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Long-term AT₁ receptor blockade improves metabolic function and provides renoprotection in Fischer-344 rats

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Many normotensive rat strains exhibit increases in systolic blood pressure (SBP) (42), insulin resistance, body weight (13, 30, 31, 41), and serum leptin (30, 39) as they age. Similar changes occur in the aging human population, and a clustering of these factors is consistent with the metabolic syndrome (MetS) (22, 41a). MetS, a collection of cardiovascular risk factors, is an intermediate state between normal metabolism and Type 2 diabetes (22, 43). Clinical trials have shown that renin-angiotensin (ANG) system (RAS) blockade, either by ANG-converting enzyme (ACE) inhibitors (4, 57) or ANG II type 1 (AT₁) receptor blockers (17, 27), may substantially lower the risk for Type 2 diabetes in hypertensive subjects. However, the exact mechanism underlying this effect is unknown. Moreover, long-term ACE inhibition or AT₁ receptor blockade in rodents protects against most of these age-related changes, including the increase in blood pressure (5, 18), weight gain (11), and decline in cognitive, mitochondrial, cardiac, and renal function (19).

Circulating, cardiac, and intrarenal RASs are dissimilarly regulated in the aging rat (9, 21, 28, 29) and during short- and long-term RAS blockade (9, 29). Previous studies have suggested that plasma renin concentrations and kidney renin mRNA are decreased in aging rats (28) and associated with a decrease in plasma ANG peptides in aging Sprague-Dawley (SD) rats (42); plasma renin is decreased in aging humans (54). In contrast, there is an increase in intrarenal tissue ANG II with aging (3) as well as an increase in the excretion of ANG peptides (42), an indicator of activation of the intrarenal RAS. Cardiac ANG peptides are also elevated in aging animals (21). These observations, suggesting activation of the cardiac and intrarenal RAS during aging in the face of a decline in the circulating RAS, raise questions about the mechanisms underlying the beneficial effects of RAS blockade during aging.

The activation of the intrarenal RAS during aging occurs over the same time frame as many of the features of MetS, but it is not clear to what extent the different components of MetS are interdependent. The Fischer-344 (F344) rat represents an interesting model since insulin resistance and kidney damage occur without an increase in blood pressure during the aging process (53). Previous studies in normotensive rats have indicated that RAS blockade will prevent many age-related renal pathologies, including the increase in blood pressure that occurs with age (5, 18, 19). Furthermore, urinary ANG peptides remained low in animals treated long term (22 mo) with RAS blockers (29). However, the extent to which the protective effects on the kidney are a result of the maintenance of low blood pressure are unclear. Therefore, we assessed the time course of changes in indexes of metabolic function, blood pressure, and activation of the intrarenal RAS in older animals compared with young F344 rats and in the presence of long-term (1 yr) RAS blockade to provide information on the interdependence of many of the features of MetS.

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RESULTS

Mean SBP was not different between older control and treated rats, but both were significantly lower ($P < 0.001$) than the young group (Fig. 1). We observed similar food intake between the two older groups of rats, and both were statistically higher than that in young rats ($P < 0.05$). While both older groups gained weight over the course of the study, the older treated group maintained a significantly lower body weight than the older control group. *$P < 0.05$ vs. young rats; $+P < 0.001$ vs. young rats; $\#P < 0.001$ vs. control rats.

As expected in this model of aging, there was a significant increase ($P < 0.001$) in serum leptin in older control rats relative to the young and treated groups with a similar trend for serum insulin (Fig. 4). Serum glucose in the older control group was significantly higher ($P < 0.001$) than the young group, whereas values in the treated group were not different from the younger group (Fig. 4). The older control group had a significantly lower QUICKI value and higher HOMA value compared with the young group, and there were no differences between young and treated rats (Fig. 5). This suggests that older control animals have insulin resistance and that blockade of the AT1 receptor may delay this from occurring.

Plasma ANG I ($P < 0.05$) and ANG II ($P < 0.001$) were higher in older treated animals than in young or older control animals (Fig. 6). However, plasma ANG-(1-7) was not significantly different among the three groups (data not shown). Creatinine excretion was similar among the groups (young: $23 \pm 1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ vs. control: $19 \pm 2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ vs. treated: $25 \pm 3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). Serum creatinine was not...
different among the groups (young: 0.5 ± 0.0 mg/dl vs. control: 0.6 ± 0.1 mg/dl vs. treated: 0.7 ± 0.1 mg/dl). GFR, calculated from serum and urinary creatinine levels, was similar among the groups (young: 1.0 ± 0.2 ml/min vs. control: 1.2 ± 0.3 ml/min vs. treated: 1.1 ± 0.3 ml/min). Urinary excretion of ANG I \((P < 0.001)\), ANG-(1-7) \((P < 0.01)\), and ANG II \((P < 0.05)\) was greater in the older control group compared with the young group. Urinary ANG peptide excretion in the older treated group was not different from the young group (Fig. 7). The urinary ANG-(1-7)-to-ANG I ratio revealed that the young group had a significantly higher ratio compared with the two older groups (young: 4.4 ± 1.1 vs. control: 0.95 ± 0.16 vs. treated: 2.5 ± 0.64, \(P < 0.05\)). Protein excretion was markedly elevated in older rats \((P < 0.05)\) compared with young animals, and this was completely prevented in AT\(_1\) receptor-blocked animals (Fig. 8).

**DISCUSSION**

Long-term AT\(_1\) receptor blockade prevented the increases in insulin, leptin, and glucose observed in older F344 rats associated with reduced weight gain. It is important to note that there were no significant differences in blood pressure between the older control and treated groups, associated with the improvements in metabolism. Proteinuria, a marker of renal damage, was completely prevented in older treated rats compared with older controls, and, in treated rats, ANG peptide excretion, an indicator of intrarenal RAS activity, was maintained at levels not different from those seen in younger animals. The results are consistent with previous studies showing protective effects of long-term RAS blockade on age-related changes in kidney and metabolic function (5, 10, 11, 19, 29, 40, 52) in rat strains where aging is accompanied by increases in SBP.

There are reports that RAS blockade improves components of, or reduces the onset of, MetS and Type 2 diabetes (4, 17, 23, 57). Our study demonstrates the advantageous effects of RAS blockade independent of blood pressure-lowering actions in accordance with previous studies (48, 50). However, the precise mechanisms underlying the beneficial effects are not entirely known, and mechanisms may involve actions at the skeletal muscle (24, 48), brain, and autonomic nervous system (30, 31) or other organs such as the liver and pancreas (18, 19, 51).
As expected, AT₁ receptor blockade led to increases in plasma ANG I and ANG II, consistent with previous findings (29) and the known feedback mechanism of the system. This implies that the dosing of the blocker was effective, even with the absence of a blood pressure-lowering effect in these nor-motensive animals. Plasma ANG-(1-7) levels were not different between the two older groups of animals. Although a previous study (29) has shown an increase in the levels of this peptide with AT₁ receptor blockade or ACE inhibition, the failure to increase ANG-(1-7) levels in the circulation may result from the reported decline in neprilysin activity in the circulation and kidney of older animals (44). Interestingly, plasma levels of ANG I and ANG II were not lower in older F344 rats relative to younger animals. This was a surprising finding since older F344 rats exhibited proteinuria with increased age, and renal damage has been suggested to contribute to the loss of renin-producing cells in the kidney. However, there was no reduction in GFR in older rats in this study, and the reduction in circulating levels of ANG II in other models of aging occurs coincident with the increase in SBP (31) rather than the onset of kidney damage as assessed by proteinuria or activation of the intrarenal RAS. As F344 rats do not have an increase in blood pressure, the maintenance of normal circulating levels of RAS peptides may reflect the absence of a pressure-mediated inhibition of renin release in these animals.

ANG II excretion was lower in older treated animals relative to older controls, suggesting a role for increased intrarenal ANG II production in the deleterious changes seen in renal and metabolic function with aging. The lower level of ANG II excretion in treated animals is similar to previous studies of long-term RAS blockade in older animals (29) or tissue ACE knockout mice (38). Our findings further suggest that the systemic and kidney RASs are differentially regulated, given increases in circulating peptides in this and a previous study (29) persisting for the duration of treatment while urinary peptides remain low. The decrease in ANG peptide excretion in the treated group may reflect tubular RAS suppression (29), consistent with the differential regulation of the systemic and kidney RASs (9, 38). GFR was similar among the groups. F344 rats develop age-related nephropathy while serum creatinine levels do not change until the latter stages of nephropathy (8, 16, 35, 56). However, F344 rats develop proteinuria, which may manifest as protein overload nephropathy, and there may be slight changes in the glomeruli in the first couple of stages (grades) of renal disease (8, 56). Proteinuria is decreased in the presence of AT₁ receptor blockers (37, 47): our study suggests that this may result from reduced activation of the intrarenal RAS as well as effects to correct hyperinsulinemia and elevated serum glucose.

Serum glucose in the older control group was significantly higher than the young group and the profile for serum insulin was similar, suggesting insulin resistance is present at this time point in older control rats. The older treated group was indistinguishable from the young group. QUICKI and HOMA values were significantly different in the older control group
Compared with the young group, suggestive of insulin resistance, which is a known feature of the F344 rat at this age. Both are established methods used to assess insulin resistance and correlate well with the glucose-clamp technique (7, 25, 32). Rats with decreased glial angiotensinogen (ASrAogen rats) have lower levels of glucose and insulin during aging compared with age-matched SD rats associated with reduced sympathetic nervous system activity, suggesting a role for glial-produced ANG II in the metabolic impairments that occur during aging (30). Insulin and leptin interact in the rat hypothalamus at the phosphatidylinositol 3-kinase (PI3K) and MAPK pathways to decrease feeding and maintain an appropriate metabolism (12). Aged Fischer × Brown Norway rats on ad libitum and restricted diets showed a greater tendency to develop insulin resistance in association with greater adiposity compared with younger animals, suggesting that abdominal obesity during aging may contribute to insulin resistance (13). While the exact mechanisms of the beneficial effects of RAS blockade on insulin and glucose are not known, it may be partly due to the decreased actions of ANG II at the kinase pathways as well as actions in the central nervous system on autonomic pathway controlling insulin and glucose metabolism.

In the F344 rat, aging is associated with an increase in serum leptin (39), as is the case with other rat strains (30). Our study confirms this as there was a significant increase in serum leptin in older control rats relative to young and L-158,809-treated animals. Leptin regulates weight loss by suppressing the appetite and food intake and enhancing energy expenditure (36, 55). Food intake was similar between the two older groups of rats, and both were statistically higher than that in young rats, but the treated group maintained a significantly lower body weight than the control group. This is consistent with a report (55) showing that leptin is primarily released from adipose tissue and serves as an index of the amount of body fat mass. We did not measure physical activity in this study, and it is possible that changes in activity levels may accompany the overall improvement in metabolic function. In ASrAogen rats, leptin levels and body weight are lower and food intake higher than in SD and (mRen2)27 rats (30), suggesting that interruption of the brain RAS may be part of the beneficial effects seen with RAS blockade. Leptin, like insulin, via actions in the brain, activates the sympathetic nervous system. Activation of the sympathetic nervous system can lead to a reduction in GFR and salt and water retention. This may lead to activation of the renal RAS and kidney damage. F344 rats are known to have leptin and insulin resistance (33, 34), as appears to be the case with our animals. Thus, elevated levels of insulin and leptin may also contribute to the declining renal function that occurs during aging.

L-158,809 is a competitive and specific AT1 receptor antagonist (14, 49), and, in this study, it decreased indexes of MetS and Type 2 diabetes after treatment for 1 yr, similar to the
effects of other RAS antagonists or inhibitors and consistent with what has been shown in previous studies (18, 19, 24, 29, 40, 48, 52). The mechanisms behind the antidiabetic effects are not well understood, and there are reports that a certain subset of AT1 receptor antagonists may have such effects by acting as a partial agonist of peroxisome proliferator-activated receptor-γ (PPAR-γ) (6, 45, 46). Other AT1 receptor antagonists tested, including valsartan, had no effect on PPAR-γ. However, in the Valsartan Antihypertensive Long-term Use Evaluation trial (27), valsartan reduced new onset diabetes, suggesting that the antidiabetic effect of this AT1 receptor antagonist is mediated indeed through the AT1 receptor, not necessarily by interactions with PPAR-γ. Valsartan treatment in KK-Ay mice, a model of Type 2 diabetes, did not affect SBP but improved insulin sensitivity, P3K activity, and glucose transporter 4 translocation while decreasing TNF-α expression in skeletal muscle (48). It is also important to note that interruption of the RAS at levels other than AT1 receptor blockade has proven beneficial in reducing the new onset of diabetes (57).

We conclude that aging is associated with activation of the intrarenal RAS independent of age-related increases in SBP. Moreover, the decline in circulating RAS during aging may be a consequence of the age-related increase in SBP, since animals without the increase in SBP have no reduction in ANG II in plasma, despite the development of MetS. Long-term blockade of the RAS reduces the onset of indexes of MetS and kidney damage that occur during aging in F344 rats independent of blood pressure-lowering effects. Moreover, intrarenal RAS activity was decreased in the presence of the blockade, suggesting that local tissue RAS blockade may contribute to the protective effects on metabolic, kidney, and cardiovascular function during aging. Further investigation is needed to determine the full complement of mechanisms and other tissue RSAs that may be involved in the deleterious changes that occur in the normotensive population as they age and may contribute to the beneficial effects of RAS blockade.

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GRANTS

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