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SHORT COMMUNICATION

Comparative Effects of Englitazone and Glyburide on Gluconeogenesis and Glycolysis in the Isolated Perfused Rat Liver

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ABSTRACT. Englitazone (CP 68,722, Pfizer) is a member of a family of drugs known as thiazolidinediones. One member of this family, troglitazone (Rezulin), is currently utilized in the treatment of Type 2 diabetes. Previous studies have focused on the ability of englitazone to increase insulin sensitivity in various tissues. However, little information is available regarding the direct effect of englitazone on hepatic glucose metabolism in the absence of insulin. Therefore, the following studies were conducted to comparatively evaluate the effect of englitazone and glyburide (a representative sulfonylurea) on gluconeogenesis and glycolysis from various substrates in the isolated perfused rat liver (IPRL). In isolated perfused rat livers of 24-hr fasted rats infused with lactate (2 mM), englitazone (6.25 to 50 μM) produced a concentration-dependent decrease (32–93%) in hepatic gluconeogenesis. When dihydroxyacetone (1 mM) and fructose (1 mM) were used as metabolic substrates, englitazone inhibited gluconeogenesis by 31 and 15%, respectively, while increasing glycolysis by 42 and 50%. Similar effects on gluconeogenesis and glycolysis were observed with glyburide, even though the effects with glyburide were more acutely evident, reversible, and of a greater magnitude. Such data suggest alterations in hepatic glucose production may contribute to the decrease in plasma glucose concentrations observed in individuals treated with englitazone and glyburide. These alterations may include effects on several regulatory enzymes (e.g. fructose-1,6-bisphosphatase, pyruvate kinase, and phosphoenolpyruvate carboxykinase), which warrant further investigation. BIOCHEM PHARMACOL **55**;11:1915–1920, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. englitazone; glyburide; thiazolidinedione; sulfonylurea; gluconeogenesis; glycolysis

Type 2 diabetes, formerly known as NIDDM†, affects over 15 million people in the United States. This disease is characterized by hyperglycemia due to increased hepatic glucose production and peripheral insulin resistance [1]. Currently, sulfonylureas, biguanides, and thiazolidinediones are used in the treatment of Type 2 diabetes. Sulfonylureas act primarily as insulin secretagogues to increase insulin secretion from the pancreatic β cells. In addition, sulfonylureas have extrapancreatic effects that enhance their ability to maintain blood glucose homeostasis. Such extrapancreatic effects include decreased hepatic gluconeogenesis [2] and stimulated synthesis of glucose transporters [3]. While all sulfonylureas address the insulin secretory defects of Type 2 diabetes, only specific sulfonylureas possess the limited capacity to improve insulin sensitivity in peripheral tissues [4,5]. Therefore, alternate pharmacological therapies are being utilized clinically to treat Type 2 diabetes, including biguanides, glucosidase inhibitors, and thiazolidinediones [6].

Thiazolidinediones address a major physiological defect in Type 2 diabetes by attenuating insulin resistance in diabetic animal models [7–9]. Englitazone (CP 68,722, Pfizer), a representative thiazolidinedione, enhances the action of insulin without producing hypoglycemia [9–11], stimulates glucose transport in adipocytes [10, 11], and blocks excess glucose from entering cells and being converted to triglycerides [12]. Blackmore *et al.* [13] suggested that the primary effect of englitazone is to improve the response of peripheral tissues to insulin via post-binding events. Their studies compared the actions of englitazone to those of insulin, and the authors concluded that the post-binding actions of the two are similar. Bowen *et al.* [14] have shown that englitazone acts on the liver as well as on peripheral tissues, such as fat and skeletal muscle tissue, to augment insulin sensitivity. Other studies have demonstrated that englitazone has "insulin-independent" or insulinomimetic effects on 3T3-L1 adipocytes [10] and rat hepatocytes [15]. Indeed, studies in isolated rat hepatocytes have shown englitazone to inhibit basal and glucagon-stimulated gluconeogenesis in the absence of insulin [13, 15]. Because such effects on hepatic glucose production have also been observed with certain sulfonylureas [2], the

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† *Abbreviations:* NIDDM, non-insulin-dependent diabetes mellitus; IPRL, isolated perfused rat liver; and F-2,6-P_2, fructose-2,6-bisphosphate.

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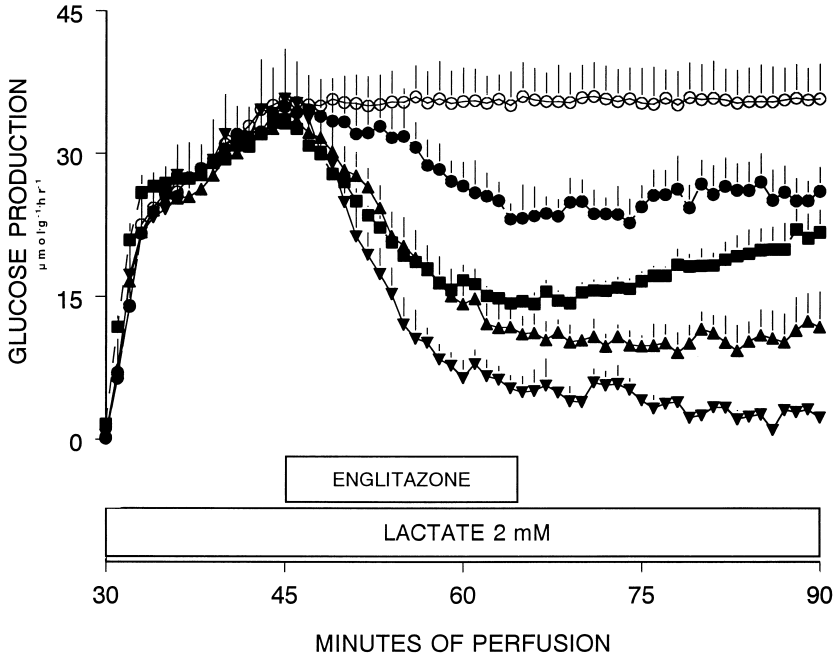

FIG. 1. Effect of various englitazone concentrations (6.25 μM, closed circles; 12.5 μM, squares; 25 μM, upright triangles; or 50 μM, inverted triangles) and vehicle (open circles) on the rates of gluconeogenesis in the livers of 24-hr fasted rates perfused with lactate (2 mM). After a 30-min, equilibration period, lactate and vehicle or englitazone were infused into the liver for periods indicated by the horizontal bars. Data represent means ± SEM for 3 separate perfusions.

aim of this study was to comparatively examine the effects of englitazone and glyburide on gluconeogenesis and glycolysis in the IPRL. Glucose, lactate, and pyruvate levels were measured when the IPRL was subjected to various gluconeogenic substrates, including lactate, fructose, glycerol, and dihydroxyacetone.

MATERIALS AND METHODS

Chemicals

All enzymes and cofactors were purchased from Boehringer Mannheim. The substrates, chemicals, and glyburide were purchased from the Sigma Chemical Co. Racemic englitazone [(±)-5-[(3,4-dihydro-2-phenylmethyl-2*H*-1-benzopyran-6-yl) methyl] thiazolidine-2,4-dione, sodium salt] was the gift of Dr. Ralph Stevenson (Pfizer). All chemicals were of the highest purity commercially available.

Animals

Male Sprague–Dawley rats (250–280 g body weight) were used in these studies. Animals were fasted 24-hr prior to perfusion of their livers.

IPRL

After pentobarbital sodium anesthesia (50 mg/kg), rat livers were perfused employing the non-recirculating perfusion technique described by Scholz *et al.* [16]. The hemoglobin-free perfusion medium Krebs-Henseleit bicarbonate buffer (KHBC) was saturated with 95% oxygen/5% carbon dioxide. The buffer was maintained at 37° (pH 7.40) and a flow rate of 30 mL/min. The various substrates and drugs were infused into the livers with a syringe pump at a point just before the hepatic portal vein cannula. After a 30-min equilibration period, the perfusate was collected at 30-sec intervals for an additional 60 min. Englitazone and glyburide were dissolved in DMSO immediately prior to infusion. The final concentration of DMSO to which the liver was exposed was always less than 0.5% (v/v) and had no effect on the parameters measured. The same concentration of DMSO was utilized in the vehicle perfusions.

Enzymatic Assays

Glucose, lactate, and pyruvate were assayed in the effluent samples according to previously described enzymatic methods [17–19]. To determine the rate of gluconeogenesis from a given substrate, the rate of hepatic glucose production was measured in the absence and presence of the gluconeogenic substrate. The rate of glucose output by the liver was corrected for the endogenous rate of glucose production.

Statistical Analysis

All perfusion experiments illustrated are representative of three separate experiments. Data from all experiments are expressed as means ± SEM.

RESULTS

Initially, a concentration–response relationship for englitazone was established, evaluating the effects of various concentrations of englitazone on hepatic gluconeogenesis from lactate. Figure 1 demonstrates that an increase in the

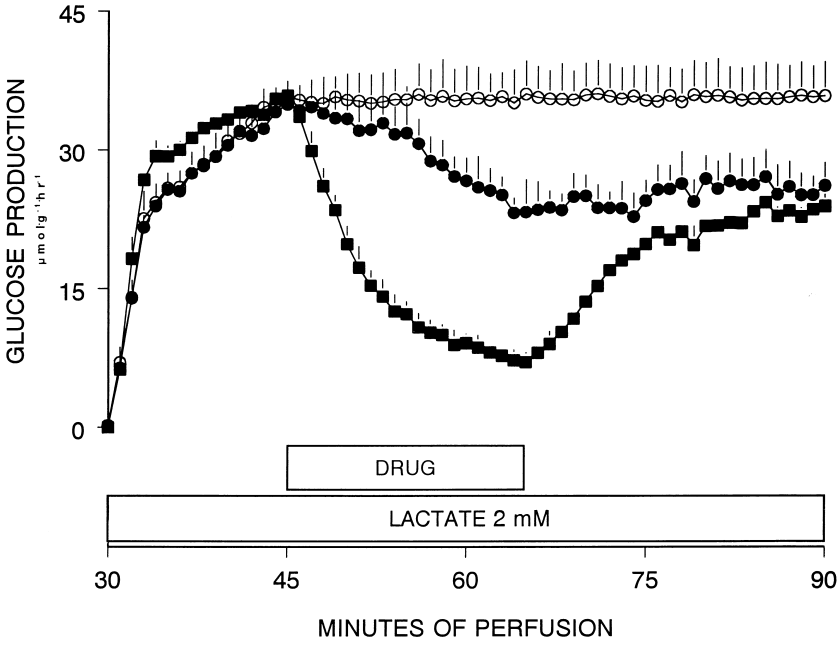

FIG. 2. Effect of englitazone (6.25 μM, closed circles), glyburide (20 μM, squares), and vehicle (open circles) infusion on rates of glucose production in livers of 24-hr fasted rats perfused with lactate (2 mM). After a 30-min equilibration period, lactate and vehicle, englitazone, or glyburide were infused into the liver for periods indicated by the horizontal bars. Data represent means ± SEM for 3 separate perfusions. (NOTE: Englitazone data are reproduced from Fig. 1).

concentration of englitazone (6.25 to 50 μM) resulted in a concentration-dependent decrease in the rate of gluconeogenesis in livers perfused with 2 mM of lactate. Specific percentage inhibitions were 50 μM, 93%; 25 μM, 70%; 12.5 μM, 52%; and 6.25 μM, 32%. Subsequent studies were conducted with the 6.25-μM concentration, since this concentration was equal to or less than blood levels of englitazone measured in the hepatic portal vein during animal studies [9]. Figure 2 compares englitazone (6.25 μM) and a representative sulfonylurea, glyburide (20 μM), using lactate (2 mM) as the gluconeogenic substrate. Both of these agents inhibited lactate-stimulated gluconeogenesis, glyburide by 80% and englitazone by 32%. The rate and magnitude of the inhibition, however, were different between the two agents. In the case of englitazone, inhibition was slow (within 16 min) and not reversible during the 90-min perfusion time period. Glyburide, on the other hand, produced a more rapid and greater inhibition (within 11 min), which was reversible to a certain degree. In Fig. 3, hepatic gluconeogenesis from dihydroxyacetone (1 mM), a substrate that does not involve the pyruvate-oxaloacetate-phosphoenolpyruvate cycle for glucose synthesis, was reversibly inhibited by 31 and 46% upon initiation of englitazone and glyburide infusion, respectively. In addition to the profound effects on dihydroxyacetone-stimulated gluconeogenesis, both compounds stimulated lactate and pyruvate production (glycolysis; Fig. 3). However, with glycerol (1 mM) as the gluconeogenic precursor (data not shown), neither englitazone nor glyburide had a significant effect on the rate of gluconeogenesis or glycolysis. Results of experiments with fructose (Fig. 4), which enters the gluconeogenic pathway at the triosephosphate level, were similar to those of dihydroxyacetone. Englitazone inhibited gluconeogenesis by approximately 15% and increased glycolysis by approximately 50%. Glyburide had no effect on fructose-stimulated gluconeogenesis, while stimulating glycolysis by 1.9-fold. A paradoxical rebound increase in fructose-stimulated gluconeogenesis was observed at the termination of both drug infusion periods. This increase was likely the result of a reduction in the drug-induced inhibition of fructose-1,6-bisphosphatase and glucose-6-phosphatase, thereby allowing increased conversion of fructose-1,6-bisphosphate and glucose-6-phosphate to glucose.

DISCUSSION

Recent studies have demonstrated that the thiazolidinediones, typified by englitazone, pioglitazone, and troglitazone, enhance insulin action in skeletal muscle, adipose tissue, and the liver. These compounds bind to and activate a nuclear peroxisome proliferator-activated receptor (PPAR) that specifically regulates gene transcription, eventually resulting in the expression of specific proteins that improve insulin action in the cell [20]. In addition, thiazolidinediones increase the level of glucose transporter protein expression, resulting in the transport of more glucose into the cell for utilization and storage [20]. Some studies have suggested that the thiazolidinediones may have insulinomimetic attributes in addition to their insulin-sensitizing properties [10, 15, 21]. The data presented in this study clearly demonstrated that a representative thiazolidinedione, englitazone, acutely inhibits gluconeogenesis and stimulates glycolysis from a variety of substrates in the absence of insulin. The inhibition of lactate-stimulated gluconeogenesis was concentration-dependent, but not reversible during the designated perfusion time period (Fig. 1). In comparison, glyburide, a representative sulfonylurea, rapidly and reversibly inhibited gluconeogenesis from lactate (Fig. 2).

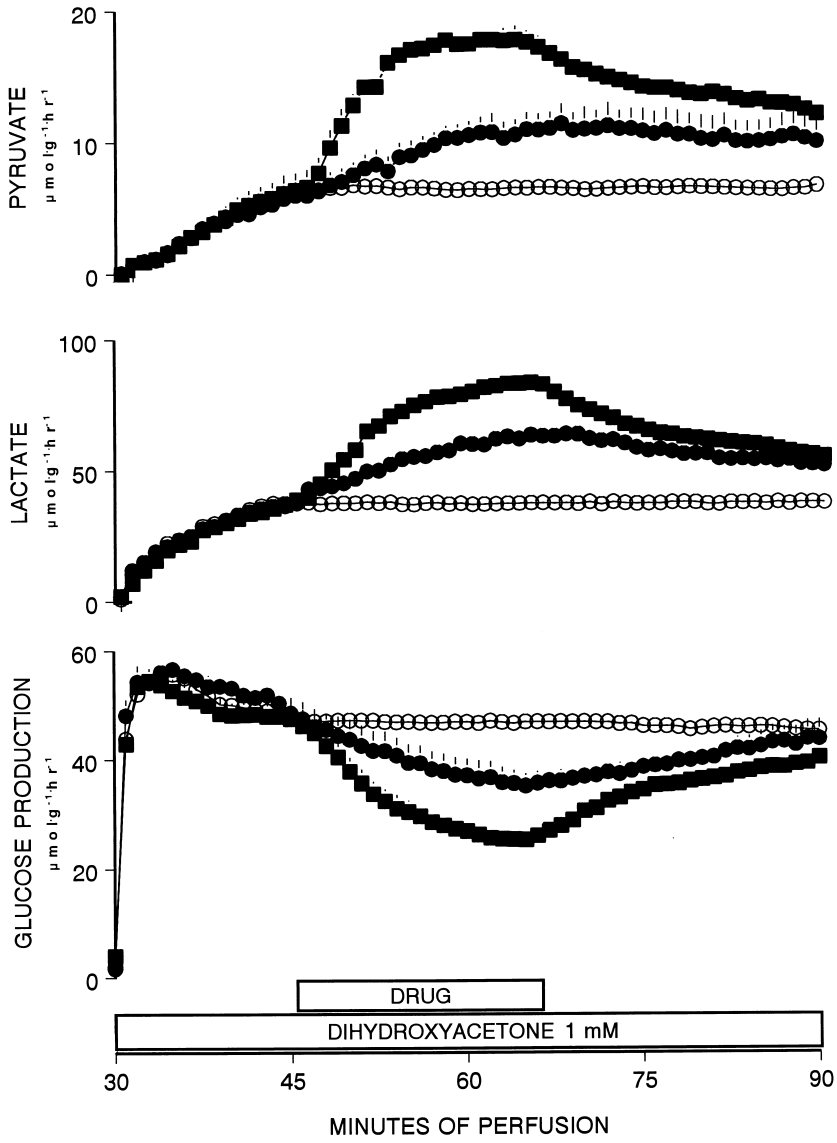

FIG. 3. Effects of englitazone (6.25 μM, closed circles), glyburide (20 μM, squares), and vehicle (open circles) infusion on rates of glucose, lactate, and pyruvate production in livers of 24-hr fasted rats perfused with dihydroxyacetone (1 mM). After a 30-min equilibration period, dihydroxyacetone and vehicle, englitazone, or glyburide were infused into the liver for periods indicated by the horizontal bars. Data represent means ± SEM for 3 separate perfusions.

Such data may help explain the decrease in hepatic glucose production observed upon thiazolidinedione or sulfonylurea administration. The differences in the onset and duration of the effect are likely due to differences in the chemical properties of the two drugs [22, 23].

Because both compounds inhibited glucose production from lactate, studies were subsequently conducted to determine which specific regions of the gluconeogenic/glycolytic pathways were responsible for this inhibition. Data from studies using dihydroxyacetone (Fig. 3), fructose (Fig. 4), and glycerol (data not shown) suggest that there may be multiple metabolic sites of action for these compounds. First, it is possible that the inhibition of gluconeogenesis observed with both englitazone and glyburide could be at least partially explained by increased F-2,6-P_2 levels during the drug-infusion periods. Such an increase in F-2,6-P_2 would stimulate phosphofructokinase activity and inhibit fructose-1,6-bisphosphatase activity, thus leading to an inhibition of gluconeogenesis. Indeed, studies in isolated rat hepatocytes have demonstrated that troglitazone [24–26], glipizide [27], and tolbutamide [26] increase the concentration of F-2,6-P_2, and this increase was correlated inversely with the rate of gluconeogenesis. However, such an increase in F-2,6-P_2 would not completely explain the observed inhibition of gluconeogenesis, since it has been demonstrated in the liver that alterations in F-2,6-P_2 do not have dramatic effects on gluconeogenesis from lactate [28]. Because both englitazone and glyburide stimulate glycolysis from dihydroxyacetone and fructose, regulatory steps that do not involve the phosphofructokinase reaction may also be involved. Therefore, besides inhibiting gluconeogenic reactions at the level of fructose-1,6-bisphosphatase, englitazone and glyburide may be stimulating glycolytic reactions. It may be speculated that these structurally dissimilar compounds activate the pyruvate kinase reaction, perhaps by elevating levels of the potent allosteric activator of this enzyme, fructose-1,6-bisphosphate. Such an activation of the pyruvate kinase reaction not only would explain the

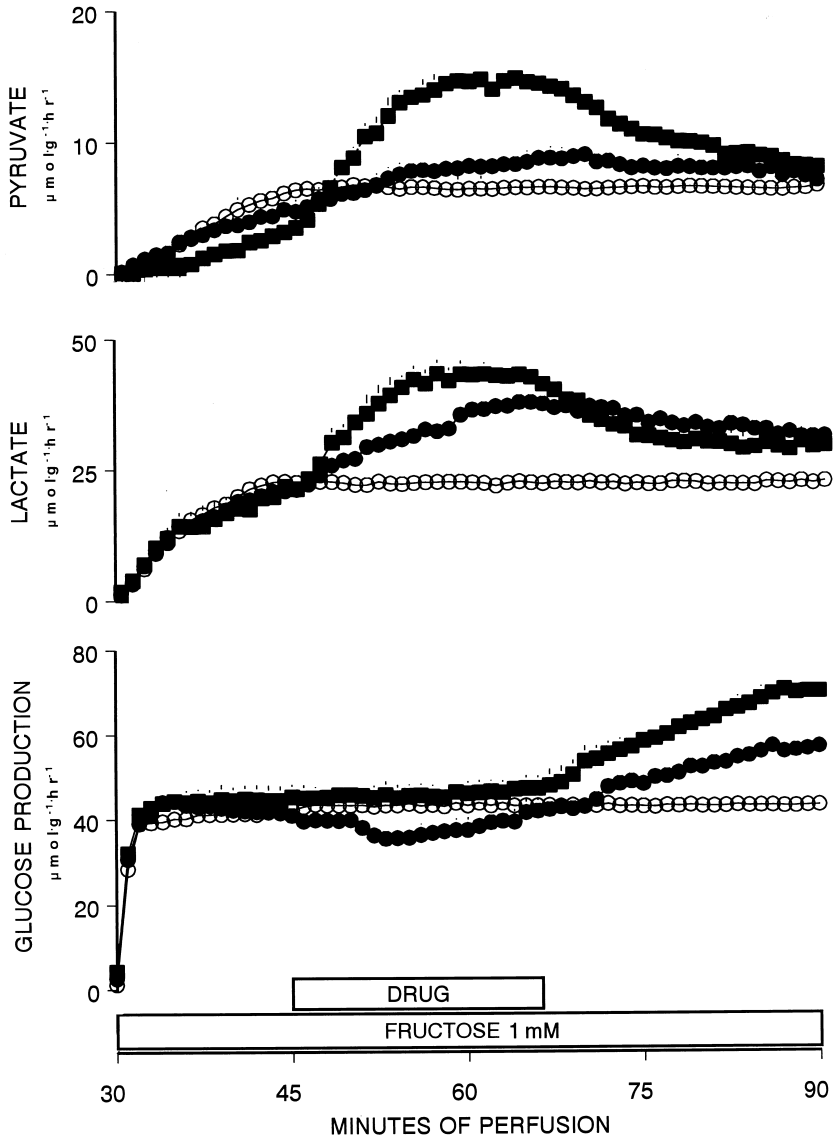

FIG. 4. Effects of englitazone (6.25 μM, closed circles), glyburide (20 μM, squares), and vehicle (open circles) infusion on rates of glucose, lactate, and pyruvate production in livers of 24-hr fasted rats perfused with fructose (1 mM). After a 30-min equilibration period, fructose and vehicle, englitazone, or glyburide were infused into the liver for periods indicated by the horizontal bars. Data represent means ± SEM for 3 separate perfusions. [NOTE: Englitazone inhibited fructose-stimulated gluconeogenesis by 15% ($P < 0.05$), whereas glyburide did not have an effect on gluconeogenesis during the drug-infusion period].

stimulation of lactate and pyruvate production from dihydroxyacetone and fructose, but also would explain inhibition of gluconeogenesis from lactate. Studies in isolated rat hepatocytes, however, have not supported a direct or indirect effect of glyburide on pyruvate kinase flux, while demonstrating that tolbutamide can stimulate pyruvate kinase flux, depending on the metabolic state of the cell [29]. In addition to pyruvate kinase, other gluconeogenic and glycolytic regulatory enzymes may be involved in the effects produced by englitazone and glyburide, including pyruvate carboxylase [29], phosphoenolpyruvate carboxykinase [30], and glucokinase [30]. Since glucose production from dihydroxyacetone was inhibited by glyburide (not englitazone) to a lower extent than from lactate (46% from dihydroxyacetone vs 80% from lactate), additional enzymatic steps located downstream from the reaction catalyzed by glycerol 3-phosphate dehydrogenase may be involved. Englitazone is serving as a model compound for the development of other antidiabetic agents in the thiazolidinedione family [31]. Troglitazone [Rezulin, Parke–Davis] was approved recently by the FDA for patients with Type 2 diabetes currently on insulin therapy. Studies in our laboratory have demonstrated similar insulinomimetic effects between englitazone and troglitazone using a variety of rat hepatic model systems ([25]; unpublished data).

In summary, the data presented here demonstrate that englitazone and glyburide, at concentrations reported to be therapeutically effective, inhibited the rate of gluconeogenesis from lactate and dihydroxyacetone, while stimulating glycolysis from lactate, dihydroxyacetone, and fructose. It is most interesting that the effects of both agents on gluconeogenesis/glycolysis were produced in the absence of insulin. Thus, even though englitazone and glyburide are considered to function primarily as insulin sensitizers and insulin secretagogues, respectively, both compounds have direct effects on hepatic glucose metabolism. Although activation of phosphofructokinase and inactivation of fructose-1,6-bisphos-

phatase reaction may be one of the mechanisms involved in decreased hepatic gluconeogenesis, other regulatory steps may also be involved.

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