Regulation of the renal thiazide-sensitive Na-Cl cotransporter, blood pressure, and natriuresis in obese Zucker rats treated with rosiglitazone

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Khan, Osman, Shahla Riazi, Xinqun Hu, Jian Song, James B. Wade, and Carolyn A. Ecelbarger. Regulation of the renal thiazide-sensitive Na-Cl cotransporter, blood pressure, and natriuresis in obese Zucker rats treated with rosiglitazone. Am J Physiol Renal Physiol 289: F442–F450, 2005. First published April 5, 2005; doi:10.1152/ajprenal.00335.2004.—Previously, we showed an increase in protein abundance of the renal thiazide-sensitive Na-Cl cotransporter (NCC) in young, prediabetic, obese Zucker rats relative to lean age mates (Bickel CA, Verbalis JF, Knepper MA, and Ecelbarger CA. Am J Physiol Renal Physiol 281: F639–F648, 2001). To test whether this increase correlated with increased thiazide sensitivity (NCC activity) and blood pressure, and could be modified by insulin-sensitizing agents, we treated lean and obese Zucker rats (9 wk old) with either a control diet or this diet supplemented with 3 mg/kg body wt rosiglitazone (RGZ), a peroxisomal proliferator-activated receptor subtype γ agonist and potent insulin-sensitizing agent, for 12 wk (n = 9/group). The rise in blood pressure, measured continuously by radiotelemetry, was significantly blunted in the RGZ-treated obese rats. Similarly, blood glucose and urinary albumin were markedly decreased in these rats. RGZ-treated rats whether lean or obese excreted a NaCl load faster but excreted less sodium in response to hydrochlorothiazide, applied as a novel in vivo measure of NCC activity. Obese rats had increased renal protein abundance and urinary excretion of NCC; however, this was not significantly reduced by RGZ (densitometry in cortex homogenate – %lean control): 100 ± 9, 93 ± 4, 124 ± 9, and 141 ± 14 for lean control, lean RGZ, obese control, and obese RGZ, respectively. Subcellular localization, as evaluated by confocal microscopy and immunoblotting following differential centrifugation, of NCC was not described (4, 28), to continuously measure blood pressure (Data Sciences International, St. Paul, MN). Rats were housed singly in microfilter-topped, plastic cages with a normal 12:12-h light-dark cycle according to protocols approved by the Georgetown Animal Care and Use Committee. After a week of baseline blood pressure recording (at about 9 wk of age), nine rats from each body type were randomly assigned to either a control diet (Purina 5001 Rodent chow, Purina Mills, St. Louis, MO) in agar with 70% water or the same base diet containing the peroxisomal proliferator-activated receptor subtype γ (PPARγ) agonist, rosiglitazone (RGZ), an insulin-sensitizing agent. Our goal was to determine the effects of insulin resistance on blood pressure, as measured by radiotelemetry, pressure-natriuretic capability, as assessed by acute NaCl load test, thiazide sensitivity, and the regulation of NCC.

METHODS

Animals, study design, and blood pressure monitoring. Thirty-six male Zucker rats (18 lean and 18 obese) were obtained from Charles River Laboratories (Wilmington, MA) at 6 wk of age. Twenty-four were implanted with radiotelemetry transmitters, as previously described (28), to continuously measure blood pressure (Data Sciences International, St. Paul, MN). Rats were housed singly in microfilter-topped, plastic cages with a normal 12:12-h light-dark cycle according to protocols approved by the Georgetown Animal Care and Use Committee. After a week of baseline blood pressure recording (at about 9 wk of age), nine rats from each body type were randomly assigned to either a control diet (Purina 5001 Rodent chow, Purina Mills, St. Louis, MO) in agar with 70% water or the same base diet containing 0.0145 mg of RGZ incorporated per gram of diet (wet wt). This resulted in an approximate dose of 3 mg/kg body wt –1 ·day –1 of RGZ in the treated rats. Rats were weighed weekly and fed diets and received water ad libitum. Urine was collected periodically in metabolic cages for measurement of sodium, aldosterone, albumin (Nephrat kit, Exocell, Philadelphia, PA), and NCC. Rats were fed diets for 12 total wk. NaCl load test. To assess natriuretic capability, after 9 wk on the diet, all rats were gavaged with 1.9 ml of 0.9% NaCl/mol urine creatinine excreted daily (to adjust for body size). Three urine collections were obtained, 0–1 h, 1–2 h, and 2–4 h, and measured for sodium (ion-selective electrode, EL-ISE, Beckman, Fullerton, CA). Thiazide response test. As a measure of NCC activity, sodium excreted in response to hydrochlorothiazide (HCTZ) was measured at 8 wk. Twenty-four-hour baseline urine was collected with the lean rats given only 70 g of feed (wet wt) and obese rats 110 g (an amount

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approximating 75% of their ad libitum intakes) to produce slight underfeeding and thus standardize sodium intakes (within body types). Rats consumed all feed given. In the morning, rats were treated orally with 3 mg/kg body wt HCTZ (Sigma, St. Louis, MO). Urine was collected for the first 6 h, the second 6 h, and a third 12-h period (overnight). Rats were refed ad libitum after the first 6 h. Urine in each collection was measured for volume and sodium. Sodium excretion above baseline was considered thiazide sensitive.

Glucose tolerance test. A glucose tolerance test (GTT) was given to all rats at 10 wk to assess their ability to rapidly regulate blood glucose (a function of insulin sensitivity). Rats were given a 50% dextrose solution (3 ml/kg body wt) intraperitoneally. Glucose was measured in tail blood (glucometer) by pricking the tail after 15, 30, 60, 90, and 120 min. Blood glucose concentration over time was plotted, and the areas under the curves were calculated and statistically compared.

Kidneys and blood collection. Rats were killed after being kept on diet for 12 wk. The left kidneys from 12 rats (n = 3/group) were fixed with 2% paraformaldehyde via retrograde perfusion through the aorta following anesthetization with inhaled isoflurane, as previously described (25). Before perfusion, the right kidney was clamped off, removed, and rapidly frozen on dry ice. Twenty-four rats (n = 6/group) were decapitated in a conscious state (necessary to obtain blood for hormonal analyses). Trunk blood was collected into both K$_2$-EDTA- and Na$^+$-heparin-containing Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ). The right and left kidneys were rapidly removed. The left kidney was processed as a whole kidney homogenate for immunoblotting. The right kidney was dissected into inner medulla, outer medulla, and cortex and processed separately as whole homogenates for immunoblotting. In addition, the cortical homogenate was spun twice at 17,000 g to attain the plasma mem-

Fig. 1. A: weekly average mean arterial pressures (MAP; n = 5–7 per group per time point). B: delta MAP, i.e., change from their own respective baselines, calculated biweekly. Obese rats had increased MAP throughout the study. Rosiglitazone (RGZ) decreased MAP from the second week. *Obese groups significantly different from lean (P < 0.05). RGZ-treated groups significantly different from control by 2-way ANOVA (body type × treatment).

Fig. 2. A: body weights (n = 9 per group). Obese rats were heavier than lean rats throughout the study. Obese rats treated with RGZ gained weight at a faster rate than untreated obese rats. B: kidney weight normalized by body weight. C: urine NOx excretion was increased by RGZ in both lean and obese rats. *Obese groups significantly different from lean. RGZ-treated groups significantly different from control by 2-way ANOVA (P < 0.05).
brane-enriched fraction from the pellet. The resulting supernatant was spun at 200,000 g to obtain the intracellular vesicle-enriched pellet. These were resuspended into a small amount of isolation solution and similarly prepared for immunoblotting. The procedures for whole homogenate preparation and subcellular differential centrifugation to enrich plasma vs. vesicular membranes have been previously described in greater detail (8).

**Plasma and urine analyses.** Aldosterone, renin activity, and insulin were analyzed in blood collected at death by RIA kits as previously described (3, 25). Triglycerides were analyzed by a colorimetric assay kit (Sigma). Urinary NOX (nitrates plus nitrites) were measured by chemiluminescence (Sievers Instruments, Boulder, CO) in urine collected at 11 wk, according to previously described methods (35). Plasma creatinine was determined by the Jaffe rate method (Creatinine Analyzer 2, Beckman Diagnostics Systems Group, Brea, CA).

**NCC in urine and tissue.** Urine (at 7 wk) was collected over 24 h in 15 ml of ice-cold 1 M Tris-HCl (pH 6.8) containing 1 mg/ml leupeptin, 1 mM sodium azide, and 0.1 mg/ml phenylmethylsulfonyl fluoride as previously described (20). The urine samples were centrifuged at 1,000 g for 5 min to spin down whole cells. Then, the supernatant was spun at 200,000 g for 120 min to obtain a membrane fraction. The resulting membrane pellets were resuspended in isolation solution and the protein concentrations were measured using the BCA Protein Assay reagent kit (Pierce). Laemmli was added to the protein solution and the protein concentrations were measured using the BCA Protein Assay reagent kit (Pierce). Laemmli was added to the protein solution and the protein concentrations were measured using the BCA Protein Assay reagent kit (Pierce). Laemmli was added to the protein solution and the protein concentrations were measured using the BCA Protein Assay reagent kit (Pierce). Laemmli was added to the protein solution and the protein concentrations were measured using the BCA Protein Assay reagent kit (Pierce).

**Immunolocalization.** The Envision + System (DakoCytomation, Carpinteria, CA) was used to conduct peroxidase labeling, while Alexa 488 and 568 fluorochrome secondary antibodies (Molecular Probes, Eugene, OR) were used to conduct immunofluorescence on paraformaldehyde-fixed paraffin sections of the left kidney. An Olympus IX-70 inverted confocal microscope was used for fluorescence detection located in the Lombardi Cancer Center Imaging Core, Georgetown University.

**Primary antibodies.** For NCC immunoblotting and immunoperoxidase labeling, we used either our own (25, 27) or Dr. M. A. Knepper’s (15) rabbit peptide-derived polyclonal antibody. For immunofluorescence, for NCC we used guinea pig peptide-derived polyclonal antibody (7), and for calbindin-D we used commercial mouse monoclonal antibody (Sigma).

**Statistics.** Data were analyzed by two-way (body type x treatment) ANOVA (SigmaStat, Chicago, IL). P < 0.05 was considered significant.

**RESULTS**

**Blood pressure.** Blood pressure increased over the course of the 12 wk only in the obese control rats (Fig. 1A). RGZ treatment of these rats normalized blood pressure until the final weeks. RGZ treatment also caused a modest fall in blood pressure in the lean rats (Fig. 1B). Delta mean arterial pressure from baseline was decreased by 5 mmHg in the obese RGZ rats after only 2 wk of treatment.

**General physiology.** Obese rats were heavier than lean from the outset and gained weight faster (Fig. 2A). RGZ-treated obese rats were heavier than obese control rats after 6 wk of treatment or at 15 wk of age. Obese rats had heavier kidneys than lean, overall (not shown), but significantly lighter kidneys when normalized by body weight (Fig. 2B). RGZ treatment reduced kidney weight (Fig. 2B). Urine NOX excretion was increased by RGZ in both lean and obese rats (Fig. 2C).

Final circulating insulin levels were lower in RGZ-treated obese rats, although not reduced to lean rat levels (Table 1). Plasma renin activity was reduced in obese vs. lean, but not affected by RGZ. Plasma aldosterone was not significantly different between treatments. However, urinary excretion of aldosterone was increased in RGZ-treated rats, and the urine potassium to sodium ratio, an indicator of aldosterone activity, was increased in both obese rats and in RGZ-treated rats. Plasma creatinine, an indicator of renal function, was significantly increased only in the obese controls, suggesting reduced glomerular filtration rate. Plasma triglyceride levels were significantly reduced by RGZ.

**Blood glucose and urine protein.** Six weeks into the study, there were no differences in fasting blood glucose levels, but by 9 wk obese controls had significantly higher blood glucose while RGZ treatment reduced them to normal (Fig. 3A). A GTT was administered 10 wk into the study. The area under the curve was calculated to be higher for the obese rats compared with the lean, while RGZ treatment managed to bring it down, although not equal to the lean rats (Fig. 3B). Twenty-four-hour urinary albumin excretion (UAE) was mildly elevated in obese rats 2 wk into the study. At 8 wk, UAE in obese control rats was markedly elevated while RGZ treatment had reduced it significantly (Fig. 3C).

**NaCl load test.** How rapidly and completely the rats were able to excrete a NaCl load was used as an index of natriuretic capability (Fig. 4A). RGZ-treated rats, whether lean or obese, excreted a greater percentage of the sodium load in all three time points collected.

**Thiazide sensitivity tests of sodium excretion.** Sensitivity to HCTZ, with regard to sodium excreted above baseline, was used as an index of NCC activity (9) (Fig. 4B). RGZ-treated rats, both lean and obese, excreted less sodium in response to HCTZ, signifying decreased NCC activity.

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Factors

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Values are means ± SE; n = 5 or 6 RGZ, rosiglitazone. *Generated ANG I. Bold P values < 0.05 by 2-way ANOVA (significant).
Urine and tissue NCC. The renal abundance of NCC was increased in obese vs. lean rats, but not significantly affected by RGZ treatment. Figure 5A shows an immunoblot of whole homogenates prepared from the cortex of the right kidney. Each lane is loaded with 5 μg of a different rat’s sample (n = 6/group except for the obese control group, where one rat died before the study ended). Surprisingly, the obese rats also excreted a relatively large amount of NCC in their urine (Fig. 5B), i.e., band density was about 14-fold higher on average for the obese rats. A summary of the densitometric analysis for kidney cortex and urine is shown in Fig. 5, C and D, respectively. Furthermore, the relative amount of NCC excreted in urine for the obese rats correlated positively with the relative amount measured in their renal cortices (Fig. 5E).

Densitometric analysis of NCC immunoblots of the 17,000-g fraction (plasma membrane enriched) of the kidney cortex also revealed a significant increase in the obese rats relative to lean (Fig. 6A). However, no differences between groups were observed for the 200,000-g fraction (intracellular vesicle enriched; Fig. 6B) and the ratio of the two fractions to each other (Fig. 6C).

Fig. 3. A: fasting blood glucose levels at 6 and 9 wk (n = p per group). B: glucose tolerance test (10 wk). Area under the curve was calculated to be higher for the obese rats compared with the lean, whereas RGZ treatment brought it down, although not equal to the lean rats. C: urinary albumin excretion at 2 and 8 wk. *Obese groups significantly different from lean. *RGZ-treated groups significantly different from control by 2-way ANOVA (P < 0.05).

Fig. 4. Urinary sodium excretion in response to a NaCl load test (A) or a single oral dose of hydrochlorothiazide (HCTZ; n = 9 per group; B). Rats treated with RGZ, whether lean or obese, excreted a greater percentage of the NaCl load. RGZ-treated rats, whether lean or obese, excreted lesser amounts of sodium in response to the HCTZ. *Significant effect of RGZ as analyzed by 2-way ANOVA (P < 0.05).
Immunoperoxidase labeling with NCC antibody in the rat kidneys showed strong apical labeling of distal convoluted tubules (DCT) in all groups (Fig. 7). Shown are images of the cortex of one rat from each group. No clear differences in subcellular distribution of NCC were detected by this means. Fluorescent dual labeling of NCC (red) expressed in early and late DCT (DCT-1 and DCT-2) (22) and calbindin-D (green) expressed in DCT-2 and connecting tubule (5) is shown in Fig. 8. Figure 8, top, shows sample DCT in the four rat groups identified to be DCT-1, as there is a clear presence of red, but not green labeling. Figure 8, bottom, shows sample DCT identified to be DCT-2, as they show the presence of dual labeling. Using this more sensitive technique, we saw stronger labeling for NCC in both DCT-1 and DCT-2 in the obese rats, relative to lean. RGZ-treated rats had strong apical labeling, as did the control rats. Control obese rats may have had more subapical NCC labeling than did lean rats or obese RGZ-treated rats, especially in DCT-1.

DISCUSSION

Diabetes and prediabetes (insulin resistance) are rapidly growing diseases closely associated with weight gain in the human populace (13, 29). The lifetime risk of developing diabetes for someone born in 2000 may be as high as 1 in 3 (36). Both diabetes and insulin resistance are strongly associated with hypertension and are the leading cause of end-stage renal disease (23).

RGZ, a drug of the class thiazolidinedione (TZD), is a potent agonist of the PPARγ (12, 37) and highly effective in lowering blood glucose in type II diabetes (21). PPARs are nuclear receptors that regulate gene transcription affecting a number of pathways involved in energy metabolism, in particular for PPARγ, pathways involved in adipocyte differentiation and adipogenesis. PPARγ is highly expressed in adipose tissue, but also in kidney (24, 38). Thus some of its effects may be directly via its renal receptors. RGZ may also have PPARγ-independent effects including anti-inflammatory actions (12, 17).

Although they have a much milder increase in blood pressure than several other strains of genetically hypertensive rats, we find that the obese Zucker rat (fa/fa) has increased blood pressure relative to lean age mates at a fairly young age (9 wk), i.e., at the start of our study. Although this is in the prediabetic phase, when renal damage would not be expected, our previous work (4) shows that they are substantially hyperinsulinemic, even at this age. Treatment with RGZ rapidly reduced blood pressure (fall of 5 mmHg in 2 wk) in the obese rats, a decrease that was sustained throughout most of the study, although it did begin to rise above the lean rat level after about 6 wk on the diet. It is unclear whether increasing the RGZ dose at this time would have again reduced the blood pressure or whether the mechanism for the rise at this point was other than insulin resistance. Others (31, 33) showed reduced blood pressure with RGZ in the obese Zucker rat, but ours was the first study to measure blood pressure continuously via telemetry, and thus we were able to develop the time course of the response. In general, these data are supportive of the hypothesis that insulin resistance or hyperinsulinemia itself is the direct cause of the increased blood pressure in this model, at least initially, rather

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Fig. 5. Na-Cl cotransporter (NCC) protein in whole kidney cortex and urine (n = 6 per group except for obese controls where n = 5). Immunoblots of kidney cortex homogenates (A) and urine NCC excretion (B) probed with rabbit polyclonal anti-NCC antibody and a summary of average band densitometry for kidney cortex (C) and urine (D) expressed as percent of lean controls are shown. E: correlation of cortex with urine NCC band density in obese rats. *Obese groups significantly different from lean (P < 0.05).
than hyperglycemia, obesity, weight gain, or gradual damage to the kidneys or vasculature.

Interestingly, RGZ treatment of the lean rats also led to a modest fall in blood pressure sustained over the course of the 12 wk. Whether this was due to improvement in insulin sensitivity, direct effects of RGZ on the vasculature, or on sodium transporters expressed in the kidney is not known. For example, TZDs have been shown to increase nitric oxide release in endothelial cells (6), as well as inhibit L-type Ca\(^{2+}\) channels in smooth muscle (16). Previously, we (28) showed a fall in blood pressure (~3–4 mmHg) in young Sprague-Dawley rats treated with a higher dose of RGZ, i.e., ~8 mg·kg body wt\(^{-1}\)·day\(^{-1}\) for only 3 days. This fall in blood pressure coincided with increased NOS III (endothelial nitric oxide synthase) protein abundance in the kidney.

RGZ treatment also slowed the progression of the diabetes and related pathology itself, as demonstrated by decreased fasting glucose levels, urinary albumin, plasma creatinine, and increased glucose tolerance (Fig. 2). However, it clearly was not a cure for the insulin resistance, as demonstrated by the GTT in which the RGZ-treated obese rats still had a lower clearance rate of glucose than did either of the lean groups of rats.

RGZ reduced circulating levels of triglycerides in obese rats, but it had no effect on renin activity, which was decreased, in agreement with other groups (1, 10). Nevertheless, several
investigators showed upregulation of other components of the renin-angiotensin system in the obese Zucker rat including increased AT₁ angiotensin II binding (2), and a blood pressure-lowering response to a AT₁ antagonist, losartan (1). We found no differences between groups for plasma aldosterone levels in agreement with our previous findings (3, 4). However, we did find an increase in daily urinary excretion of aldosterone in RGZ-treated rats and an increase in the urinary potassium-to-sodium ratio in both RGZ-treated rats and in obese rats, which suggests increased aldosterone activity. It is not clear why RGZ might increase aldosterone activity; however, it may be due to indirect effects of lower blood pressure, especially in the lean rats.

In our studies, not only did RGZ reduce blood pressure, but it also appeared to improve both lean and obese rats’ ability to rapidly excrete a NaCl load or natriuretic capability (Fig. 4A). There were no significant differences between the lean and obese rats, however. This contrasted some with what we previously found (4), which was decreased natriuretic ability in the obese rats. We believe this may be due to the fact that in this study we gave a larger volume of saline to the obese rats to normalize for increased body size, not done in the previous study.

It is also interesting that the RGZ-treated rats excreted the NaCl load faster given the fact that aldosterone activity appeared somewhat increased in these rats. Under certain conditions, this may play a role in the edema and sodium retention that many times accompany RGZ therapy (11, 30). We did not notice appreciable edema in these treated rats; however, our dose was fairly moderate. Our previous work (28) did show modest, significant increases in several proximally expressed sodium transport proteins including the sodium phosphate cotransporter (NaPi-2) and the sodium hydrogen exchanger (NHE3) with short-term RGZ treatment to young, healthy rats. These are not thought to be “aldosterone-regulated” proteins. However, NCC is aldosterone regulated (15, 32). Indeed, NCC is one candidate protein that has been proposed to have a critical role in the manifestation of pressure-natriuresis (34). Thus, perhaps counterintuitively, we found a decrease in thiazide sensitivity (Fig. 4B) in both lean and obese rats treated with RGZ, which might suggest that NCC activity was reduced by RGZ. Furthermore, there was a trend for increased thiazide sensitivity in the control obese vs. lean rats, but it did not reach statistical significance.

Utilizing confocal imagery of immunofluorescent labeling, immunoperoxidase labeling, and immunoblotting of whole homogenates and fractions prepared by differential centrifugation of the cortex homogenate, we assessed whether there might be differences in protein abundance and the relative cellular distribution of NCC in the four rat groups. We did see a modest yet significant increase in NCC abundance in obese rats’ kidneys, relative to lean, an observation that confirmed our previous immunoblotting work (4). However, RGZ did not seem to reduce NCC abundance, urinary excretion, or subcellular distribution.

Furthermore, our preliminary findings (Song J and Ecelbarger C, unpublished observations) using array-based (Affymetrix) mRNA determination suggest that mRNA for NCC is also increased in the obese rats’ kidneys relative to lean (about 1.8-fold), but not affected significantly by RGZ therapy, although this is yet to be confirmed by real-time polymerase chain reaction or similar technical approach.

The confocal microscopy also allowed us to assess whether the treatments or the body type of the rats might affect NCC expression in the early (DCT-1) vs. late (DCT-2) DCT. Animals of both treatments and body types had labeling in both of these cell types, and no differences could be clearly determined.

Thus the apparent decrease in thiazide sensitivity appears not to be due to decreased protein abundance or subcellular distribution of NCC. Perhaps NCC activity is reduced by increased NO bioavailability. Urine NOx was increased in both lean and obese Zucker rats treated with RGZ (Fig. 2C). Additional studies are warranted in this area.

![Fig. 8. Immunofluorescent colocalization of NCC (red) and calbindin-D (green) in the kidney cortex. NCC is found only in the distal convoluted tubules (DCT), whereas calbindin is found in both the connecting tubule and the distal portion of the DCT. Early DCTs are shown at the top, whereas late DCTs are shown at the bottom. Obese rats show stronger labeling for NCC in both early and late DCT.](http://ajprenal.physiology.org/10.1152/ajprenal.00779.2004)
Also remarkable was the finding of a relatively large amount of NCC in the urine from the obese rats. The urinary NCC correlated fairly well with the relative amount of NCC in the obese rats’ kidneys at the time of death. Excretion of NCC into the urine is likely due to exocytic delivery of NCC-containing vesicles to the apical surface of the DCT cells and rather than insertion into the membrane, some of the protein molecules are excreted into the lumen and end up in the urine. This process apparently is enhanced in the obese rats. Urinary excretion of the water channel protein aquaporin-2 (14) has been suggested to be a marker of the vasopressin responsiveness of the renal collecting duct. Similarly, urinary NCC may be a useful non-invasive marker of renal NCC abundance.

Overall, these results demonstrate that chronic treatment of prediabetic obese Zucker rats with a common insulin-sensitizing drug, RGZ, will maintain blood pressure in the normal range, reduce hyperglycemia and albuminuria, and increase natriuretic ability. The abundance and excretion of NCC are increased in obese rats, whereas the activity appears to be reduced by RGZ. Thus the mechanism for reduced activity does not seem to involve a decrease in renal protein abundance, localization, or turnover. Furthermore, whether the decrease in NCC activity accounts for, or is at least a contributory factor in, the blood pressure fall with RGZ is not yet clear. Another factor to consider is that RGZ-treated obese rats gained weight faster and were fatter than untreated obese rats, especially as the study progressed. This may have blunted some of the benefits of the RGZ on insulin sensitivity, blood pressure, and DRGZ on insulin sensitivity, blood pressure, and sodium excretion, and NCC expression and activity.

Although serious side effects such as liver toxicity and edema have been associated with some members of the class of PPARγ agonists, TZDs, these drugs are remarkably effective in treating pathology associated with both insulin resistance and diabetes. Thus low-dose therapy in prediabetic, insulin-resistant subjects should be considered. Furthermore, the use of urinary NCC, as a noninvasive diagnostic tool to assess NCC turnover, has promise, although its relationship to obesity, NCC activity, and insulin resistance needs to be further elucidated.

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REFERENCES


