

## PPAR $\gamma$ Agonists, Modulation of Ion Transporters, and Fluid Retention

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Peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) agonists, also known as thiazolidinediones (TZDs), are used as insulin-sensitizing agents to treat type 2 diabetes. Examples include pioglitazone (Actos<sup>®</sup>), rosiglitazone (Avandia<sup>®</sup>), and others such as troglitazone, farglitazar (GI262570), and GW7845. Side effects of TZD therapy include fluid retention and peripheral edema. The weight gain from fluid retention is undesirable from a cosmetic standpoint but more seriously can adversely affect comorbidity in the diabetic state.<sup>1</sup>

Reported effects of PPAR $\gamma$  agonists on cardiovascular function are mixed. One recent high-profile study found that rosiglitazone increases the risk of death from cardiovascular disease,<sup>2</sup> although other studies observe beneficial effects of pioglitazone on major cardiovascular events in humans<sup>3</sup> and protection against ischemia–reperfusion injury and reduction of myocardial infarct size in animal models.<sup>4</sup> Regardless of the risk–benefit relationships of PPAR $\gamma$  agonist therapy, it is clear that fluid retention exacerbates compromised cardiac function and lessens patient compliance with drug treatment. Unfortunately, PPAR $\gamma$ -agonist-induced fluid retention is refractory to most first-line diuretic therapies.<sup>5</sup>

The nature of PPAR $\gamma$ -agonist-induced fluid retention suggests an integrated physiologic response that includes a primary effect on renal regulation of salt and water balance. PPAR $\gamma$  is expressed in the collecting duct, suggesting that ligands for this receptor modulate salt and water homeostasis through ion transport systems in this nephron segment. The epithelial Na<sup>+</sup> channel (ENaC) expressed in collecting duct principal cells is regulated by steroid (aldosterone) and peptide hormones (insulin, IGF-1, and vasopressin) and represents a key control point for the modu-

lation of salt reabsorption and, consequently, BP. It is logical, therefore, to postulate that ENaC is a likely target of a PPAR $\gamma$  agonist effect. Unfortunately, studies performed to substantiate this relationship are contradictory, and it is difficult to establish a consensus as to potential mechanisms.

Two different collecting-duct-specific PPAR $\gamma$  null mouse models showed significant reductions in PPAR $\gamma$ -agonist-induced weight gain and plasma volume expansion compared with those of wild-type littermates,<sup>6,7</sup> substantiating the notion that the collecting duct plays a primary role in the development of PPAR $\gamma$ -induced volume expansion. Primary cell cultures derived from the collecting duct of wild-type, but not from collecting-duct-specific PPAR $\gamma$  null mice, show increased amiloride-sensitive <sup>22</sup>Na<sup>+</sup> flux in response to PPAR $\gamma$  agonist challenge.<sup>6,7</sup> In contrast, in separate studies, neither pioglitazone nor rosiglitazone increased ENaC activity in three well-characterized culture models of principal cells as measured by short-circuit current electrophysiology.<sup>8</sup> Interestingly, 24-h treatment with either GI262570 or pioglitazone decreased vasopressin-stimulated, amiloride-sensitive current in Madin–Darby canine kidney clone 7 (MDCK-C7) cells.<sup>9</sup>

Biochemical investigations demonstrate agonist-mediated changes in ENaC subunit mRNA or protein abundance or in proteins known to regulate ENaC with little consensus across studies. Several groups found no change in ENaC subunit mRNA concentrations in rodents treated with PPAR $\gamma$  agonists, whereas other studies found increases in ENaC $\alpha$ , decreases in ENaC $\beta$ , and either increases or decreases in ENaC $\gamma$ . Serum glucocorticoid-induced kinase, an intracellular mediator that regulates ENaC expression, increases upon exposure to PPAR $\gamma$  agonists in some studies but not in others.

*In vivo* studies using ENaC inhibitors have been unable to clarify the question of the importance of the ENaC in PPAR $\gamma$ -mediated fluid retention. The data regarding the efficacy of amiloride, a selective ENaC blocker, in alleviating fluid retention are contradictory, with one study showing no effect<sup>10</sup> and another showing complete reversal of water-induced weight gain.<sup>7</sup>

To more definitively address the involvement of ENaC, Vallon *et al.* used a mouse model containing a collecting-duct-specific gene inactivation of ENaC $\alpha$ . In these mice, functional inactivation of ENaC did not protect animals against rosiglitazone-induced weight gain, fluid retention, or decreased hematocrit.<sup>11</sup> These data argue against a primary role for ENaC in agonist-mediated fluid retention.

Taken together, the composite data are consistent with the collecting duct as the site at which PPAR $\gamma$  agonists act to induce fluid retention. The data do not, however, make a strong case for ENaC as the primary target of the agonists. In

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light of what is currently understood about renal physiology and the role of ENaC in BP and volume regulation, several anomalies in physiologic principles also argue against stimulation of ENaC as the initial target of PPAR $\gamma$ -agonist-mediated fluid retention.

Clinical data show that stimulation of ENaC activity leads to an increase in Na<sup>+</sup> and water reabsorption, resulting in an increase in BP. Human gain-of-function mutations in ENaC (Liddle's syndrome) cause severe hypertension early in life. As mentioned previously, aldosterone and insulin also increase ENaC-mediated Na<sup>+</sup> absorption. Hyperaldosteronism arising from pituitary tumors is not an uncommon cause of hypertension. We and others postulate that the hyperinsulinemia seen in prediabetic states contributes to the development of the accompanying hypertension. In contrast, ENaC inhibitors, such as amiloride, or naturally occurring loss-of-function mutations in the channel lead to salt wasting and a decrease in BP.

A meta-analysis of 37 clinical trials examining correlations between PPAR $\gamma$  agonists and BP shows that these drugs lower BP.<sup>12</sup> Consistent with these results, human loss-of-function mutations in endogenous PPAR $\gamma$  are associated with severe insulin resistance and with early onset hypertension.<sup>13</sup> Thus, there is a consensus that PPAR $\gamma$  agonists decrease BP and loss of PPAR $\gamma$  increases BP.

This presents an interesting quandary when evoking ENaC-mediated mechanisms to simultaneously explain PPAR $\gamma$ -agonist-mediated increases in fluid retention and decreases in BP. The correlation between Na<sup>+</sup> reabsorption and BP in PPAR $\gamma$ -agonist-treated subjects is the converse of what would be predicted if the effects were mediated by increases in ENaC abundance or activity. Accumulating data raise the possibility that changes in Na<sup>+</sup> balance during PPAR $\gamma$  agonist therapy may be secondary to other, more immediate responses.

Although the role of Cl<sup>-</sup> in the regulation of body fluid homeostasis is still speculative, regulation of fluid balance by this ion in individual organ systems is well accepted. Cystic fibrosis is characterized by dehydration of airway surface liquid and pancreatic, salivary, seminal, and vaginal secretions. The pathology of cystic fibrosis is more complex than dehydrated secretions, although the basic presentation of the disease underscores the importance of Cl<sup>-</sup> in the regulation of local fluid balance.

The paradigm that changes in renal Cl<sup>-</sup> flux can alter whole-body salt and fluid homeostasis is supported by naturally occurring mutations. The ClC-Kb Cl<sup>-</sup> channel is expressed in the thick ascending limb of Henle's loop and the distal convoluted tubule. Loss of function of this channel results in Bartter syndrome type III, an autosomal-recessive, salt-wasting tubulopathy.<sup>14</sup> Conversely, a gain-of-function genetic variant of ClC-Kb is associated with salt-sensitive increases in BP, predisposing people with the polymorphism to hypertension.<sup>14</sup> This link between BP regulation and Cl<sup>-</sup> transport substantiates a heretofore unappreciated role for anions in salt and fluid homeostasis.

In the MDCK-C7 cell line, a model of principal cells, PPAR $\gamma$  agonists inhibit vasopressin-stimulated Cl<sup>-</sup> secretion with agonist dose-response relationships that mirror receptor

transactivation profiles.<sup>9</sup> The PPAR $\gamma$ -agonist-induced decrease in anion secretion is the result of decreases in levels of mRNA encoding CFTR (cystic fibrosis transmembrane regulator). There are numerous reports describing changes in renal and plasma Na<sup>+</sup> and K<sup>+</sup> concentrations in response to treatment with PPAR $\gamma$  agonist. Remarkably, one study shows a statistically detectable increase in plasma Cl<sup>-</sup> concentration after a 4-d challenge with GI262570.<sup>10</sup> These data are consistent with those describing decreased secretion of the anion in response to PPAR $\gamma$  agonists in continuous cell lines.

The paradigm of PPAR $\gamma$ -mediated decreases in CFTR expression also is validated by studies in intestinal cells. Oral administration of rosiglitazone to mice for 8 d reduces intestinal forskolin-stimulated anion secretion and substantially inhibits cholera-toxin-induced intestinal fluid accumulation.<sup>15</sup> In HT29 intestinal cells, 5 d of treatment with rosiglitazone inhibits cAMP-dependent Cl<sup>-</sup> secretion concomitantly with a decrease in the protein expression of CFTR, Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup>, and KCNQ1.<sup>15</sup> Thus, the strongest and most consistent evidence to date suggests that the primary effect of PPAR $\gamma$  agonists in polarized epithelia is a decrease in the expression of Cl<sup>-</sup> channels.

In summary, a compendium of data suggests that PPAR $\gamma$  agonists cause fluid retention through effects on the renal collecting duct. Because hormonal regulation of ENaC plays a major role in electrolyte and fluid homeostasis, it is logical to hypothesize that this transporter is the target of PPAR $\gamma$  action. However, physiologic principles argue against a stimulation of ENaC simultaneously with a decrease in BP. We postulate that the initial flux of fluid from the vasculature may be driven by the changes in Cl<sup>-</sup> balance. Na<sup>+</sup> and fluid retention would be a compensatory response to the loss of fluid from the vasculature and into the interstitial space. Additional *in vivo* investigations are needed to fully substantiate the hypothesis of regulation of Cl<sup>-</sup> transport in PPAR $\gamma$ -agonist-induced changes in volume homeostasis and to elucidate the specific role of the CFTR Cl<sup>-</sup> channel in normal regulation of salt and fluid balance.

## DISCLOSURES

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## Harnessing Transporters to Clear Uremic Toxins

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The constancy of the *milieu intérieur* described by Claude Bernard is an absolute prerequisite for the health of the organism. The primacy of the kidney in whole organism homeostasis is best exemplified by the disturbance in the composition and amount of body fluid and solute when this organ fails to function. In simplistic terms, one can envision renal failure as the inability to remove what should be excreted and inability to add what should be added to the body. The inability to excrete the appropriate amount of water and various solutes is well studied and serves as a marker in clinical practice to evaluate the degree of dysfunction and adequacy of therapy. There are also many additional substances that are not properly excreted in renal failure, the accumulation of which contributes to the uremic state, but these are less well studied in terms of their metabolism, mechanism of action, or mode of excretion.

The expanding group of proven and putative uremic toxins has recently been highlighted by the European Uremic Toxin Work Group (<http://EUTox.info>) and the count has reached over 110 moieties.<sup>1</sup> This highly diverse group of substances ranges from inorganic solutes to organic substances including acids, guanidine, peptides, indoles, nucleotides, peptides, and others.<sup>2</sup> The chemical properties of these moieties are as expansive as their identities, with a broad range of molecular weight (outside the 10- to 30-kD middle molecule class), hydrophobicity, protein binding, a host of post-translational modifications, and a myriad of target organs and mechanisms to impart damage. Many of these molecules are normal constituents of the *milieu intérieur* when their levels are maintained within discreet ranges. Clearly no single biologic system will possess the broad span to handle the excretion of this vast group of molecules. Excretion of these molecules in health includes some contribution from hepatic conjugation, but they largely rely on glomerular filtration or tubular secretion by a host of transporter proteins.

In general, one can devise two broad categories of countermeasures to combat pernicious uremic toxins. First is attempting to directly block their actions (e.g., angiotensin receptor blockade) or neutralize downstream effects on target organs (e.g., alkali replacement), and a second is their enhanced removal either by improvement of dialytic clearance (e.g., large-pore dialysis membranes), inhibition of production (e.g., calcimimetics for parathyroid hormone), or enhancement of endogenous clearance perhaps by pharmacologic means. All of these approaches have been attempted for various uremic toxins over the years. In this issue of *JASN*, the paper by Toyohara and coworkers<sup>3</sup> demonstrates success of one of the methods—enhancement of endogenous clearance—which has been attempted the least.

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