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Paradoxical Antidiuretic Effect of Thiazides in Diabetes Insipidus: Another Piece in the Puzzle

JOHANNES LOFFING
Department of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland

Diabetes insipidus (DI) is a temporary or chronic disorder characterized by the excretion of excessive quantities of very dilute, but otherwise normal urine. The disease results either from impaired synthesis and secretion of antidiuretic hormone (ADH, vasopressin) by the hypothalamus and posterior pituitary, respectively (central DI), or from an unresponsiveness of the kidneys to the hormone itself (nephrogenic DI) (1–3). The renal water transport defect is located in the collecting system (*i.e.* the connecting tubule – CNT and the collecting duct – CD) in which vasopressin normally controls the expression and cell surface targeting of the apical water channel aquaporin-2 (AQP2) (4). The CNT and CD are also the site of amiloride-sensitive sodium reabsorption via the epithelial sodium channel (ENaC) (5). Sodium transport across ENaC may osmotically drive transepithelial water reabsorption. Consistently, both AQP2 and ENaC are regulated by vasopressin via V2-receptor-dependent cAMP production (4,5). While exogenous application of vasopressin efficiently corrects the reduced AQP2 expression and the urinary concentration defect in central DI (4), the treatment of nephrogenic DI is usually less obvious and may include different approaches such as dietary sodium restriction, prostaglandin synthesis inhibitors, potassium-sparing diuretics and/or thiazide diuretics (3,6). The use of diuretics for the treatment of a polyuric disease appears paradoxical, but the beneficial effect of thiazides has now been proven for more than 45 yr. In 1959, Crawford and Kennedy showed in a seminal paper that thiazides reduce polyuria and increase urine osmolality in DI (7). Since then, thiazides have become an important component in the therapeutic repertoire for treatment of DI. Nevertheless, the precise mechanisms by which thiazide diuretics elicit their paradoxical anti-diuretic effect in DI are still largely elusive.

Thiazide diuretics inhibit the NaCl co-transporter (NCC/TSC) in the renal distal convoluted tubule (DCT) (8). The DCT is water impermeable and considered to be part of the diluting segment (8). Therefore, the water-preserving effect of thiazides is unlikely related to a direct effect on the DCT. In fact, the most widely accepted hypothesis suggests that the antidiuretic action of thiazides is secondary to increased renal sodium excretion (1,2,6). The renal sodium loss causes extracellular volume contraction leading to lowered GFR and increased proximal tubular sodium and water reabsorption. Hence, less water and solutes are delivered to the distal tubule and collecting duct and are lost as urine (1,2,6). Indeed, micropuncture and renal clearance studies on normal rats (9–11) or rats with DI (12,13) support this hypothesis. However, there is also increasing evidence that sodium depletion and increased proximal water reabsorption does not solely account for the antidiuretic effect. Spannow *et al.* demonstrated that the replacement of renal sodium losses by a servo-controlled system does not prevent the acute antidiuretic effect of bendroflumethiazide (BFTZ) in a rat model with hereditary central DI (Brattleboro rats) (14). Moreover, studies by Walter and Shirley indicated that in Brattleboro rats sustained antidiuresis depends on continued application of HCTZ. After withdrawal of the drug, urinary flow rate returns to high values despite maintained sodium depletion (15). Furthermore, functional studies by Shirley *et al.* (12) and Gronbeck *et al.* (16) suggested that water reabsorption downstream of the proximal tubule must also contribute to the antidiuresis seen after prolonged thiazide treatment of central DI. Cesar and Magaldi reported that hydrochlorothiazide (HCTZ) is able to directly increase the water permeability in inner medullary collecting ducts isolated from normal or Brattleboro rats and perfused *in vitro* (17). Interestingly, in these experiments, addition of prostaglandins diminished the effect of thiazides, possibly explaining why prostaglandin synthesis inhibitors (*e.g.* indomethacin) augment the effect of thiazides in DI (17).

In this issue of JASN, Kim *et al.* (18) add another important piece to the puzzle of the paradoxical antidiuretic effect of thiazides. In a rat model of lithium-induced nephrogenic DI, the authors describe that HCTZ treatment is able to modulate the expression of renal proteins important for water and sodium reabsorption in the collecting system (18). The employed experimental model is of major clinical importance since nephrogenic DI is a frequent side effect of lithium therapy (*e.g.* for bipolar mood disorders) (6). The mechanism by which lithium causes nephrogenic DI is not precisely clear but appears to involve altered expression levels of water and sodium transporting proteins in the kidney. Marples *et al.* established by immunoblotting, immunofluorescence, and immunogold electron microscopy that chronic (for weeks) lithium treatment of rats induces nephrogenic DI that goes along with a marked

Correspondence to Dr. Johannes Loffing, Department of Pharmacology and Toxicology, University of Lausanne, Rue du Bugnon 27, CH-1005 Lausanne, Phone: +41–21–692 53 68; Fax: +41–21–692 53 55; E-mail: johannes.loffing@ipharm.unil.ch

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reduction of AQP2 in the apical plasma membrane of CNT and CD cells (19). Moreover, prolonged lithium treatment decreases the expression of NCC in the DCT and of ENaC in the CD, possibly explaining the increased renal sodium excretion under lithium therapy (20).

Now, Kim and co-workers show that HCTZ partially recovers AQP2 abundance from lithium-induced down-regulation. In addition, the investigators describe that HCTZ increases the abundance of NCC, ENaC α-subunit and a lower molecular weight form of ENaC γ-subunit in kidneys of lithium treated rats (18). The latter changes are ascribed, at least in part, to elevated plasma aldosterone levels (18) since similar changes in the expression profile of NCC and ENaC are found in normal rats in response to aldosterone application (21,22). The stimulatory effect of HCTZ on NCC protein abundance is surprising in view of previous data showing DCT cell apoptosis and reduced NCC mRNA and/or protein abundance after several days of thiazide (HCTZ or metolazone) treatment of normal rats (23,24). Differences in the experimental protocols may explain these discrepant results. The mechanism by which HCTZ partly restores AQP2-expression in lithium-induced nephrogenic DI is unclear. It is explicitly not related to an unintentional effect of HCTZ on renal lithium clearance since the lithium serum levels did not significantly differ between vehicle and HCTZ-treated rats with lithium-induced DI (18). Whatever the underlying mechanism, the data of Kim *et al.* provide an additional and novel explanation (*i.e.* partially restored AQP2 expression) for the antidiuretic effect of HCTZ in lithium-induced nephrogenic DI. However, it is important to note that up-regulation of AQP2 is not necessarily involved in the antidiuretic effect of thiazides in all forms of DI. At least in Brattleboro rats with central DI, chronic thiazide (BFTZ) treatment reduces urine volumes without any detectable effect on the expression and subcellular localization of AQP2 in the epithelial cells lining the collecting system (16). Future studies will need to define whether thiazides improve AQP2 expression also in other forms of nephrogenic DI with reduced AQP2 levels (*e.g.* chronic hypokalemia (25), hypercalcemia (26), postobstructive diuresis (27), cisplatin-induced polyuria (28)) or whether the effect of HCTZ might be peculiar to lithium-induced DI.

Lithium-induced DI can present with peculiar features as indicated by recent studies of Christensen and co-workers (29). These authors showed that chronic lithium administration profoundly changes the cellular composition of the renal CD in a way that has not been reported for any other model of nephrogenic or central DI. In healthy mammals, the CD is composed by two major cell types (*i.e.* principal and intercalated cells). While the principal cells contain AQP2 and ENaC and are responsible for transepithelial water and sodium transport, the intercalated cells are mainly involved in the renal control of acid-base homeostasis and lack AQP2- and ENaC-expression. Cristensen *et al.* found that chronic lithium treatment profoundly decrease the fraction of principal cells and increased the abundance of intercalated cells in the CD. This epithelial remodeling was most pronounced in the medullary CD. The authors speculated that the decreased water reabsorption in the collecting system of lithium-treated rats is probably the result of both a reduced density of AQP2-expressing cells and a decrease in AQP2 expression in remaining principal cells (29). In reverse, these data raise the intriguing possibility that the profound effect of HCTZ on the renal AQP2- abundance in lithium-induced DI might involve, at least partly, a reversal of the lithium-induced epithelial remodeling in the CD. The higher density of AQP2-expressing CD profiles in the renal inner medulla of HCTZ- *versus* vehicle-treated rats with lithium-induced DI [Figure 3 of the paper of Kim *et al.* (18)] may support this hypothesis. Remarkably, chronic treatment with acetazolamide, a carbonic anhydrase inhibitor, has been reported to significantly reduce the fraction of intercalated cells in the renal inner medulla (30). HCTZ has carbonic anhydrase-inhibiting properties beside its effects on NCC (31). Obviously, the paradoxical effect of thiazides in DI is complex and the puzzle is still not completely solved.

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See related article, "Antidiuretic Effect of Hydrochlorothiazide in Lithium-Induced Nephrogenic Diabetes Insipidus Is Associated with Upregulation of Aquaporin-2, Na-Cl Co-transporter, and Epithelial Sodium Channel?," on pages 2836–2843.