Spironolactone Prevents Chlorthalidone-Induced Sympathetic Activation and Insulin Resistance in Hypertensive Patients


Abstract—Recent studies from our laboratory indicate that chlorthalidone triggers persistent activation of the sympathetic nervous system and promotes insulin resistance in hypertensive patients, independent of serum potassium. Mechanisms underlying these adverse effects of chlorthalidone remain unknown, but increasing evidence in rodents suggests the role of angiotensin and aldosterone excess in inducing both sympathetic overactivity and insulin resistance. Accordingly, we conducted studies in 17 subjects with untreated stage 1 hypertension, measuring sympathetic nerve activity at baseline and after 12 weeks of chlorthalidone alone (25 mg/d), chlorthalidone plus spironolactone, and chlorthalidone plus irbesartan, using randomized crossover design. We found that chlorthalidone alone decreased 24-hour ambulatory blood pressure from 135±3/84±2 to 124±2/78±2 mm Hg and significantly increased sympathetic nerve activity from baseline (from 41±3 versus 49±4 bursts per minute; P<0.01). The addition of spironolactone to chlorthalidone returned sympathetic nerve activity value to baseline (42±3 bursts per minute; P>0.05), whereas the addition of irbesartan failed to alter the sympathetic nerve activity response to chlorthalidone in the same subjects (52±2 bursts per minute; P<0.01) despite a similar reduction in ambulatory blood pressure (121±2/75±2 and 121±2/75±2 mm Hg, respectively). Chlorthalidone alone also increased indices of insulin resistance, which was not observed when used in combination with spironolactone. In conclusion, our study demonstrates beneficial effects of spironolactone in attenuating both chlorthalidone-induced sympathetic activation and insulin resistance in humans, independent of blood pressure reduction. Because sympathetic overactivity and insulin resistance contribute to the poor prognosis in patients with cardiovascular disease, combination therapy of chlorthalidone with mineralocorticoid receptor antagonists may constitute a preferable regimen than chlorthalidone alone in hypertensive patients.

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Key Words: diuretics ▪ sympathetic nervous system ▪ insulin resistance ▪ hypertension

Chlorthalidone has been proposed as the preferred diuretic for treatment of hypertension, given its superiority in reducing blood pressure (BP) and hypertensive target organ complications when compared with other thiazide-type diuretics. Previous studies demonstrated that chlorthalidone-induced BP reduction was accompanied by reflex sympathetic activation and detrimental indices of insulin resistance in hypertensive patients. Furthermore, the magnitude of the chlorthalidone-induced increase in insulin resistance was found to be correlated with the increase in sympathetic nerve activity (SNA) and independent of serum potassium in our previous study. Despite increasing popularity of chlorthalidone use in the United States, mechanisms underlying chlorthalidone-induced sympathetic excitation and insulin resistance remain unknown, and effective therapy in preventing these adverse effects of chlorthalidone has not been identified.

Chlorthalidone is known to induce a sustained activation of the renin-angiotensin-aldosterone system in hypertensive patients. In animal experimental models, both angiotensin II (Ang II) and aldosterone cross the blood-brain barrier and directly stimulate central sympathetic outflow to the heart and peripheral circulation. In addition, Ang II further triggers aldosterone synthesis in the brain, thereby amplifying central neuronal activation and hypertension even with a small

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elevation in circulating Ang II. Both Ang II and aldosterone have also been implicated in the pathogenesis of insulin resistance by inhibiting the insulin signaling pathway in the adipocytes and skeletal muscle, resulting in impaired insulin-mediated glucose uptake. Whether the addition of angiotensin receptor blockers (ARBs) or mineralocorticoid receptor (MR) antagonists prevents chlorthalidone-induced sympathetic activation and insulin resistance has not been investigated previously.

Accordingly, the goal of the present investigation is to determine whether the addition of MR antagonists or ARBs constitutes an effective strategy in reducing both chlorthalidone-induced sympathetic activation and insulin resistance in hypertensive patients. In untreated hypertensive patients, we performed a randomized crossover trial in which we recorded postganglionic sympathetic action potentials with intraneural microelectrodes and assessed indices of insulin resistance at baseline, after chlorthalidone alone, after chlorthalidone plus spironolactone, and after chlorthalidone plus ARB irbesartan. Because Ang II and aldosterone have been shown to impair baroreflex function, which exerts inhibitory influence on the SNA, we also assessed baroreflex control of SNA and heart rate (HR) during each treatment arm.

Methods
Seventeen patients with untreated stage 1 hypertension participated in the study after providing written informed consent. The study was approved by the institutional review board of the University of Texas Southwestern Medical Center at Dallas. All of the subjects had BP between 140 and 159/90 and 99 mm Hg on 3 determinations by oscillometric technique in the seated position. The subjects had no history of heart disease, diabetes mellitus, or evidence of target organ damage, such as left ventricular hypertrophy by electrocardiography or chronic kidney disease. The patients had not received antihypertensive drugs for ≥4 weeks before the study.

Experimental Protocols
All of the subjects were randomized to receive 12 weeks of chlorthalidone, 25 mg/d, alone; chlorthalidone, 25 mg daily, plus spironolactone 25 mg daily; and chlorthalidone 25 mg daily plus irbesartan of 150 mg daily, using a single-blind crossover design without washout between treatments. Each subject was followed every 4 weeks for measurement of serum potassium (K). All of the subjects were given oral KCl supplementation to maintain serum K between 4.0 and 4.5 mmol/L. Ambulatory BP monitoring was performed at baseline and after 12 weeks of each treatment phase.

After completion of ambulatory BP monitoring, measurement of SNA by peroneal microneurography, casual BP, arterial baroreflex sensitivity, fasting plasma glucose, insulin, plasma renin activity (PRA), and serum aldosterone were performed in the supine position during the morning hours between 8:00 and 11:00 AM (see method detail in the online-only Data Supplement). All of the subjects were instructed to take the study drug 1 to 2 hours before microneurographic study. Analysis of these variables was performed without the knowledge of treatment that each subject had received.

Statistical Analysis
Mixed linear models were used to conduct the repeated-measures analysis to assess differences among baseline period, chlorthalidone alone, chlorthalidone plus spironolactone phases, and chlorthalidone plus irbesartan. Contrasts from these models were used for pairwise comparisons. Treatment order was also assessed in the models, and no effect of treatment order on any outcome variables was found. Because data sets for insulin, PRA, homeostasis model assessment-insulin resistance (HOMA-IR), and homeostasis model assessment of β-cell function data are not normally distributed, the data were analyzed after a natural logarithmic transformation. The 0.05 level of significance was used for model main effects, and the 0.02 level of significance was used for pairwise tests to adjust for multiple testing. Pearson correlation coefficient was used to assess the association between changes in SNA with changes in indices of insulin resistance and other nonmetabolic variables. Levels of insulin, PRA, HOMA-IR, and homeostasis model assessment of β-cell function are expressed as median and the interquartile range. Other variables are expressed as mean and SEM. Statistical analysis was performed with SAS version 9.2 (SAS Institute Inc, Cary, NC).

Results
Baseline characteristics of subjects who participated in the study are shown in the Table. Chlorthalidone alone caused a significant reduction in 24-hour ambulatory BP without affecting 24-hour HR (Table). When spironolactone or irbesartan was added to chlorthalidone, there was a small reduction in ambulatory and casual BP, but the reduction did not reach statistical significance when compared with chlorthalidone alone (Table). Treatment with chlorthalidone plus irbesartan, however, significantly increased the nighttime HR compared with baseline (P=0.01). Chlorthalidone alone caused a significant increase in PRA and serum aldosterone levels. The addition of spironolactone to chlorthalidone did not alter the increase in PRA and serum aldosterone induced by chlorthalidone alone. The addition of irbesartan, however, caused a further increase in PRA compared with chlorthalidone alone without affecting aldosterone responses (Table). Chlorthalidone alone significantly increased SNA and SNA per 100 RR intervals from baseline (from 41±3 to 49±4 bursts per minute and 66±4 to 74±4 bursts per 100 RR; P<0.01 versus baseline; Figures 1 and 2). The addition of spironolactone to chlorthalidone returned the SNA value to baseline (42±3 bursts per minute and 67±3 bursts per 100 RR; P value not significant versus baseline; Figures 1 and 2), whereas the addition of irbesartan failed to alter SNA response to chlorthalidone in the same subjects (52±2 bursts per minute and 75±3 bursts per 100 RR; P<0.01 versus baseline and versus chlorthalidone plus spironolactone) despite a similar reduction in 24-hour ambulatory BP (121±275/75±2 and 121±2/75±2 mm Hg, respectively; Table). Baroreflex control of SNA and HR remained unchanged during all phases of treatment (Figure 2).

Chlorthalidone alone significantly increased fasting plasma glucose, insulin, and HOMA-IR, and reduced quantitative insulin sensitivity check index from baseline (Table and Figure 3). In contrast, the addition of spironolactone returned HOMA-IR and serum insulin levels to baseline in the same subjects to values significantly lower than chlorthalidone alone (Figure 3). HOMA-IR and insulin levels during irbesartan plus chlorthalidone were not significantly different from those during chlorthalidone alone or baseline period. Neither chlorthalidone nor a combination of chlorthalidone with spironolactone or irbesartan had any detectable effect on homeostasis model assessment of β-cell function compared with baseline (111±24% versus 68±19% versus 91±26% versus 72±19%, respectively; P>0.05). With K supplementation, there were no changes in serum K after 12-week treatment of chlorthalidone from baseline (Figure 3) or during any treatment period. Percentage of changes in fasting plasma
glucose during chlorthalidone treatment alone from baseline was significantly correlated with percentage of changes in SNA in both bursts per minute \((r=0.64; \ P=0.01)\) and bursts per 100 RR interval \((r=0.76; \ P=0.001)\), which was not observed when chlorthalidone was combined with spironolactone or irbesartan \((P>0.05)\). There were no correlations between changes in insulin, HOMA-IR, quantitative insulin sensitivity check index, serum aldosterone, or PRA with changes in SNA during all of the treatment phases (data not shown).

**Discussion**

There are 2 major new findings of this study. First, the addition of spironolactone to chlorthalidone prevents adverse effects of chlorthalidone on both the sympathetic nervous system and insulin sensitivity in hypertensive patients. Second, these beneficial effects of spironolactone were not observed with the ARB irbesartan despite similar reductions in BP.

Previous work from our laboratory has indicated that chlorthalidone triggers sustained sympathetic activation in hypertensive patients, but the underlying mechanism(s) of this potentially detrimental response remains unknown. Activation of Ang II receptor subtype 1 by Ang II in the central nervous system after chlorthalidone-induced volume depletion might be one mechanism. The effects of ARBs on SNA in humans, however, are inconsistent, and studies have found SNA to be unchanged, increased, or decreased after ARB treatment. An increasing body of evidence in rodents suggests that circulating aldosterone penetrates the blood-brain barrier and directly stimulates central sympathetic outflow via activation of central MRs. This central sympathoexcitatory and pressor action of aldosterone is attenuated by intracerebroventricular infusion of MR antagonists at doses that had no systemic spillover. Furthermore, aldosterone can be produced locally in the brain on stimulation by circulating Ang II, which may contribute to chlorthalidone-induced volume depletion. In humans, recent study from our laboratory provided additional support for the animal data, because patients with primary aldosteronism (PA) from aldosterone-producing adenomas were found to have sympathetic overactivity, which was reversible after surgical resection of the tumor.
tension, suggesting that the sympathoinhibitory action of the treatment. In our previous study, spironolactone alone was shown to provide more definitive evidence for sympathoinhibitory effects of spironolactone when added to chlorthalidone treatment. Our data from the randomized crossover study, which requires demonstration of sustained elevation in plasma aldosterone level or adrenal aldosterone production despite increased sodium intake according to the current Endocrine Society guidelines, whereas the PA subjects in 2 previous studies were identified by only elevated random levels of aldosterone.

Despite variability in the SNA data among these cross-sectional studies comparing PA subjects with normal controls, our data from the randomized crossover study provide more definitive evidence for sympathoinhibitory effect of spironolactone when added to chlorthalidone treatment. In our previous study, spironolactone alone was not shown to alter SNA in patients with essential hypertension, suggesting that the sympathoinhibitory action of MR antagonists is more apparent in the setting of renin-angiotensin-aldosterone excess. Furthermore, suppression in SNA during chlorthalidone-spironolactone therapy occurred without any detectable changes in baroreceptor control of SNA or HR, suggesting direct effects of spironolactone on the central sympathetic outflow.

In contrast to spironolactone, the addition of the ARB irbesartan failed to attenuate the increase in SNA in the same subjects, suggesting that Ang II is less important than aldosterone in mediating a chlorthalidone-induced increase in SNA. Alternatively, limited oral bioavailability of irbesartan may be responsible for failure of irbesartan to inhibit central Ang II type 1 receptors, as suggested by a previous study by Leenen and Yuan in the rat model of Ang II–induced hypertension. Thus, the results of our study may differ with higher dose of irbesartan or other ARBs. Incomplete Ang II receptor subtype 1 blockade during ARB treatment or failure to suppress aldosterone release, that is, aldosterone escape or breakthrough phenomenon, is another potential mechanisms underlying the failure of irbesartan to prevent chlorthalidone-induced increases in sympathetic nerve discharge. Nevertheless, the results of our study are consistent with a previous study by Fu et al, which showed markedly increased SNA when hydrochlorothiazide was administered in combination with losartan.

In addition to stimulation of the sympathetic nervous system, chlorthalidone is well known to increase plasma glucose and the risk of progression to diabetes mellitus. Although hypokalemia is thought to be the main mechanism of thiazide diuretic-induced dysglycemia, possibly by impairing pancreatic release of insulin, previous studies from our group and others demonstrated a component of insulin resistance that is independent of serum potassium. Activation of the renin-angiotensin system is thought to worsen insulin sensitivity, because Ang II has also been shown to both inhibit the insulin signaling pathways in the adipocyte and skeletal muscle and to impair pancreatic β-cell function. Treatment with angiotensin-converting enzyme inhibitors or ARBs has been shown to reduce the development of diabetes mellitus in patients with hypertension or impaired glucose tolerance. In contrast, previous studies have shown variable effects of angiotensin-converting enzyme inhibitors and ARBs on the development of thiazide diuretic-induced insulin resistance. Although the combination of losartan with
hydrochlorothiazide and the combination of captopril with bendroflumazide failed to prevent the detrimental effect of thiazide diuretics on glucose homeostasis, a combination of valsartan with hydrochlorothiazide was shown to prevent the increase in fasting plasma glucose and to restore glucose-induced insulin secretion to pretreatment values in obese patients with hypertension. This variability in study results might derive from variability in the dose and potency of various diuretics, as well as the specific type of angiotensin-converting enzyme inhibitors or ARBs. Nevertheless, the ARB irbesartan failed to prevent the adverse metabolic effects of chlorthalidone in our study.

Like Ang II, aldosterone has been implicated in the pathogenesis of insulin resistance in large populational studies and in patients with PA. In vitro studies demonstrate that aldosterone inhibits insulin signaling pathways both in adipocytes and in vascular smooth muscle cells. In vivo studies have indicated that aldosterone induces dysglycemia in rodents by impairing glucose uptake in the skeletal muscle and liver via inhibition of GLUT2 and GLUT4 gene expression. Treatment with MR antagonists improves insulin sensitivity and skeletal muscle glucose transport in rodents with aldosterone excess; however, there are no previous studies that have addressed the impact of MR blockade during thiazide therapy to insulin sensitivity in humans. Thus, our study represents the first demonstration that spironolactone prevents the chlorthalidone-induced insulin resistance in hypertensive patients. Furthermore, this beneficial effect of spironolactone was not explained by changes in serum potassium, suggesting direct effects of MR blockade.

Our study is limited by lack of a placebo arm, but at least the randomized crossover design allows us to compare SNA and indices of insulin resistance during each combination therapy and during chlorthalidone treatment alone in the same subjects. Our study is also limited by the small sample size, which may explain failure to detect a significant reduction in the ambulatory or casual BP when irbesartan or spironolactone was added to chlorthalidone. However, the nighttime HR was found to be significantly increased during the chlorthalidone plus irbesartan phase compared with baseline, consistent with increased sympathetic drive to the sinus node. Changes in insulin sensitivity were calculated from the HOMA-IR and quantitative insulin sensitivity check index equations rather than directly obtained from the hyperinsulinemic-euglycemic clamp method. Both HOMA-IR and quantitative insulin sensitivity index have been validated against the hyperinsulinemic glucose clamp and shown to be reliable indices of insulin sensitivity, supporting our findings. Only irbesartan was tested in this study, and results may not be applicable for all ARBs. Only SNA targeted to the skeletal muscle vasculature was measured, and our study results may not be applicable to other regional sympathetic outflow. Lastly, we cannot ascertain whether the ability of spironolactone to improve insulin sensitivity in chlorthalidone-treated subjects is the cause or consequence of sympathoinhibitory effect of spironolactone. Exogenous infusion of insulin has been shown to acutely increase muscle SNA in normotensive subjects during a euglycemic clamp. Conversely, activation of the sympathetic nervous system has been directly implicated in the pathogenesis of insulin resistance by reducing skeletal muscle glucose uptake both by flow-dependent and flow-independent mechanisms. This latter hypothesis is supported by one recent study, wherein catheter-based renal sympathetic denervation improved insulin sensitivity in patients with resistant hypertension.

Perspectives
Regardless of the mechanisms underlying the beneficial effects of spironolactone on glucose metabolism and SNA, it is known that both insulin resistance and sympathetic overactivity contribute to the poor prognosis of patients with cardiovascular diseases. Thus, the addition of spironolactone might maximize the long-term cardiovascular benefit of chlorthalidone therapy in hypertensive patients by reducing the adverse metabolic consequences and neurohormonal activation. Additional large clinical trials are needed to determine whether the combination of chlorthalidone with spironolactone is superior to chlorthalidone alone or other combination therapy in reducing cardiovascular outcomes in hypertensive patients.

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Disclosures
None.

References

**Novelty and Significance**

**What Is New?**
- Chlorthalidone, a thiazide-like diuretic, is known to cause insulin resistance and activation of the sympathetic nervous system in hypertensive patients, but effective measures to prevent these adverse effects have not been identified.
- The present study demonstrates that spironolactone, another diuretic that reduces BP by blocking actions of the aldosterone hormone, prevents chlorthalidone-induced insulin resistance and sympathetic overactivity.

**What Is Relevant?**
- Chlorthalidone is widely accepted to be the preferred diuretic for treatment of hypertension, but many associated metabolic adverse effects, particularly increased risk of diabetes mellitus, limits its use in clinical practice.

**Summary**
The addition of spironolactone to chlorthalidone may allow hypertensive patients to receive BP-lowering benefit from chlorthalidone with minimal metabolic adverse effects.
Spironolactone Prevents Chlorthalidone-Induced Sympathetic Activation and Insulin Resistance in Hypertensive Patients
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ONLINE SUPPLEMENT
SPIRONOLACTONE PREVENTS CHLORTHALIDONE-INDUCED SYMPATHETIC ACTIVATION AND INSULIN RESISTANCE IN HYPERTENSIVE PATIENTS


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Measurement of sympathetic nerve activity by microneurography
All experiments were performed with the subjects in the supine position. Casual BP was measured by the oscillometric technique with the Welch Allyn CE00050 Monitor (Tyco Instruments, Inc., Arden, NC). Heart rate (HR) was monitored by R wave of an ECG lead. Postganglionic efferent sympathetic nerve discharge, HR, and respiratory rate were recorded continuously using a multi-channel digital data recorder (MacLab/8S ML780, AD Instruments Inc., Mountain View, CA).

Multiunit recordings of postganglionic SNA was obtained with tungsten microelectrodes inserted into muscle nerve fascicles of the peroneal nerves using the microneurographic technique of Valbo et al. SNA was analyzed by an investigator (Z.W) without the knowledge of the treatment phase assigned to each subject. The interobserver and intraobserver variations in identifying bursts are <10% and < 5%.

In our laboratory, the intra-subject variability of SNA when measured on repeated occasions without any interventions is less than 15% with the correlation coefficient of reliability in the measurement of SND of 0.91.

Arterial baroreflex testing
Arterial baroreflex sensitivity was assessed by measuring muscle SNA and HR during BP changes induced by bolus infusion of 100 mg of nitroprusside and 150 mg of phenylephrine, using a modified Oxford technique. BP during baroreflex testing was continuously monitored with a finger arterial plethysmograph (Finometer, FMS). The baroreflex control of SNA was calculated as the slope of the curve relating increases or decreases in diastolic BP to SNA. The baroreflex control of HR was calculated as the slope of the curve relating systolic BP to HR.

24-hour ambulatory BP recording
Ambulatory BP (ABP) was monitored continuously every 20 minutes, using a SpaceLabs model 90207 (SpaceLabs Inc., Issaquah, Washington, USA). Values between 07:00 to 23:00 were considered as day and between 23:00 to 07:00 were assigned as night according to previous studies.

Biochemical and Hormonal Assays
Blood samples were collected after fasting for 12 hours with the subjects in the seated position for more than 5 minutes. The plasma samples were then separated and stored at -80°C until analysis. Plasma glucose and insulin levels were measured as previously described. Insulin sensitivity was estimated through the use of the homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI). Pancreatic β-cell function was estimated through the use of the homeostasis model assessment of b-cell function (HOMA-BF). Plasma renin activity (PRA) was assayed in duplicates using the GammaCoat™ kit (DiaSorin, Stillwater, MN) according to the
manufacturer’s instructions incorporating a 3 h incubation at 37C. The lower limit of quantitation was 0.1 ng/mL/h, and samples that assayed below this limit were assigned a value of 0.05 ng/mL/h. Serum aldosterone was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an API-5000 triple quadrupole mass spectrometer and a Shimadzu HPLC front end. Serum samples were mixed with deuterated internal standards (IS, [2,2,4,6,6,21,21-2H7]-aldosterone (using 2H6 species, Sigma-Aldrich).

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