Protective Role of Angiotensin II Subtype 2 Receptor in Blood Pressure Increase in Obese Zucker Rats

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Abstract—Earlier, we reported that there was an increase in angiotensin II type 2 (AT$_2$) receptor expression in the renal proximal tubule, and selective activation of the AT$_2$ receptor by AT$_2$ agonist inhibits Na,K-ATPase activity in the proximal tubules and increases urinary Na excretion in obese Zucker rats. We hypothesized that the AT$_2$ receptor has a protective role against blood pressure increase in obese Zucker rats. To test this hypothesis, we treated obese Zucker rats with the AT$_2$ receptor antagonist PD123319 (PD; 30 μg/kg per minute) using osmotic pumps. Age-matched lean rats and vehicle-treated obese Zucker rats served as controls. On day 15 of the treatment with PD, arterial blood pressure was measured by cannulation of the left carotid artery under anesthesia. Control obese rats exhibited higher mean arterial pressure (122.0 ± 3.4 mm Hg) compared with lean control rats (97.0 ± 4.8 mm Hg). The PD treatment of obese rats raised mean arterial pressure further by 13 mm Hg. The plasma renin activity was significantly increased in the PD-treated obese compared with control-obese or lean rats. Western blot analysis revealed that the PD treatment in obese rats caused an ∼3-fold increase in the renin expression in the kidney cortex but had no effect on the expression of the cortical angiotensin II type 1 and AT$_2$ receptors. The present study suggests that the renal AT$_2$ receptors provide a protective role against blood pressure increase in obese Zucker rats, and this protective effect, in part, could be because of the ability of the AT$_2$ receptors to keep the kidney renin expression low in obese rats.

Key Words: angiotensin II receptors ■ renin ■ kidney ■ obesity ■ hypertension

Of the angiotensin (Ang II) receptors, the Ang II type 1 (AT$_1$) receptor is known to mediate most of the Ang II actions, including vasoconstriction, antinatriuresis, aldosterone secretion, increased sympathetic outflow, and cellular growth/proliferation. Abnormal regulation and function of the AT$_1$ receptor contribute to development and maintenance of various forms of hypertension. On the other hand, the Ang II type 2 (AT$_2$) receptor is suggested as a functional antagonist of AT$_1$ receptors. However, the AT$_2$ receptor has been implicated in cardiovascular functions such as vasodilatation, depressor effect on blood pressure, and cardioprotection. The AT$_2$ receptor is involved in blood pressure regulation in various animal models, such as the renal wrap hypertension model, AT$_2$ knockout mice, and diet-induced hypertension. After the discovery of the AT$_2$ receptor in various parts of the kidney, including in tubules, attempts have been made to establish a link among the renal AT$_2$ receptor, renal Na excretion, and blood pressure regulation. The AT$_2$ receptor null mouse develops hypertension associated with an inhibition in pressure natriuresis. Rats with selective intrarenal reduction of the AT$_2$ receptors produced by antisense oligonucleotides exhibit increased blood pressure. However, these studies involved either genetic manipulations of the AT$_1$ receptor or blockade of the AT$_1$ receptor and subsequent infusion of an AT$_2$ agonist or antagonist to produce acute changes in the arterial blood pressure of these animal models. Although these studies suggest a role for AT$_2$ receptors in blood pressure regulation, there exists a gap in our understanding of the role of AT$_2$ receptors in long-term blood pressure control.

Recently, we have reported an increase in the AT$_2$ receptor expression in the kidney cortex, which, on activation, inhibits the Na,K-ATPase activity in the proximal tubules of obese Zucker rats. The obese Zucker rat is a model of insulin resistance and develops hypertension. An impaired pressure natriuresis is believed to be a cause of hypertension in obese Zucker rats and other animal models of obesity. Hyper-tension in obese Zucker rats is associated with an enhanced renal AT$_1$ receptor function. We hypothesized that, whereas enhanced AT$_1$ receptor function may contribute to increased renal Na retention and hypertension, AT$_2$ receptor-mediated inhibition of Na,K-ATPase and enhanced renal Na excretion may be beneficial by limiting the blood pressure increase in obese rats. Therefore, to test this hypothesis, we measured blood pressure in obese Zucker rats after 2 weeks of treatment with a selective AT$_2$ receptor antagonist.

Materials and Methods

Animal Models and Drug Treatment

Male obese and lean Zucker rats (10 to 11 weeks of age) were purchased from Harlan (Indianapolis, Ind). Animals were housed...
in the University of Houston animal care facility. Food and water were supplied ad libitum, and their daily consumption was recorded. The institutional animal use and care committee approved the animal experimental protocols. Body weight of the animals was recorded at the start and the end of the drug treatments. For drug treatment, the obese rats were divided into 2 subgroups (n = 6 to 7 per group), i.e., the obese-control rat group and the obese-PD123319 (PD) rat group. The obese-control group was treated with normal saline as vehicle, and the obese-PD group was treated with PD (30 μg/kg per minute), an AT₁R antagonist, for 2 weeks using Alzet osmotic pumps implanted SC (model 2ML-2, Alza). Lean Zucker rats (n = 6) served as a normal control. Because of a limited supply of PD, only obese rats were treated to investigate the effect of AT₂ blockade on the blood pressure.

General Parameters
During the course of treatment, daily food and water intake was recorded. On day 12, blood glucose was measured using a Glucometer (AccuChek-Compact, Roche Diagnostics) after 6 hours of fasting. On day 13, the rats were placed in metabolic cages for 48 hours. After an initial 24 hours of acclimatization, urine was collected over the next 24-hour period. Urinary sodium was measured using a flame photometer (Cole Parmer, model 2655-10).

Blood Pressure and Heart Rate Measurements
On day 15 of the treatment period, the rats were anesthetized using Inactin (100 to 150 mg/kg IP) for measuring blood pressure. After tracheotomy, the right carotid artery was cannulated with PE10 and attached to a data acquisition system (PolyView, Grass Ins), via Grass pressure transducer PT300. Heart rate and blood pressure were continuously monitored. After 30 to 45 minutes of a stabilization period, the systolic and diastolic blood pressures and heart rate were recorded. At the end of the blood pressure measurement, blood sample was collected for plasma renin activity (PRA). Kidneys were excised, patted dry to weight, and stored frozen at −80°C for measuring the expression of AT₁ receptors, AT₂ receptors, and renin in the kidney cortex.

Western Blotting
The expression of AT₁ receptor, AT₂ receptor, and renin in the kidney cortex of various rat groups was determined by Western blotting. For this purpose, the kidney cortices were homogenized in the buffer containing (in mmol/L): Tris 50, EDTA 10, PMSF 1, and a mixture of protease inhibitors (aprotinin, calpain inhibitors, leupeptin, pepstatin, and trypsin inhibitor). Proteins in the homogenates were determined by BCA method using a kit (Pierce). Equal amounts of protein (30 μg for AT₁, 60 μg for AT₂ receptors, and 30 μg for renin) from various rat groups were subjected to SDS-PAGE and electroblotting onto Immobilon P (blot). The blot was incubated with primary polyclonal antibodies for the AT₁ receptor, AT₂ receptor, or renin. After the incubation with the primary antibodies, the blots were incubated with horseradish peroxidase–conjugated anti-IgG, and enhanced chemiluminescence substrates were obtained from Alpha Diagnostics Intl. All of the other chemicals used in the study were purchased from Sigma Aldrich.

Statistical Analysis
Results were expressed as means±SEMs. All of the data were subjected to statistical analyses using GraphPad Prism 4. One-way ANOVA and Student t tests were performed to determine the significance of differences between different groups. Statistical significance was set at P < 0.05.

Results
General Parameters
Compared with lean Zucker rats, obese Zucker rats had greater body weight and consumed more food and water. The kidney weight of obese rats was higher compared with those from lean rats. The PD treatment did not affect the food intake in obese animals. Plasma glucose was greater in obese than in lean rats, and the PD treatment modestly, but statistically insignificantly, increased fasting blood glucose in obese rats. Compared with control obese rats, PD-treated obese rats exhibited higher PRA (Table).

The urinary volume and urinary Na excretion over a 24-hour period in obese rats was greater than in lean rats. The PD treatment caused a significant increase in urinary volume but had no effect on urinary Na in obese rats. It should be noted that the extent of increase in urinary volume was similar to the extent of increase in water intake in PD-treated obese rats (Table).

Effects of PD on Blood Pressure
Compared with lean rats, obese Zucker rats exhibit higher systolic and diastolic blood pressures (lean: 114 ± 5/88 ± 5 mm Hg versus obese: 135 ± 5/105 ± 6 mm Hg; P < 0.05). The 2-week treatment of obese Zucker rats with PD caused a significant increase by 13 mm Hg in mean arterial blood pressure (Figure 1A). The heart rates were similar in lean and obese rats and were not affected by the PD treatment (control-obese: 389 ± 12 bpm versus PD-obese 391 ± 7 bpm; Figure 1B).
Effect of PD on the Expression of AT₁ and AT₂ Receptors and Renin in Kidney Cortex

Western blot shows the presence of the AT₂ receptor in multiple bands (44 and 39 kDa) in the renal cortex. These multiple bands of the AT₂ receptor are likely attributable to various degree of glycosylation, as reported earlier. Densitometric analysis of the AT₂ bands revealed that the cortical AT₂ receptor expression was significantly elevated in obese compared with lean rats, as reported earlier. The PD treatment did not affect either the AT₂ receptor (Figure 2A) or the AT₁ receptor (Figure 2B) expression in the kidney cortex of obese rats. The cortical AT₁ receptor expression was modestly but significantly greater in the obese rat compared with lean Zucker rats (Figure 2B). These AT₁ receptor expression data are consistent with earlier reports.

Western blot demonstrates the presence of renin as ≈41 kDa band in the renal cortex. Densitometric analysis of the bands suggests that the cortical renin expression in obese rats was significantly lower compared with lean rats, which is consistent with an earlier study. The PD treatment of obese rats caused a significant increase in the cortical renin expression (Figure 3).

Discussion

In the present study, we demonstrated the protective role of the AT₂ receptor in long-term blood pressure regulation in obese Zucker rats. Treatment of obese Zucker rats with the AT₂ receptor antagonist PD for 2 weeks caused a significant elevation in blood pressure. The PD treatment did not affect the expression of the AT₂ receptor or the AT₁ receptor in the kidney cortex. However, the PD treatment of obese rats caused an increase in the PRA and the kidney renin expression.

Earlier we showed that the AT₂ receptor expression in the proximal tubules and renal cortical membranes is increased in obese compared with lean Zucker rats. A selective activation of the AT₂ receptors promotes natriuresis via a tubular mechanism, possibly by inhibiting Na⁺-K⁺-ATPase activity. The obese Zucker rat is a model of insulin resistance that exhibits hyperglycemia and high blood pressure. Impaired renal function and, consequently, abnormal renal Na handling are believed to be factors that contribute to the development of hypertension in this animal model. Based on the enhanced renal AT₂ receptor expression and natriuretic function in obese rats, we predicted a compensatory and protective role of the AT₂ receptor against blood pressure increase in obese Zucker rats. Earlier studies using a genetic manipulation of the AT₂ receptor, including the selective renal AT₂ receptor knockout approach, have suggested the role of the

Figure 1. A, Mean arterial blood pressure and (B) heart rate of lean, obese vehicle, and obese Zucker rats treated with PD for 2 weeks. *Significantly different compared with lean rats. †Significantly different compared with obese control (1-way ANOVA followed by Neuman-Keuls test; P<0.05; n=5 rats in each group).

Figure 2. A, AT₂ receptor expression and (B) AT₁ receptor expression in the kidney cortex of lean, obese vehicle, and PD-treated obese rats. Top, Representative Western blots for AT₂ and AT₁ receptor expression. Bottom, Bar graphs represent the densities of AT₂ and AT₁ receptor bands normalized with β-actin and GAPDH, respectively, as loading controls. Values are represented as means±SEM. *Significantly different from lean rats (1-way ANOVA; P<0.05; n=5 in each group).
AT2 receptor in blood pressure regulation. In our study, we used a direct pharmacological approach to test the role of the AT2 receptor in the long-term blood pressure control in a disease animal model. The 2-week treatment with the AT2 antagonist clearly elevated systolic and diastolic blood pressures in obese Zucker rats, suggesting a protective effect.

Because treatment with PD was systemic, the precise mechanism responsible for BP increase by PD treatment is not known. It could be the global blockade of AT2 receptors, as well as the unopposed action of AT1 receptors in the central and peripheral organs/tissues, that contributed to the BP increase by PD treatment. The BP increase in PD-treated rats was also associated with water intake and proportional urinary excretion. Although the increase in water intake in the present study may be related to the blockade of the central AT2 receptor, the role of the central AT2 receptors in thirst is not clear. Some studies have suggested that AT2 receptors are not involved in thirst regulation, whereas other studies reported that the AT2 receptor may have a role in thirst in response to water deprivation. Higher blood pressure could be responsible for an increase in diuresis in the PD-treated obese rats. Another plausible explanation for the increase in water intake and urinary volume could be related to a modest increase in blood glucose by PD treatment of obese rats, which already are hyperglycemic compared with lean rats. Although the increase in fasting blood glucose did not reach statistical significance in PD-treated obese rats compared with control obese, the nonfasting blood glucose (measured on day 15 of the treatment) was significantly higher, at 160±10 mg/dL in PD-treated compared with 136±24 mg/dL in control-obese rats. The positive relationship among hyperglycemia, water consumption, and polyuria is well known. However, interesting observation is that the blockade of AT2 receptors contributed to hyperglycemia in obese rats. This could be because of an enhanced action of AT1 receptors and/or inactivation of AT2 receptors affecting insulin sensitivity in PD-treated rats. There is evidence implicating AT1 receptors in the development of insulin resistance in obese rat models and in humans. On the other hand, AT2 receptors have been shown to stimulate peroxisome proliferation activator receptor-γ in PC12 cells. The peroxisome proliferation activator receptor-γ is a transcription factor known to enhance insulin sensitivity. If AT2 receptors are linked to peroxisome proliferation activator receptor-γ stimulation in insulin-dependent tissues, such as muscles and adipose tissue, the AT2 receptor antagonism would potentially affect insulin sensitivity. Therefore, it is possible that blocking of the AT2 receptor and unopposed action of the AT1 receptors by PD treatment contributed to insulin resistance, further elevating blood glucose, which, in turn, induced thirst and increased the urinary excretion. This notion warrants further systematic study. Earlier, it was shown that AT2 receptor knockout in mice causes a shift in pressure-natriuresis and an increase in blood pressure. Whether BP elevation by long-term pharmacological blockade of the AT2 receptor, as seen in our present study, is a consequence of a shift in pressure natriuresis is yet to be investigated. We found that PD treatment caused a modest increase in PRA but a profound increase in kidney renin expression. Higher Ang II in the circulation and in the kidney and subsequent unopposed function of the AT1 receptor could have a differential role in regulating blood pressure in obese animals. However, the enhanced contractile response mediated by the AT1 receptor in obese rats might have been compensated by higher endothelial AT1 receptor and endothelial NO synthase expression, as shown in earlier studies. Therefore, it is unlikely that the vascular AT1 receptor might have contributed to the blood pressure increase in PD-treated obese rats. Higher kidney renin expression and unopposed action of tubular AT1 receptor function might have contributed to the blood pressure increase in PD-treated rats. This notion is consistent with other studies suggesting abnormal tubular handling of Na as the cause of hypertension in this and other animal models of obesity. The AT2 receptors have been implicated in inhibiting the renin synthesis. An increase in the cortical renin expression in PD-treated obese rats is a remarkable observation, suggesting that the AT2 receptor maintains kidney renin expression at a lower level, and provides a protective and compensatory role in limiting blood pressure increase in obese rats. Because the dose of PD used in the present study, when given acutely, does not affect BP, it further supports the notion that long-term blocking of the AT2 receptor may reset the blood pressure–raising mechanisms. Because this study does not include experimental protocols to determine the renal function parameters, such as glomerular filtration rate, blood flow, and fraction of sodium excretion, a definite role of the kidneys in the blood pressure increase by PD treatment of obese rats cannot be established. Some investigators have reported a decrease in the renal expression of AT2 receptors in diabetic animal models. Contrary to these reports, we found enhanced renal AT2 receptor expression as reported earlier and in the present study. Our findings are supported by other reports, showing that AT2 receptor expression is increased in the human...
diabetic kidney and in the diabetic rat aorta. Reasons for the discrepancy in AT2 receptor expression could be because of the difference in renal preparations, strain of rats, and the methods of AT2 receptor measurements. In our studies, we measured AT2 receptor expression by Western blotting (and RT-PCR; unpublished data in proximal tubules) in the isolated proximal tubules and purified basolateral and brush-border membranes prepared from the kidneys of either Zucker rats or streptozotocin-treated Sprague-Dawley rats. In addition, the increase in AT2 receptor expression was supported by the enhanced AT2 receptor functions, in terms of Na,K-ATPase inhibition in the proximal tubules, urinary sodium excretion, and vascular tone in these animals models. On the other hand, Webb et al measured AT2 receptors by Western blotting in glomeruli and by immunostaining in the kidney from streptozotocin-treated rats. Bonnet et al measured AT2 receptors by RT-PCR, immunostaining, and autoradiography in streptozotocin-treated spontaneously hypertensive rats and Wistar-Kyoto rats. These investigators did not extend the studies to demonstrate whether a reduction in AT2 receptor expression was associated with a reduction in AT2 receptor-mediated functions in their animal models.

Protective effects of the AT2 receptor against various pathophysiological conditions have been documented. For example, treatment with an AT2 receptor antagonist caused an increase in blood pressure in the renal-wrap hypertensive rats. In another study, cardiac-specific overexpression of the AT2 receptor provides protection against AT1 receptor-mediated pressure and chronotropic effects. The AT2 receptor provides protection against NO-nitro-L-arginine methyl ester–induced cardiac hypertrophy and fibrosis. In brief, our study demonstrates that long-term blockade of the AT2 receptor results in an increase in blood pressure in obese Zucker rats, suggesting a protective and possibly compensatory role of the AT2 receptor in blood pressure increase in this animal model. This AT2 receptor role could be important in light of the reports showing that the various hormone and enzyme inhibitors are used for improving renal function in diabetes mellitus and treating hypertension. The preference for one over the other or a combination of both is based on the idea that AT1 receptor blockers selectively block AT1 receptors and leave AT2 receptors unopposed to function, whereas angiotensin-converting enzyme inhibitors reduce Ang II production, leading to attenuation of function of both the AT1 and AT2 receptors. The present study supports the notion that a beneficial role for AT2 receptors in protecting against blood pressure elevation should be a part of modalities for treating hypertension.

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Disclosures
None.

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