Troglitazone inhibits glutamine metabolism in rat mesangial cells

ROBERT ROUTH, KEVIN McCARTHY, AND TOMAS WELBOURNE
Departments of Pathology and Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, Shreveport, Louisiana 71130

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Routh, Robert, Kevin McCarthy, and Tomas Welbourne. Troglitazone inhibits glutamine metabolism in rat mesangial cells. Am J Physiol Endocrinol Metab 282: E231–E238, 2002.—Troglitazone is a peroxisome proliferator-activated receptor-γ agonist that has been shown to halt mesangial expansion in experimental models of type 2 diabetes mellitus and to act directly on rat mesangial cells. Because glutamine serves as the precursor for cellular biosynthetic processes, we asked whether troglitazone would inhibit mesangial cell glutamine metabolism under these conditions. Confluent monolayers of rat mesangial cells were incubated in RPMI medium in the presence of troglitazone or vehicle (DMSO). Troglitazone effected a dose-dependent reduction in glutamine utilization and in alanine formation, associated with a decrease in monolayer collagen-glycosaminoglycan content. Despite the reduced glutamine uptake, ammonium formation did not decrease, consistent with increased glutamate flux through the deamination pathway. Assayable activity of the alanine aminotransferase decreased by 63%, whereas assayable glutamate dehydrogenase remained unchanged. In control monolayers, the sum of ammonium plus alanine plus glutamate nitrogen released accounted for <75% of the glutamine nitrogen uptake. In troglitazone-treated monolayers, all of the glutamine nitrogen taken up could be accounted for as ammonium nitrogen released into the medium. These results are consonant with troglitazone reducing glutamine metabolism and specifically the transamination pathway in rat mesangial cells associated with a reduction in collagen-glycosaminoglycan content.

Troglitazone inhibits glutamine metabolism in rat mesangial cells because of troglitazone's effect of inhibiting matrix expansion in vivo (10, 12, 14) and in vitro (24) and because glutamine supports protein synthesis (9, 20). However, it is not known whether mesangial cells utilize glutamine, because arginine is present as the other major nitrogenous precursor in the standard RPMI medium (16). Thus there are two potential substrates available for protein synthesis in vitro, and it is not known whether troglitazone inhibits utilization of these precursors. Therefore, our purpose was to determine whether troglitazone inhibits amino acid utilization under in vitro conditions in which matrix protein production is limited (24), and if so, whether this effect is limited to glutamine and to a specific pathway.

The major fuels both in vivo for the intact functioning kidneys (21) and for in vitro culture conditions (22) are glutamine and glucose, and the putative metabolic pathways involved in their utilization by mesangial cells in culture are shown in Fig. 1. Although troglitazone has been shown to directly enhance glucose uptake and accelerate glycolysis (7), no previous information is available concerning an effect of troglitazone on amino acid metabolism. Thus monitoring glucose uptake and lactate production provides a positive control for troglitazone’s only known metabolic action. Troglitazone-enhanced glucose uptake in muscle (7) is coupled to the formation of lactate with the possibility of 2 mol lactate/mol glucose utilized. Whether and how glutamine is metabolized by mesangial cells in culture are not known; however, these cells do not express the phosphatase-independent glutaminase found in most epithelial cells so that glutamine hydrolyzed to glutamate and ammonium is by the intracellular glutaminase (Fig. 1, reaction 1). The pivotal intermediate is glutamate, because it can either be transaminated (Fig. 1, reaction 2) by alanine aminotransferase (ALT), present in both the cytosol and mitochondria (13), or deaminated (Fig. 1, reaction 3) by the mitochondrial glutamate dehydrogenase (GDH) (17, 27). Nevertheless, glutamate metabolized by both pathways yields the same carbon product, α-ketoglutarate (AKG$^{2-}$), which can be

Address for reprint requests and other correspondence: T. Welbourne, Dept. of Molecular and Cellular Physiology, LSUHSC, Shreveport, LA 71130 (E-mail: twelbo@lsuhsc.edu).

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TROGLITAZONE IS A THIAZOLIDINEDIONE that exhibits insulin-sensitizing activity and has been shown to attenuate hyperinsulinemia and hyperglycemia in human subjects with type 2 diabetes mellitus (5). In addition to its insulin-sensitizing activity, troglitazone halts mesangial expansion and reduces proteinuria in insulin-resistant diabetic animals (10, 14), as well as reducing production of extracellular matrix in isolated mesangial cells (24). These findings point to troglitazone actions other than its insulin-sensitizing activity in ameliorating the expansion of extracellular matrix occurring in diabetic states.

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transamination pathway associated with a reduction in glutamine metabolism by virtually eliminating the results to follow demonstrate that troglitazone reduces glutamine uptake as well as the nitrogenous products formed when incubated with troglitazone, and glutamine may support mesangial cell protein synthesis either as an energy fuel or nitrogen source or both.

To determine whether troglitazone inhibits glutamine utilization and to assess the site(s) of inhibition, confluent rat mesangial monolayers were incubated with troglitazone, and glutamine uptake as well as the nitrogenous products formed was determined. The inhibitory effect of troglitazone on glutamine metabolism was then related to the monolayer total protein and collagen-glycosaminoglycan content. The results to follow demonstrate that troglitazone reduces glutamine metabolism by virtually eliminating the transamination pathway associated with a reduction in mesangial cell collagen-glycosaminoglycan content.

MATERIALS AND METHODS

Cell culture. Glomerular mesangial cells were cultured as previously described (11, 24). Briefly, glomeruli were isolated by differential sieving from kidneys obtained from male Sprague-Dawley rats weighing 300 g. Isolated glomeruli were then cultured in RPMI 1640 medium (BioWhitaker, Walkersville, MD) containing 17% fetal calf serum and antibiotics. Mesangial cells were identified by their stellate shape and their ability to form hillocks in culture. The cells were seeded in either 24- or 12-well plates and used for experiment after gaining confluence. After confluence, the cells were exposed to serum-free RPMI medium (16) containing 5 mM glucose and either 5, 10, or 20 μM troglitazone (gift of Dr. Hardwick Johnson, Parke-Davis, Ann Arbor, MI) dissolved in DMSO or DMSO alone. The medium was then harvested and the cells washed with ice-cold PBS (3×) and lysed in 50 mM Tris buffer containing 0.1% Triton X-100 and the protease inhibitors (in mM): 0.2 phenylmethylsulfonyl fluoride, 5 benzamidine, and 10 EDTA, pH 7.2. Aliquots of the medium were processed for amino acid analysis by HPLC (30) or used directly for enzymatic analysis of glucose and lactate or microdiffusion analysis of ammonia. Cell lysates were used for ALT, GDH, total protein, and collagen assays.

Amino acid analysis. Medium samples were promptly treated with an equal volume of ice-cold 5% trichloracetic acid, left on ice for 10 min, and then centrifuged at 10,000 g for 10 min. Aliquots of the protein-cleared supernatant containing free amino acids were then treated with O-phthalaldehyd e (FLUKA, Buchs, Switzerland) for precolumn derivatization and injected onto a C18 4.6 × 250 mm column (Microsorb, Varian, Walnut Creek, CA) for separation of the derivatized amino acids. The column effluent was passed through a fluorescence detector, with peaks for the major amino acids at characteristic retention times as shown in Fig. 2. Figure 2A shows the retention times for standards: aspartate, 7.85; glutamate, 11.2; serine, 15.7; glutamine, 16.4; homoserine (internal standard), 17.8; arginine, 18.3; and alanine, 20.5 min (Asp and Arg at 0.5 mM; all others 1 mM). Figure 2B shows the amino acid profile of the serum-free RPMI 1640 medium incubated for 72 h in the absence of cells. Note the absence of an alanine peak at 20.5 min in the serum-free RPMI 1640 medium. Figure 2C shows the effect of incubating mesangial cells for 72 h on the amino acid profiles. Figure 2D shows the effect of incubating mesangial cells plus 10 μM troglitazone on the same amino acid profile; note the ability to monitor the major amino acids involved in nitrogen balance as well as to detect the diminished alanine peak. The medium concentration of the major amino acids was obtained from the peak areas in B, C, and D divided by the peak areas shown in A for their respective standards.

Utilization or production rates for the respective amino acids were obtained from the concentration differences observed between B and either C (control) or D (troglitazone treated) times the medium volume, 1–2 ml. These rates were then expressed as milligrams of total protein.

Total protein, collagen, and glycosaminoglycan assays. Total protein in the monolayers was determined in triplicate on the cell lysates by using the dye-binding assay with albumin as the standard (1). Total monolayer collagen content was determined as the total soluble collagen by using the Sircol Collagen Assay kit (Biocolor, Belfast, Northern Ireland). Aliquots of the cell lysates were added to 1 ml of Sircol dye reagent that binds to collagen, followed by 30 min of mixing. After centrifugation at 10,000 g for 10 min, the supernatant was carefully decanted, and 1 ml of 0.5 M NaOH was added. Samples and collagen standards were then read at 540 nm on a spectrophotometer. Collagen concentration in the lysate buffer was then read from a standard curve with the use of acid-soluble type 1 collagen. Collagens contain ~14% hydroxyproline, and collagen concentration obtained using Sircol binding correlates well with the hydroxyproline content (Sircol Collagen Assay, Biocolor). The sulfated proteoglycan and glycosaminoglycan content of mesangial cell monolayers was determined in the aforementioned cell lysates with a quantitative dye-binding assay (Blyscan Proteoglycan & Glycosaminoglycan Assay, Biocolor). The assay uses 1,9-dimethylmethylene blue as a dye label, with sample concentrations determined using a calibration curve prepared with glycosaminoglycan standards (0–5.0 μg).

Ammonium determination. Ammonium concentration was determined by the microdiffusion method (30), and formation rate was determined as described by subtracting the medium blank and expressing it as milligrams of protein.
**Glucose and lactate determination.** Glucose concentration was measured enzymatically with hexokinase (Sigma, St. Louis, MO) and glucose-6-phosphate dehydrogenase (Sigma), the latter catalyzed reaction converting glucose 6-phosphate and NAD to 6-phosphogluconate and NADH; coupled NAD conversion to NADH was monitored at 340 nm. Lactate concentration was determined enzymatically using lactate dehydrogenase (Sigma) coupled to the reduction of NAD to NADH monitored as described. The assay conditions included a hydrazine trap for pyruvate to force the reaction to completion. Utilization of glucose and production of lactate were calculated as for the amino acids and are expressed as milligrams of protein.

**Enzymatic assays.** Both of the enzymes catalyzing the conversion of glutamate to AKG, Fig. 1, reactions 2 and 3, were assayed using the cell lysate and carried out under optimal conditions as follows. ALT activity was assayed in the reverse direction to that shown in Fig. 1, reaction 2, by use of 1 M alanine and 0.3 M AKG to produce glutamate and pyruvate (32); conversion of pyruvate to lactate was then coupled to NADH oxidation in the presence of lactate dehydrogenase, with the rate of decrease in absorbency at 340 nm being directly proportional to the amount of cell lysate buffer protein added. GDH activity was assayed in the reverse direction to that shown in Fig. 1, reaction 3, using 3.3 M NH₄Cl and 0.23 mM AKG and being coupled to the oxidation of NADH measured as a decrease in absorbency at 340 nm as described (25); the rate of decrease in absorbency was directly proportional to the amount of cell lysate protein added. Results are expressed in units per milligram of protein (1).

**Statistical analysis.** Differences between control and troglitazone-treated monolayers were analyzed using either Student’s *t*-test or ANOVA and a corrected *t*-test (Bonferroni) for multiple doses with differences considered significant at *P* < 0.05.
RESULTS

As shown in Fig. 2 C, glutamine, and not arginine, was the major nitrogen source for mesangial cell protein synthesis, and its uptake was reduced in response to troglitazone (Fig. 2, C vs. D). Therefore, we focused on glutamine uptake and specifically the products of the pathways depicted in Fig. 1. The response of glutamine uptake and alanine and ammonium formation to 5, 10, and 20 μM of troglitazone for 24 h is shown in Fig. 3, A, B, and C, respectively. Glutamine uptake (Fig. 3A) was decreased at 10 (P < 0.01) and 20 μM (P < 0.01) troglitazone by 32 and 37%, respectively (961 ± 67 and 884 ± 68, respectively, vs. 1,406 ± 69 nmol/mg protein for control), whereas the 20% decrease at 5 μM troglitazone did not achieve statistical significance (1,125 ± 78, P < 0.0.1). In contrast, alanine formation (Fig. 3B) was reduced by 72, 84, and 95% (from 868 ± 110 to 240 ± 35, 135 ± 11, and 40 ± 12 nmol/mg protein) at 5, 10, and 20 μM troglitazone, respectively (all P < 0.01, ANOVA and corrected t-test). Ammonium production (Fig. 3C) increased 1.6-fold at 5 μM troglitazone (2,036 ± 242 vs. 1,262 ± 113 nmol/mg protein, P < 0.05 vs. control), followed by a decline to values not different from the controls at 10 and 20 μM despite the reduced glutamine utilization. These results show that troglitazone markedly inhibits alanine formation in a dose-dependent manner, with a reduction in overall glutamine utilized (significant at the higher doses, 10 and 20 μM, of troglitazone).

Figure 4 shows the formation of alanine by control monolayers and by monolayers exposed to 10 μM troglitazone over a 72-h time course. At 12 h, troglitazone decreases the formation of alanine by 73% (6.8 ± 0.2 vs. 25 ± 2 nmol·h⁻¹·mg⁻¹ for control, P < 0.001), whereas glutamine uptake was unchanged (81 ± 12 vs. 75 ± 10 nmol·h⁻¹·mg⁻¹, respectively). After an additional 12 h, the alanine formation decreases to only 9% of the control monolayer (28 ± 4 vs. 2.4 ± 2 nmol·h⁻¹·mg⁻¹, P < 0.004). At 48 and 72 h, troglitazone still suppresses alanine formation to a rate that is less than 20% of the control monolayer alanine formation rate.

Figure 5 shows the total protein and collagen content of mesangial monolayers incubated for 48 h with vehicle or 5, 10, and 20 μM troglitazone. Total protein content decreased at 20 μM troglitazone by 38%, but the total protein content in monolayers treated with 5 and 10 μM was not significantly reduced. In contrast to the total protein, collagen content significantly decreased (29%, P < 0.05; 48%, P < 0.01; 52%, P < 0.01) at 5, 10, and 20 μM troglitazone, respectively. These results show that troglitazone markedly reduces the collagen content of mesangial cells and can do so at a concentration of troglitazone (5 μM) that inhibits the transamination but has little inhibitory effect on glutamine utilization.

The effects of incubating mesangial cells with 10 μM troglitazone on glutamine and glucose uptake are shown in Fig. 6. Mesangial cells readily take up glucose and released lactate, whereas troglitazone increased both glucose uptake (10,684 ± 198 vs. control, 8,671 ± 50 nmol/mg, P < 0.01) and lactate release (9,851 ± 1,150 vs. control, 6,344 ± 413 nmol/mg, P < 0.05). This confirms that troglitazone’s enhancement of glucose utilization observed in other cells (7) can also be dem-
onstrated in cultured mesangial cells. Noteworthy, troglitazone increased lactate formation relatively more than the glucose uptake (55 vs. 23%), consistent with a shift to anaerobic glycolysis as previously noted (7). In contrast to the increase in glucose uptake, troglitazone decreased glutamine uptake by 30% (1,525 ± 6231 vs. 2,176 ± 6376 nmol/mg, \( P < 0.01 \)) and glutamate release by 41% (245 ± 35 vs. 413 ± 60 nmol/mg, \( P < 0.005 \)).

Table 1 presents the balance between nitrogen taken up as glutamine’s amino and amide nitrogen (glutamine \( \triangleq \)) and the nitrogen released as the sum of ammonium, glutamate, and alanine. In the absence of troglitazone, only 73% of the glutamine nitrogen removed could be accounted for as the sum of ammonium, alanine, and glutamate nitrogen (3,192/4,350 nmol/mg \( \times 100 \)). Therefore, in the control monolayers there is a large positive nitrogen balance. In contrast, with 10 \( \mu \)M troglitazone, all of the glutamine nitrogen could be accounted for, predominantly as ammonium (3,106/3,050 nmol/mg). As shown in Fig. 2, C and D, serine was taken up in the control monolayers (383 ± 20 nmol/mg), and this uptake reversed to a small release with troglitazone (63 ± 18 nmol/mg, \( P < 0.0001 \) vs. control uptake). After 72 h, the total protein content remained unchanged (1,229 ± 32 and 1,132 ± 42 mg/monolayer), whereas collagen content was decreased by 40% (40 ± 2 to 24 ± 2 \( \mu \)g/monolayer, \( P < 0.001 \)), and glycosaminoglycan content decreased by 38% (5.8 ± 0.3 to 3.6 ± 0.3 \( \mu \)g/monolayer, \( P < 0.01 \)).

The decrease in alanine production shown above was associated with a 63% reduction (6 ± 1 vs. 16 ± 2 U/mg, \( P < 0.05 \)) in the assayable alanine aminotransferase activity, as shown in Fig. 7. Addition of troglitazone (10–100 \( \mu \)M) directly to the assay medium did not inhibit ALT activity of the control homogenates. On the other hand, cellular alanine content decreased \( (P < 0.01) \) in troglitazone-treated monolayers from 232 ± 20 nmol/mg protein in control to 72 ± 6 nmol/mg protein at 10 \( \mu \)M troglitazone \( (P < 0.01) \). Although ammonium formation increased by 47% (3,106 ± 336 vs. 2,115 ± 402 nmol/mg, \( P < 0.05 \)), the assayable glutamate dehydrogenase activity remained unchanged (13 ± 1 vs. 14 ± 2 U/mg; Fig. 7).

DISCUSSION

Thiazolidinediones are agonists (2) for the nuclear receptor peroxisome proliferator-activated receptor-\( \gamma \) (PPAR\( \gamma \)), which plays an important regulatory role in the metabolism of lipids and glucose (5, 6). An effect of troglitazone on glutamine metabolism is novel and, if through PPAR\( \gamma \), would provide the first evidence that this pluripotential receptor regulates glutamine metabolism. Because troglitazone has been shown to limit extracellular matrix formation by mesangial cells in vivo in diabetic (10, 14) and nondiabetic animal models (12), and because matrix protein synthesis is, in part, dependent on glutamine (3, 28, 29), we monitored the effect on both glutamine metabolism and extracellular matrix production as reflected in the collagen and glycosaminoglycan content. Our results suggest a relationship between glutamine metabolism and mesangial cell matrix formation, specifically through troglitazone inhibition of the transamination pathway(s). We will discuss first the evidence for the inhibition of glutamine transamination by troglitazone and then
the consequence of this effect for overall glutamine metabolism; finally, we will relate this, as far as possible, to the decrease in extracellular matrix protein.

These results demonstrate a novel action of troglitazone of inhibiting glutamine-dependent alanine formation. The evidence for this is that troglitazone decreases alanine formation in a dose-dependent manner (Fig. 3B) associated with a decreased glutamine utilization (Fig. 3A; Table 1). We know that this represents a reduction in glutamate transamination, because parallel studies in monolayers incubated with [2-15N]glutamate show that troglitazone inhibits 15N alanine production (31). We know that this effect of reducing glutamate transamination is not secondary to the reduction in glutamine uptake, because the inhibition of alanine formation quantitatively exceeded the decrease in glutamine uptake (Fig. 3, A and B); furthermore, the decrease in alanine formation occurring at 12 h (Fig. 4) preceded any decrease in glutamine uptake. These results indicate that the site of inhibition was not primarily at the glutamine uptake or glutaminase step but point to the transamination site. In addition, troglitazone reduced the activity of the enzyme carrying out this reaction, ALT activity (Fig. 7), supporting the flux measurement. Taken together, these findings are supportive of troglitazone’s primary site(s) of action being at the transamination pathway.

Because the inhibition of alanine formation exceeded the reduction in glutamine uptake, the question arises as to the fate of glutamate formed by the glutaminase reaction (Fig. 1). Clearly, there was not an increased efflux of glutamate from these cells in the presence of troglitazone, rather just the opposite (Fig. 6). However, there was a large increase in ammonium formation (nearly 800 nmol/mg at 5 μM; Fig. 3C) that approximates the decrease in alanine formation (Fig. 3B). These findings are consistent with an accelerated flux of glutamate through the deamination pathway (18, 27). At higher concentrations, troglitazone inhibited glutamine utilization but not the ammonium formation (Fig. 3C), suggesting that the flux through the GDH pathway was maintained despite the reduced overall glutamine utilization. In support of this, studies with [2-15N]glutamine have shown that troglitazone enhances the flux of glutamate through the GDH pathway, leading to the formation of labeled ammonium (31). However, unlike the inhibition of the alanine formation, assayable GDH activity was not increased (Fig. 7). These results are therefore consistent with troglitazone acting to inhibit the transamination pathway and also accelerating glutamate flux through the deamination pathway (Fig. 1). Whether this reflects multiple sites of troglitazone action or a single action that inhibits flux through the transamination while accelerating flux through the deamination pathway remains to be determined.

Troglitazone’s effect of inhibiting glutamine metabolism is novel, although its non-insulin-like enhancement of glucose uptake has been demonstrated in vitro (7). Because glucose utilization may be reciprocally related to glutamine utilization (34), enhanced glucose uptake might primarily inhibit glutamine utilization and secondarily reduce the flux through the transamination pathway. However, as discussed above, alanine formation was decreased prior to and was quantitatively greater than glutamine utilization, so that the effect of glucose uptake would have to be primarily at the transamination site. A mechanism for glucose utilization to specifically inhibit the transamination reaction is not readily apparent. Increased lactic acid formation, if it led to an intracellular acidosis, might account for the increase in glutamate flux through the deamination pathway (18, 27). If the transamination reaction was also inhibited with a cellular acidosis, then much of what is observed could be explained by a decrease in intracellular pH.

Other mechanisms to explain the reduced transamination flux could be considered. Because ALT operates at near equilibrium in cells (13), a rise in intracellular alanine might occur, and this would drive the reaction in reverse. However, cellular alanine concentration actually decreased with troglitazone and therefore favors the transamination of glutamate, pointing to a reduction in the activity of the enzyme. Note that this is not a direct effect, because adding up to 100 μM troglitazone to the assay medium did not inhibit the

Table 1. Glutamine nitrogen uptake and recovery as NH₄⁺, Ala, and Glu⁻ nitrogen vs. collagen and GAG content

<table>
<thead>
<tr>
<th></th>
<th>Gln × 2</th>
<th>NH₄⁺</th>
<th>Ala</th>
<th>Glu⁻</th>
<th>Sum</th>
<th>Coll</th>
<th>GAG</th>
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<tr>
<td>Control</td>
<td>4,350 ± 754</td>
<td>2,115 ± 401</td>
<td>+</td>
<td>664 ± 119</td>
<td>413 ± 60</td>
<td>=</td>
<td>3,192 ± 571</td>
</tr>
<tr>
<td>Tro (10 μM)</td>
<td>3,050 ± 460*</td>
<td>3,106 ± 335*</td>
<td>+</td>
<td>37 ± 16*</td>
<td>245 ± 35*</td>
<td>=</td>
<td>3,388 ± 369</td>
</tr>
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Results are means ± SE in nmol nitrogen/mg protein or µg/monolayer for collagen and glycosaminoglycan (GAG) with n = 6 per group. Tro, troglitazone. *Difference from control with P < 0.05.

Fig. 7. ALT and GDH activities from control and troglitazone-treated cell homogenates. Results are means ± SE from 6 control and 6 troglitazone-treated monolayers. *Different from control, P < 0.05.

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activity. On the other hand, assayable ALT activity is reduced after exposure to troglitazone, consistent with a reduction in the amount of ALT protein. One possible mechanism for troglitazone to downregulate ALT is through PPARγ, suggesting that troglitazone may act as a partial agonist (2), possibly by competing for this receptor’s ligand-binding site with a true agonist (19). Note that serine/pyruvate/alanine glyoxalate aminotransferase plays an important role in serine metabolism (33), and although we did not assay for this activity, troglitazone virtually eliminated serine uptake. The observation that aminotransferase activities may be upregulated by PPARγ activation raises the possibility that transamination reactions are important pathways through which troglitazone exerts its anti-diabetic action (as antagonists, with reduced gluconeogenesis) and, considering the importance of hepatic alanine uptake, possibly contributing to hepatic injury.

The increase in ammoniagenesis promoted by troglitazone was not associated with an increase in assayable GDH activity, suggesting that cell pH may be decreased (31). One potential pathway for troglitazone to reduce the intracellular pH would be to decrease protein kinase C activity (10), which in turn limits the activity of sodium/hydrogen ion exchange (31). Another mechanism for inducing an intracellular acidosis might be through an increased acid load, resulting from enhanced lactic acid production (Fig. 6). Further studies designed to test these putative mechanism(s) involved in regulating both alanine and ammonium formed are necessary.

Glutamine has been reported to influence the synthesis of matrix protein through both the amino and amide nitrogens (3, 20, 28, 29) and to support the energy demands for protein synthesis as an oxidizable fuel (9). Thus glutamine potentially plays multiple roles as a provider of precursor amino acids through transamination reactions as well as by providing the amide nitrogen for glycosaminoglycan synthesis and more generally by providing energy for protein synthesis. Our observations show that matrix protein content is reduced by as much as 30% at 5 μM troglitazone concentration without a corresponding reduction in either total protein content (Fig. 4) or glutamine uptake (Fig. 3A). These observations argue against a role for glutamine solely as a source of energy for overall protein synthesis. Because both collagen and sulfated proteoglycans plus glycosaminoglycans fall almost 40% with >80% inhibition of the transamination pathway, this suggests that troglitazone inhibition of transamination may be coupled in some manner to the reduction in matrix protein formation. However, we have not measured collagen synthesis, so that the decrease in total collagen might reflect either a decreased synthesis or an increased catabolism, or both. Under hyperglycemic and even normoglycemic conditions, collagen synthesis is associated with increased glucose transporter (GLUT-1) activity (4, 8), and agents that enhance glucose uptake also increase the synthesis of extracellular matrix components (4). In this regard, increased glucose uptake coupled to the hexosamine pathway has been shown to stimulate transforming growth factor-β (TGF-β), production (26), and although we did not assay for TGF-β, others have shown that production of this proinflammatory cytokine is downregulated by troglitazone in vivo (10, 12). TGF-β may up-regulate glutaminase activity and transamination reactions and thereby provide precursors for subsequent matrix protein synthesis, e.g., proline, glutamate, and aspartate. Regardless of the mechanism, a reduction in glycosaminoglycan content of mesangial cells may, in turn, limit the availability and activation of associated positively charged chemokines (15, 26) that modulate numerous cell functions through effects on matrix proteins. Thus troglitazone actions on these cellular functions may be, in part, through effects on glutamine metabolism and secondarily may contribute to the modulation of pathophysiological processes.

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