drinking SHRsp rats were left untreated or received either 50 mg/L enalapril in the drinking fluid or had a subcutaneous 200-mg, sustained time-release (3.3 mg/d) pellet of aldactone (Innovative Research of America). Systolic BP monitoring in these four groups was continued for the next 8 weeks. Rats were placed in metabolic cages for 24-hour urine collections for determination of proteinuria at baseline, at 4 weeks, and during the final 1 to 2 weeks before euthanasia and removal of the kidneys for histological analysis. Sodium and potassium excretion rates were also measured during the final collection. Blood was obtained at the time of euthanasia for serum potassium measurements. However, hemolysis in several samples precluded reliable measurements and data analysis. Protein concentration in the urine was determined by spectrophotometry, and sodium and potassium measurements were done by a flame photometer with an internal lithium standard.8,18–20

Histological Analysis

Rats were anesthetized (100 mg/kg body wt inactin IP), and the kidneys were perfusion-fixed at the ambient pressure as previously described.8,18–20 In brief, the kidneys were perfused with saline until the venous effluent cleared, followed by modified Karnovsky’s fixative for 10 minutes. Two transverse sections of the kidney through the papilla were postfixed in buffered formalin and embedded in paraffin. Sections (3 to 4 μm) were stained with hematoxylin and eosin and periodic acid Schiff. Glomerular and vascular injury were quantified separately in both of the sections from each kidney in a blinded fashion as described in detail previously.8,22 In brief, the glomerular damage score was the percentage of glomeruli exhibiting either (1) acute hypertensive injury; (2) segmental glomerular sclerosis; or (3) ischemic injury (globally shrunken glomeruli with collapsed capillary loops). The total number of vascular profiles exhibiting evidence of acute disruptive hypertensive injury was expressed per 100 glomeruli in the section as a vascular damage score. A composite renal damage score was calculated as the sum of the vascular damage score and the percent glomerular damage score.

Statistical Analyses

All data are expressed as mean±SEM. Statistical analysis was performed with ANOVA followed by Student-Newman-Keuls test or by Kruskal-Wallis nonparametric ANOVA followed by the Dunn multiple comparison test as appropriate.20 Linear regression analysis was used to calculate the slope of the relation between renal damage scores and BP.20 A probability value of <0.05 was considered significant.

Results

Body weight and protein excretion rates were not different between the groups at baseline (Table). Even at 4 weeks, statistically significant differences in proteinuria were not observed between the groups, as only mild increases in protein excretion were present in all groups except for 3 untreated saline-drinking SHRsp. As expected, sodium excretory rates were significantly greater in all saline-drinking rats as compared with the control tap water–drinking rats, but there was no difference between the 3 saline drinking groups. By contrast, no significant differences were present in potassium excretion rates between any of the groups. Similarly, no differences were observed between the groups in final body weight. However, 3 of the 10 untreated saline-drinking rats became severely hypertensive and/or symptomatic (progressive weight loss) and had to be euthanized for humane reasons, (2 during week 7 and an additional 1 during week 8 of saline drinking). The final data in these rats were obtained 2 to 3 days before euthanasia. Overt stroke was not observed. However, because histological examination of brain tissue was not performed, it is possible that these or some of the other salt-supplemented SHRsp rats that had very severe hypertension would have had histological evidence of stroke or may have had overt stroke if allowed to survive longer.

Figure 1 shows the course of weekly averages of systolic BP in the 4 groups. As can be seen, systolic BP increased progressively in the control tap water–drinking SHRsp rats, even in the absence of supplemental NaCl. However, this aging-associated BP increase was greatly and significantly exaggerated in untreated saline-drinking SHRsp. Concurrent treatment with aldactone essentially abolished the BP effects of saline drinking so that the systolic BP of the saline-drinking aldactone group did not differ significantly from that of the untreated tap water–drinking SHRsp rats. Strikingly, despite NaCl supplementation, concurrent treatment with enalapril not only prevented the increase in BP seen in untreated saline-drinking rats but even abrogated the BP increases with aging seen in untreated tap water–drinking control SHRsp rats.

Figure 2 summarizes these results and shows the overall average BP during the entire 8-week course for the 4 groups as well as the magnitude of the BP change in the 4 groups, measured as the difference in average systolic BP during the final week from that at baseline.

Figure 3 shows the differences between the 4 groups for parameters of renal damage assessed at the conclusion of the studies. Both proteinuria and histological renal damage were
renal damage and BP (increase in renal damage/mm Hg increase in BP) are observed. In animals whose average systolic BP during the final 3 weeks did not exceed 200 mm Hg (n = 25), the slope of the relation is relatively flat (0.06 ± 0.02), and although still significant (P < 0.02) has a correlation coefficient (r) of only 0.48. By contrast, in rats whose average systolic BP during the final 3 weeks exceeded 200 mm Hg (n = 16), the slope is significantly steeper (1.48 ± 0.24), with a much stronger correlation (r = 0.86, P < 0.0001). These relations are not affected substantively by the exclusion of the data from the 3 rats that were euthanized early (Figures 4A and 4B).

Discussion

The results of the present studies confirm the renoprotective efficacy of pharmacological RAAS blockade in salt-supplemented SHRsp that has been noted in previous studies.10–14 However, in contrast to those previous studies, the present data indicate that such protection can be entirely explained by the BP effects of these agents without invoking a blockade of the postulated direct BP-independent tissue-damaging effects of angiotensin or aldosterone.10–16 The dosages of the agents used in our study are similar to that used in previous studies, therefore the differences in BP observed with RAAS blockade between the present and previous studies is most likely attributable to the differences in the BP measurement methodologies. In contrast to the conventional isolated periodic tail-cuff BP measurements that have been used in previous studies,10–16 continuous BP radiotelemetry was used in the present study. In addition to the documented effects of physical restraint on BP during tail-cuff BP measurements,18,27 BP is characteristically labile in conscious animals, and such lability is further exaggerated in hypertensive states.6–9,18–25 Moreover, since rats are nocturnal, the BP effects of salt supplementation are most pronounced at night and as such are unlikely to be detected by conventional tail-cuff BP measurements.28
of isolated periodic tail-cuff measurements in assessing the ambient BP load and its relation to renal damage.

However, one difference between the present and the previous studies examining the effects of RAAS blockade in this model needs to be acknowledged. In contrast to the previous studies in which salt supplementation was performed on the background of a standard North American rodent chow, which minimizes overt stroke development, our studies do not directly address the issue of the mechanism of RAAS blockade–mediated protection against stroke. It is possible that in contrast to renoprotection, stroke protection by RAAS blockade is BP-independent. However, an exceedingly close correlation between stroke development and radiotelemetrically measured BP has been noted in non–salt-supplemented SHRsp fed a Japanese-style diet, similar to that seen for renal damage in the present study.

Given that salt supplementation would normally be predicted to suppress the RAAS, the striking success, particularly of enalapril, in reducing BP in saline-drinking SHRsp is somewhat unexpected. However, it is probably not too surprising in view of the reported lack of the expected renin and aldosterone suppression after salt supplementation in the SHRsp strain. Although the reasons for this nonsuppression of the RAAS remain unclear, the present data suggest that such nonsuppression may play a major role in the exquisite BP salt sensitivity of this strain as compared with its progenitor SHR strain, possibly through RAAS-mediated alterations in pressure-natriuresis relations. Furthermore, it is likely that as renal damage and injury to the preglomerular vasculature develops, altered intrarenal intravascular pressures and perfusion patterns result in a secondary stimulation of renin synthesis and release. Such an activation of intrarenal RAS, even if heterogeneous, is also likely to cause altered pressure natriuresis relations, further exacerbate hypertension, and thereby cause a vicious cycle to ensue. Such an interpretation is supported by the fact that at least as judged by differences in antihypertensive effects of RAAS blockade observed between our study and that in previous studies are due to the differences in dietary protocol rather than differences in BP measurement methodology. However, when BP radiotelemetry has been used in saline-drinking SHRsp fed a Japanese-style diet, sustained substantial antihypertensive effects of the ACE inhibitor benazepril and the angiotensin receptor blockers valsartan have been observed, similar to those seen in the present study.

Because of the use of the standard North American rodent chow, which minimizes overt stroke development, our studies do not directly address the issue of the mechanism of RAAS blockade–mediated protection against stroke. It is possible that in contrast to renoprotection, stroke protection by RAAS blockade is BP-independent. However, an exceedingly close correlation between stroke development and radiotelemetrically measured BP has been noted in non–salt-supplemented SHRsp fed a Japanese-style diet, similar to that seen for renal damage in the present study.

However, one difference between the present and the previous studies examining the effects of RAAS blockade in this model needs to be acknowledged. In contrast to the previous studies in which salt supplementation was performed on the background of the moderately potassium-deficient stroke-promoting Japanese style diet, only a standard North American rodent chow was used in the present study because of the focus of our investigations on renal damage, which in general tends to precede stroke development. Therefore, it is possible that the

![Figure 4. A, Correlation of histological renal damage score in individual SHRsp with their respective overall average systolic BP during the entire 8-week period of follow-up. Data points for rats that were euthanized before 8 weeks are indicated by arrow. Linear regression analysis: r=0.77, P<0.0001; slope 1.2±0.2, intercept 178 mm Hg, n=41. If the data of the 3 rats that were euthanized early are excluded (n=38), the strength of the correlation is reduced (r=0.56) but remains highly significant (P<0.0003). B, Correlation of histological damage scores in individual SHRsp with their respective average systolic BP during the final 3 weeks. Data points for rats that were terminated before 8 weeks are indicated by arrow. Linear regression analysis for SHRsp with an average systolic BP <200 mm Hg (n=25): r=0.48, slope 0.06±0.02; P<0.02, for SHRsp with an average systolic BP >200 mm Hg (n=16): r=0.86, slope 1.5±0.24; P<0.0001, and combined for all SHRsp (n=41): r=0.80, slope 0.84±0.01; P<0.0001. If the data of the 3 rats euthanized early are excluded (n=38), the strength of the correlation is reduced (r=0.67) but remains highly significant (P<0.0001). For rats with an average systolic BP >200 mm Hg after a similar exclusion of these data points: r=0.67, P<0.01 (n=13).](http://hyper.ahajournals.org/doi/10.1161/01.HYP.0000123686.74867.04)

![Figure 5. Course of systolic BP in an untreated SHRsp given a standard diet and 1% NaCl to drink after the first 7 days on tap water. Each point on the tracing represents average systolic BP during a 10-second interval, sampled every 10 minutes, 24 hours per day. Note extreme lability of BP, with fluctuations sometimes exceeding 100 mm Hg.](http://hyper.ahajournals.org/doi/10.1161/01.HYP.0000123686.74867.04)
proteinuria at 4 weeks, little evidence of renal damage was present even in the untreated saline-drinking rats at this time. The effects of aldosterone antagonism, although less impressive than that of enalapril, are probably mediated through qualitatively similar effects on the pressure-natriuresis relation and possibly sodium and potassium balance.33,34

The ability to measure BP accurately and continuously by radiotelemetry through the entire course in the present studies has also allowed a more precise characterization of the relation between BP and renal damage in saline-drinking SHRsp. Minimal damage was observed in rats whose average systolic BP did not exceed \( \approx 200 \) mm Hg during the final 3 weeks. By contrast, in rats that had more severe hypertension, florid, disruptive, and acute vascular and glomerular damage was observed in a pattern characteristic of malignant nephrosclerosis.35–38 Moreover, an extremely strong linear correlation was observed between BP and the severity of the observed renal damage. Such a pattern of relation between BP and renal damage, although not clearly demonstrated previously in this model, probably because of limitations of the tail-cuff methodology, is not unanticipated, given the postulated pathophysiology of malignant nephrosclerosis. Normally, the renal autoregulatory mechanisms in response to increases in systemic BP have proportionate glomerular vasoconstriction and thereby protect the renal microvasculature from hypertensive damage.37–41 Therefore, as long as the BP remains below a certain threshold (within the autoregulatory range) little damage is expected beyond thickening and remodeling of resistance vessels and possibly ischemic changes late in the course.37,38,41 However, when the BP exceeds a certain threshold (the autoregulatory capacity is exhausted), renal damage is predicted to result, with the severity of renal damage exhibiting a linear and proportional relation to the BP increases.41 Additionally, the histological pattern of acute disruptive injury to the preglomerular vessels and glomeruli (malignant nephrosclerosis) is characteristic of direct hypertension-mediated (barotrauma) damage.35–38,41–44 Therefore, given this barotrauma-driven pathogenesis of renal damage in models of malignant nephrosclerosis, the strong correlation observed between BP and renal damage in both treated and untreated SHRsp rats in the present study is not unexpected.

The precise BP threshold at which renal damage ensues may not, however, be fixed in a given strain or an individual animal. The rate at which increases in BP occur may be another additional and important determinant of the threshold for damage. Both experimental and clinical observations have suggested that more rapid increases in BP are associated with greater target organ damage than slower increases to comparable BP levels.35,37,38,44 Such differences have been attributed to adaptive changes in vascular structure that render it more resistant to acute disruptive hypertensive injury.35,37,38,44,45 Another potential explanation for this protective adaptation may be provided by the observations that suggest that both the lower and upper limits of autoregulation are reset and shifted to the right by chronic hypertension in both experimental animals and in humans.41,46,47 This may also account for the fact that in the aging non–salt-supplemented SHRsp, higher BP is usually seen before the development of malignant nephrosclerosis.3,23,30 Similarly, even salt-supplemented SHRsp treated with RAS blockade and slowly increasing BP with aging may be able to tolerate higher pressures than the threshold BP seen in the present study.11

Such a threshold relation between BP and renal damage has important implications with respect to the expected effectiveness of antihypertensive therapy in ameliorating renal damage in models of malignant nephrosclerosis. Even modest BP reductions to below the autoregulatory threshold that may not be detected by conventional BP measurements, are, nevertheless, likely to be successful in preventing target organ damage in such models. This is in contrast to other models such as diabetes and/or reduced renal mass, in which vasodilation and an impairment of the protective renal autoregulation results in an impaired buffering of BP increases and a linear relation between BP and renal damage across the entire hypertensive range.37,40,41,48 Moreover, the histological pattern of renal damage is expected to be dominated by glomerular rather than vascular damage in the absence of hypertension severe enough to cause vascular injury.41,48 Antihypertensive therapy is also less likely to be successful in ameliorating progressive glomerular damage in the absence of complete normalization of the systemic BP in such models.18–20,24,25,41,48 That such insights from these experimental animal models may be relevant clinically is indicated by similar qualitative observations in humans. Much greater success has been achieved clinically in preventing malignant nephrosclerosis and/or hypertensive stroke than in preventing the progression of chronic renal disease, both diabetic and nondiabetic.41,49

Perspectives

The present studies emphasize the limitations of conventional BP measurements in investigating hypertensive renal damage or in separating the relative contribution of BP-independent versus BP-dependent mechanisms in the renoprotection provided by pharmacological agents. Our data, using continuous BP radiotelemetry, show that renal damage in the saline-drinking SHRsp model of malignant nephrosclerosis shows a threshold relation with BP. Additionally, these data show that renoprotection by RAAS blockade in this model is primarily BP-dependent and is achieved by preventing the rapid and acute increases in BP above the threshold for renal damage that are observed in untreated saline-drinking SHRsp. Such observations do not exclude a role for the widely postulated RAAS blockade–mediated, BP-independent mechanisms but indicate that such effects are more likely to be demonstrable in models in which renal damage is less BP-dependent or in which hypertension is less RAAS dependent.

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