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Research Article

Differential effect of low-dose thiazides on the renin angiotensin system in genetically hypertensive and normotensive rats

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Abstract

Fifty years since thiazide diuretics were introduced, they are established as first-line antihypertensive therapy. Because the thiazide dosing profile lessened, the blood pressure lowering mechanism may lie outside their diuretic properties. We evaluated this mechanism in spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY) by examining the effects of low-dose hydrochlorothiazide (HCTZ) administration on renin-angiotensin system components. The 7-day, 1.5 mg/kg per day HCTZ did not change systolic pressure (SBP) in WKY, but decreased SBP by 41 ± 2 mm Hg ($P < .0001$) in SHR, independent of increased water intake, urine output, or alterations in electrolyte excretion. HCTZ significantly increased the plasma concentrations of angiotensin I (Ang I) and angiotensin II (Ang II) in both WKY and SHR while reducing angiotensin-converting enzyme (ACE) activity and the Ang II/Ang I ratio (17.1 ± 2.9 before vs. 10.3 ± 2.9 after, $P < .05$) only in SHR. HCTZ increased cardiac ACE2 mRNA and activity, and neprilysin mRNA in WKY. Conversely in SHR, ACE2 activity was decreased and aside from a 75% increase in AT_1 mRNA in the HCTZ-treated SHR, the other variables remained unaltered. Measures of cardiac *mas* receptor mRNA showed no changes in response to treatment in both strains, although it was significantly lower in untreated SHR. These data, which document for the first time the effect of low-dose thiazide on the activity of the ACE2/Ang-(1-7)/*mas* receptor axis, suggest that the opposing arm of the system does not substantially contribute to the antihypertensive effect of thiazides. © 2008 American Society of Hypertension. All rights reserved.

Keywords: Hydrochlorothiazide; angiotensin II; AT_1 receptor; angiotensin converting enzyme 2; angiotensin-(1-7); hypertension.

Introduction

Thiazide diuretics, the cornerstone of hypertensive and heart failure drug therapy, are purported to exert their antihypertensive and cardiac unloading effects through increased sodium and water excretion. Their mechanistic action was identified when thiazides were prescribed at doses exceeding 50 mg/day. To avoid side effects, the doses were progressively reduced to less than half of the amount considered to exert diuresis when first introduced into the therapeutic armamentarium.

Acting at the cortical diluting tubular segment, high doses of thiazide diuretics induce negative changes in sodium and fluid balance while decreasing circulating blood volume and cardiac output.[1] Initially, peripheral vascular resistance increases to partially compensate for the reduction in cardiac output and arterial pressure. Long-term, both plasma volume and cardiac output are partly restored, keeping the blood pressure low by decreasing peripheral vascular resistance.[2] Because the biphasic nature of the hemodynamic response to thiazide administration was not fully understood,[1] additional studies explored an effect of these drugs on vascular reactivity. In 1992, Calder et al[3] investigated direct effects of low-dose hydrochlorothiazide (HCTZ) on human, guinea pig, and rat arterioles. This article and work by Pickkers et al,[4] showed that HCTZ induced vascular relaxation via stimulation of Ca^{2+}-activated K^+ channels secondary to a direct effect of HCTZ on vascular carbonic anhydrase. Additional studies suggested the vascular actions of HCTZ were mediated by a vascular endothelium-dependent mechanism,[5] whereas a detailed study in spontaneously hypertensive rats (SHR) showed that HCTZ

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inhibits agonist-induced vasoconstriction by calcium desensitization in smooth muscle cells linked to the Rho-Rho kinase pathway.[6]

Earlier studies demonstrated that the saluretic action of diuretics evoked heightened renal renin release from the attendant decrease in blood volume and renal perfusion pressure.[7] As the decreased HCTZ doses (< 25 mg/day) were administered to patients, the increase in plasma renin activity (PRA) seemed necessary to augment the angiotensin II (Ang II) blockade effect, and this rationale has remained unchallenged to date.[8–10]

There is no systematic analysis of the mechanism of action for low-dose thiazides as the majority of the early investigations utilized doses exceeding 50 mg/day. Another intricacy to ponder is the paradoxical acceptance of the tenet that amplifying the effect of angiotensin-converting enzyme (ACE) inhibitors and Ang II receptor blockers required the use of low-dose thiazide diuretics as this dogma insinuates that further increasing the activity of the renin-angiotensin system (RAS) is obligatory toward lower pressure through blockade of this pressor system.

Accumulating evidence that within the RAS, two distinct biochemical pathways yielding effector peptides conveying opposing actions led us to explore the hypothesis that low-dose thiazides may veer the balance between these two axes comprised of ACE/Ang II/AT_1 and angiotensin converting enzyme 2 (ACE2)/Ang-(1-7)/*mas* receptor. Accordingly, we investigated the effect of an equivalent low-dose HCTZ administration in SHR and Wistar Kyoto rats (WKY) on hemodynamics, fluid balance, and RAS activity.

Methods

Animals

Ten-week-old male SHR (n = 24) and WKY rats (n = 24) from Charles River Laboratories (Wilmington, MA) were housed in individual cages with free access to water and rat chow (Purina Mills, Richmond, VA) providing 17 and 28 mEq of sodium and potassium per 100 g/day, respectively. The American Association for Accreditation of Laboratory Animal Care (AAALAC) approved facility was maintained on a 12-hour light/dark cycle at constant temperature and humidity. All experiments were performed in accordance with protocols of the WFUSM Institutional Animal Care and Use Committee.

Experimental Protocol

The rats were randomly divided into four groups (WKY-vehicle, SHR-vehicle, WKY-HCTZ, and SHR-HCTZ, n = 12 per group). The vehicle-treated rats served as controls and drank tap water while the HCTZ groups received 3 mg/kg per day HCTZ dissolved in water on treatment day 1, which slightly increased urine output. Therefore, the dose was decreased to 1.5 mg/kg per day for the remaining six treatment days, a dose that decreased blood pressure, but did not induce polyuria. The rats were individually housed in metabolic cages to measure water intake, food consumption, and urine production. The HCTZ solution was adjusted daily to accommodate water intake and body weight variation.

Baseline tail-cuff blood pressures were recorded for 3 days and on treatment days 1, 3, 5, and 7. Once treatment terminated, the rats were anesthetized with halothane (1.5%; Halocarbon Laboratories, River Edge, NJ) and a catheter was inserted into the abdominal aorta to acquire blood for analysis. The heart was removed by cardio-pulmonary excision. The ventricles were isolated, weighed, submerged in liquid N_2, and stored at −80°C.

Biochemistry

The aortic blood samples were collected in an inhibitor cocktail and processed as previously reported.[11] The minimum detectable levels of these assays were 0.96 fmol/mL for angiotensin I (Ang I), 0.76 fmol/mL for Ang II, and 2.78 fmol/mL for Ang-(1-7). The intra- and interassay coefficients of variation were 18% and 22% for Ang I, 12% and 22% for Ang II, and 8% and 20% for Ang-(1-7). Plasma renin concentration (PRC) and serum ACE activity were measured as reported elsewhere.[11,12] ACE2 activity in cardiac membranes was quantified using the ACE2 fluorescent enzymatic assay as previously described.[13]

RNA Isolation and Reverse Transcriptase/Real–time Polymerase Chain Reaction

Cardiac ACE, ACE2, neprilysin, and AT_1 and *mas* receptor mRNAs were measured as previously detailed.[11] The results were quantified as Ct values, where Ct was defined as the threshold cycle of polymerase chain reaction at which amplified product is first detected, and expressed as relative gene expression (the ratio of target/18S rRNA control).

Western Blot Analysis

AT_1 receptor, *mas* receptor, and β-actin in the cardiac membranes were measured by Western blot hybridization as previously described[14] using an affinity purified polyclonal anti-AT_1 antibody (1:500) (Alpha Diagnostics, San Antonio, TX), a polyclonal anti-*mas* receptor antibody (1:2000) (developed by M.C. Chappell), or a monoclonal anti-β-actin antibody (1:2000) (Sigma, St. Louis, MO).

Statistical Analysis

Values are expressed as mean ± SEM. Normality and homogeneity were assessed prior to conducting a two-way analysis of variance with Bonferroni tests for each variable. Significant interactions were analyzed via 1-way analysis of variance with Bonferroni or unpaired *t* tests. $P < .05$ was considered statistically significant.

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