Hydrochlorothiazide Potentiates Contractile Activity of Mouse Cavernosal Smooth Muscle

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ABSTRACT

Introduction: Hydrochlorothiazide has a negative influence on penile erection but little is known about the mechanism(s) involved.

Aims: To characterize the effects of this diuretic on mouse corpus cavernosum (CC) smooth muscle in vitro and ex vivo.

Methods: CC strips of C57BL/6 mice (12−16 weeks old) were mounted in organ baths containing Krebs-Henseleit solution and tissue reactivity was evaluated. Expression of genes encoding diuretic targets and enzymes involved in penile erection were evaluated by polymerase chain reaction.

Main Outcome Measures: Stimulation-response curves to phenylephrine (10 nmol/L−100 μmol/L) or to electrical field stimulation (1−32 Hz) were constructed, with or without hydrochlorothiazide. Strips of CC from mice after long-term hydrochlorothiazide treatment (6 mg/kg/day for 4 weeks) with or without amiloride (0.6 mg/kg/day for 4 weeks) in vivo also were studied. Nitric oxide and Rho-kinase pathways were evaluated.

Results: Hydrochlorothiazide (100 μmol/L) increased the maximum response to phenylephrine by 64% in vitro. This effect was unaffected by the addition of indomethacin (5 μmol/L) but was abolished by N(ω)-nitro-L-arginine methyl ester (100 μmol/L). Hydrochlorothiazide (100 μmol/L) potentiated electrical field stimulation-induced contraction in vitro, but not ex vivo. Long-term treatment with hydrochlorothiazide increased the maximum response to phenylephrine by 60% and resulted in a plasma concentration of 500 ± 180 nmol/L. Amiloride (100μmol/L) caused rightward shifts in concentration-response curves to phenylephrine in vitro. Long-term treatment with hydrochlorothiazide plus amiloride did not significantly increase the maximum response to phenylephrine (+13%). Reverse transcriptase polymerase chain reaction did not detect the NaCl cotransporter in mouse CC. Hydrochlorothiazide did not change Rho-kinase activity, whereas amiloride decreased it in vitro and ex vivo (approximately 18% and 24% respectively). A 40% decrease in Rock1 expression also was observed after long-term treatment with hydrochlorothiazide plus amiloride.

Conclusion: Hydrochlorothiazide potentiates contraction of smooth muscle from mouse CC. These findings could explain why diuretics such as hydrochlorothiazide are associated with erectile dysfunction.

Key Words: Corpus Cavernosum; Erectile Dysfunction; Adrenergic Mechanism; Diuretics

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INTRODUCTION

Hydrochlorothiazide (HTZ) and other thiazide diuretics are commonly used as monotherapy or in combination with other drugs in the treatment of arterial hypertension. Although their primary site of action is in the kidney, the major hypotensive effect of thiazides during long-term administration is due to vasodilation rather than saluresis or loss of free water.[1−3] Thiazides cause vasorelaxation in vitro and in vivo, and the mechanisms proposed for this action are not related to their natriuretic effects (inhibition of the NaCl cotransporter).[4−8] For instance,

vasodilation of the human brachial artery in vivo induced by HTZ also occurs in patients with Gitelman syndrome, which is characterized by the presence of a non-functional thiazide-sensitive NaCl cotransporter.[9] Thiazides cause alterations in ion channel homeostasis and HTZ-induced relaxations appear to be mediated by Ca^{2+}-activated K^+ channels.[7,10,11] For example, the relaxant effect of HTZ in guinea pig isolated mesenteric small arteries was associated with a decrease in intracellular calcium concentration ($[Ca^{2+}]_i$).[7,12]

Thiazide and thiazide-like diuretics can cause sexual dysfunction in humans, especially with high doses of diuretics and hypertension.[13–19] Nitric oxide (NO) released from non-adrenergic, non-cholinergic nitrergic nerve endings and from the endothelium is the most important mediator of corpus cavernosum (CC) smooth muscle relaxation.[20] In contrast, noradrenaline, released from adrenergic nerves, is the major mediator of penile CC contraction.[21] Thus, an imbalance between the contractile and relaxant mechanisms of non-vascular smooth muscle could contribute significantly to the observed erectile dysfunction (ED) after thiazide therapy.[22]

AIMS

Using CC strips from mice, the effect of HTZ in vitro on the contraction of CC smooth muscle induced by phenylephrine or electrical field stimulation (EFS) was investigated. Because amiloride is often coadministered with HTZ to prevent the occurrence of hypokalemia,[23,24] its possible interaction on HTZ effects in vitro also was evaluated. The effects of long-term treatment in vivo with HTZ, with or without amiloride, on the contractility of CC smooth muscle ex vivo were investigated. The involvement of the NO and the Rho-kinase (ROCK) pathways in HTZ-induced altered CC contractility was studied. Expression of genes encoding the main target transporter for HTZ (NaCl cotransporter) and amiloride (epithelial sodium channel [ENaC]) also was assessed in the CC of mice.

METHODS

Animals

Male adult (3–4 months old) C57BL/6 mice (25–30 g) were obtained from the animal care facility of the State University of Campinas (Campinas, SP, Brazil) and used in this study. Animals were housed in temperature-controlled facilities on a 12-hour light-and-dark cycle with food and water ad libitum. All experimental procedures were conducted in accordance with institutional guidelines and were approved by the ethical principles in animal research adopted by the Brazilian College for Animal Experimentation.

Mouse CC Preparation

The animals were rendered unconscious by inhalation of CO_2 and then decapitated. The penises were surgically removed and immediately placed in chilled Krebs solution (NaCl 118 mmol/L, $NaHCO_3$ 25 mmol/L, glucose 5.6 mmol/L, KCl 4.7 mmol/L, KH_2PO_4 1.2 mmol/L, $MgSO_4$ 1.17 mmol/L, and $CaCl_2$ 2.5 mmol/L). Cavernosal strips were obtained by dissection of the tunica albuginea and surrounding connective tissues. Each penis resulted in two strips, one for each CC, that were vertically suspended between two metal hooks in 10-mL organ baths containing Krebs solution at 37°C continuously bubbled with a mixture of 95% O_2 and 5% CO_2 (pH = 7.4). One hook was connected to a force transducer (ADInstruments, Colorado Springs, CO, USA) and the other acted as a fixed attachment point. The resting tension was adjusted to 4 mN and CC strips were allowed to equilibrate for 60 minutes.[25–27] Changes in isometric force were recorded using a PowerLab 4/30 data acquisition system (Software Chart, version 7.0; ADInstruments).

Concentration-Response Curves

Cumulative concentration-response curves for phenylephrine (10 nmol/L–100 μmol/L) were constructed for the CC strips in the absence (control) or presence of HTZ (10, 30 and 100 μmol/L) and amiloride (100 μmol/L) added 30 minutes before phenylephrine. The NO synthase inhibitor N(ω)-nitro-L-arginine methyl ester (L-NAME; 100 μmol/L) or the cyclooxygenases inhibitor indomethacin (5 μmol/L) was used to evaluate any influence of NO or prostaglandins on HTZ effects in cavernosal contractility.[28–30] Each strip was used for only one concentration-response curve. The possible pharmacologic interaction between HTZ and amiloride in the contractile response to phenylephrine also was evaluated by incubating CC strips with HTZ (100 μmol/L) in the presence or absence of amiloride (100 μmol/L). To evaluate cholinergic-mediated relaxation, cumulative concentration-response curves were obtained by adding acetylcholine (ACh; 0.001–10 μmol/L) to CC strip pre-contracted with U46619 (9,11-dideoxy-11α, 9α-epoxymethanoprostaglandin F2α; 100 nmol/L)[31] in the absence (control) or presence of HTZ (100 μmol/L). All concentration-response data were evaluated for fit to a logistics function and non-linear regression analysis was used to determine the parameters maximal response and log half-maximal effective concentration (EC_{50}) using GraphPad Prism (GraphPad Software, San Francisco, CA, USA).

Electrical Field Stimulation

For EFS experiments, CC strips were placed between two platinum ring electrodes. EFS was carried out at 50 V for 10 seconds at a range of frequencies from 1 to 32 Hz in square-wave pulses (1-ms pulse width) using a Grass S88 stimulator (Astro-Med, West Warwick, RI, USA). Frequency-response curves to EFS were constructed to evaluate changes in basal tone of strips to EFS in the absence and presence of L-NAME (100 μmol/L) and/or HTZ (100 μmol/L).