Biochemical Pharmacology, Vol. 53, pp. 67–74, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(96)00660-0

In Vitro and *In Vivo* Suppression of Gluconeogenesis by Inhibition of Pyruvate Carboxylase

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ABSTRACT. The mechanism of inhibition of gluconeogenesis by phenylalkanoic acids was studied *in vitro* and *in vivo*. *In vitro* production of $^{14}CO_2$ from labeled glucose or palmitate was not inhibited at 4 mM, a concentration of phenylacetic acid that inhibited gluconeogenesis from lactate/pyruvate. *In vitro* studies with isolated mitochondria showed that the CoA ester of phenylacetic acid was formed. The parent phenylalkanoic acid had no effect on purified pyruvate carboxylase activity, but phenylacetyl CoA ester decreased pyruvate carboxylation in a concentration-dependent manner. Phenylacetic acid inhibited gluconeogenesis in isolated rat liver cells from 10 mM lactate/1 mM pyruvate (decreased 39%, $P < 0.05$), but not 10 mM L-glutamine or [^{14}C]aspartate, showing that the inhibition of gluconeogenesis occurred at the level of pyruvate carboxylase. A 20 mg bolus with infusion of 1 mg/min of phenylpropionic acid decreased blood glucose levels of normal [110 ± 12 to 66 ± 11 mg/dL, N = 7, $P < 0.05$ (unpaired Student's *t*-test vs control)] and streptozocin diabetic rats [295 ± 14 to 225 ± 12 mg/dL, N = 7, $P < 0.01$ (paired *t*-test vs basal)]. Hepatic glucose production in control and diabetic rats was suppressed under conditions where liver glycogen was depleted, indicating that gluconeogenesis had been inhibited *in vivo*. The results suggest the possibility that the inappropriate overproduction of glucose can be controlled by inhibitors of pyruvate carboxylase. This class of inhibitors may be useful in the treatment of non-insulin-dependent diabetes mellitus. *Copyright © 1996 Elsevier Science Inc.*, BIOCHEM PHARMACOL 53;1:67–74, 1997.

KEY WORDS. pyruvate carboxylase; gluconeogenic inhibitor; hypoglycemic agents; hepatic glucose production

Diabetes mellitus is an abnormal metabolic state characterized by insulin resistance involving both liver and muscle. Insulin resistance is manifested by hepatic glucose overproduction and in muscle by non-oxidative glucose underutilization [1–4]. These defects are a function of insulin insufficiency and insulin insensitivity. Studies in diabetic animals and studies in normal animals and humans have shown that augmented gluconeogenesis is a major factor in the increased plasma glucose that appears in the fasting and postabsorptive states [1, 4–7]. The excessive gluconeogenesis in diabetics is inappropriate in the presence of fasting hyperglycemia and fasting hyperinsulinemia, both of which should suppress hepatic glucose production [1, 8–11]. The overactivity of gluconeogenesis in inadequately treated diabetics has been ascertained to be primarily responsible for fasting and postabsorptive hyperglycemia in both insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus [1–3, 6, 12–14].

PC§ catalyzes a thermodynamically irreversible reaction, which may be rate limiting in hepatic gluconeogenesis [15–17]. PC and several other gluconeogenic enzymes have their transcription regulated by insulin [18]. The activity of the enzyme increases with insulin insufficiency and is suppressed by insulin [19, 20]. Acetyl CoA derived from long-chain fatty acid oxidation aids in the allosteric stimulation of the inactive PC monomer to an active tetramer [15–17]. Both the fasting and uncontrolled diabetic states are associated with increased long-chain fatty acid oxidation and hepatic gluconeogenesis [1, 2, 4].

Pharmacological regulation of gluconeogenesis might be accomplished by controlling PC. Previous studies from this laboratory demonstrated the inhibition of glucose production from alanine, lactate, and pyruvate in the isolated perfused livers of normal fasted rats by phenylalkanoic acids [21]. The mechanism of the inhibition was not known at that time.

In this paper, *in vitro* and *in vivo* data will be presented which explain the mechanism of the inhibitory action of

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Received 4 March 1996; accepted 5 July 1996.

§ *Abbreviations:* PC, pyruvate carboxylase; Ra, rate of glucose appearance; Rd, rate of glucose disappearance.

the phenylalkanoic acids on hepatic gluconeogenesis in the rat. It has been shown the phenylalkanoic acids are activated to their acyl CoA esters, which inhibit the stimulatory action of acetyl CoA on PC probably by competition with acetyl CoA.

MATERIALS AND METHODS

In vitro studies were conducted at the University of Arizona at Tucson and *in vivo* studies were conducted at the University of Texas at San Antonio. All studies involving tissues or animals were conducted under protocols approved by the Institutional Animal Care and Use Committees of the respective Universities.

Drugs and Chemicals

Phenylacetic acid and 3-phenylpropionic acid were purchased from the Aldrich Chemical Co. (Milwaukee, WI). They were selected as being readily obtainable representatives of the class of phenylalkanoic acids. Addition of NaOH allowed preparation of stock solutions of phenylalkanoic acids at the desired pH. Phenylacetyl CoA and other chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO).

Oxidation of Palmitate and Glucose

Hepatocytes were isolated from fed rats using glucose-containing medium [22]. Viability as measured by trypan blue exclusion was greater than 90%. The cells were resuspended in DMEM with 10% fetal bovine serum (Hyclone, Logan, UT). Cells (4×10^6) were incubated in a volume of 1 mL with 0.5 μCi of either [1-^{14}C]palmitate or [U-^{14}C]glucose with or without phenylacetic acid at 37° and pH 7.4. After 30 min of preincubation at 37°, $^{14}CO_2$ was collected for 60 min with an apparatus previously described [23].

High Pressure Liquid Chromatography

Extracts of mitochondria prepared from 1.7 g rat liver [24] were prepared following incubation with 10 mM lactate/1 mM pyruvate at ambient temperature for 10 min with or without 2 mM phenylacetic acid. Separation of CoA esters was performed according to the method of Corkey *et al.* [25]. A mobile phase of 30:70 methanol:50 mM K_2HPO_4, pH 5.3, was pumped at 1 mL/min through a 10 μm, reverse phase C_{18} column with detection at 254 nm. Authentic phenylacetyl CoA (Sigma) was used as a standard to identify and quantify the peak with a retention time of 13.6 min.

PC Activity

Purified rat liver PC [26] was added to buffer (100 mM KCl, 50 mM Tris, 10 mM $MgCl_2 \cdot 6H_2O$, pH 7.8) containing malate dehydrogenase, 0.003 mM acetyl CoA, 0.2 mM NADH, 5 mM pyruvate, 2 mM ATP, and 50 mM K_2CO_3 and various concentrations of phenylacetyl CoA (Sigma). Oxaloacetic acid generated by PC was reduced to malate by NADH and malate dehydrogenase. The coupled reactions were measured spectrophotometrically by the change in absorption at 340 nm [27]. Malate dehydrogenase activity was not affected by phenylacetyl CoA.

Total and Radiolabeled Gluconeogenesis

Hepatocytes were isolated from rats fasted overnight [22] with Krebs/Henseleit buffer containing no glucose to minimize endogenous glycogen. In a volume of 1 mL, 4×10^6 hepatocytes were incubated for 1 h at 37° with two different substrates. [^{14}C]Aspartate (0.5 μCi, 2.25 μM) was added to the two non-radiolabeled glucose precursor solutions of either 10 mmol/L lactate/1 mmol/L pyruvate or 10 mmol/L glutamine. The amount of glucose produced was measured by the glucose oxidase method [28] purchased in kit from (Sigma) and the production of [^{14}C]glucose from [^{14}C]aspartate (0.5 μCi/sample) was measured on the same sample as non-charged radioactive material passing through anionic and cationic ion exchange resin columns [29].

Animals

Male Sprague-Dawley rats (250–300 g) obtained from Harlan (Indianapolis, IN) were studied. The rats were given free access to food and water, housed in individual cages in an air-controlled room, and subjected to a standard light (6:00 a.m. to 6:00 p.m.) dark (6:00 p.m. to 6:00 a.m.) cycle. Streptozotocin-induced diabetic rats were prepared by the tail vein injection of 50 mg/kg of streptozotocin (Sigma) diluted in 0.5 N citrate buffer (pH 5.5). A week after injection, plasma glucose values were obtained in the fed state. Only rats proven to have glucose values over 200 mg/dL on three different measurements were retained for this study. Four to five days before the experiments, all rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p., body weight). Indwelling catheters were inserted in the right internal jugular vein and in the left carotid artery, and both catheters were exteriorized through the skin at the back of the neck [30]. To investigate the contribution of gluconeogenesis to hepatic glucose production, rats were restrained from food for 24 h in order to deplete liver glycogen.

In Vivo *Experimental Design*

Study groups consisted of (1) group I: controls without any glucose infusion (N = 7), (2) group II: controls with exogenous glucose infusion to maintain basal euglycemia (N = 7), and (3) group III: streptozotocin diabetic rats without any glucose infusion (N = 9). In all three groups, rats received [3-^{3}H]glucose (DuPont-NEN, Boston, MA) infusion and phenylpropionic acid. After 24 h of fasting, a prime (2.4 μCi)-continuous (0.15 μCi/min) infusion of

[3-^{3}H]glucose was started 90 min prior to phenylpropionic acid administration via the arterial catheter as previously described [31–33]. Phenylpropionic acid was injected as a bolus 90 min after the start of [3-^{3}H]glucose at time 0 and continuously infused throughout the 120-min study period. To provide a control group for the effect of duration of time in group I (N = 9) and group III (N = 5), saline was infused instead of phenylpropionic acid. Otherwise, the study protocol was identical to that described above. In group II, a variable infusion of 25% glucose solution was started at time 0 and variably adjusted to maintain the plasma glucose concentration at approximately 90 mg/dL in the euglycemic clamp study. The specific activity of [3-^{3}H]glucose was determined at 5- to 20-min intervals throughout all studies. Plasma samples for determination of plasma insulin, glucagon, and lactate were obtained at −30, 0, 90, and 120 min during the study. The total amount of blood withdrawn during each study was 6.5 mL. To prevent intravascular volume depletion and anemia, an equivalent amount of normal saline plus fresh whole blood obtained by heart puncture from the sibling of the test animal was infused at a constant rate throughout the study.

Analysis and Statistics

Plasma glucose was measured by the glucose oxidase method using a Beckman Glucose Analyzer (Palo Alto, CA). Plasma [^{3}H]glucose activity was measured after deproteinization by the method of Somogyi [34]. Plasma insulin and glucagon concentrations were measured by radioimmunoassay [35] using rat insulin standards (Novo, Copenhagen, Denmark). Plasma and tissue lactate concentrations were measured by enzymatic analysis. Glycogen in the liver and muscle was measured by determining the glucose concentration after enzymatic digestion [36].

Basal glucose production was calculated as [3-^{3}H]glucose infusion rate divided by steady state plasma glucose specific activity. During non-steady-state conditions following phenylpropionic acid infusion, the Ra and the Rd were estimated using Steele's equation [37]. Hepatic glucose production in group III was determined by subtracting the rate of unlabeled glucose infusion from the total Ra.

Data are expressed as means ± SEM. Statistical significance was assessed by the unpaired (see Tables 1 and 2, Fig. 3) or paired (see Figs. 4–7) Student's *t*-test. Significance was accepted at $P < 0.05$.

RESULTS

Phenylpropionic acid has been shown to inhibit gluconeogenesis at 2–4 mmol/L concentrations [21]. Because the conversion of pyruvate to glucose by the liver is an energy-requiring series of reactions, it was necessary to assess potential effects of these compounds on cell metabolism. These studies were carried out to ascertain whether the inhibition of gluconeogenesis had occurred because of an associated decrease in the activity of the two major pathways of energy production. The effect of phenylpropionic acid on glucose and long-chain fatty acid oxidation was assessed in isolated hepatocytes prepared from fasted rats. Phenylacetic acid failed to significantly inhibit glucose or palmitate oxidation at a concentration at which it inhibited the conversion of alanine, lactate, and pyruvate to glucose [21]. These data are shown in Table 1. Similar data were collected for phenylpropionic acid (data not shown). The lack of effect of these phenylalkanoic acids on the utilization of the two major metabolic substrates (glucose and palmitate) makes it unlikely that their antigluconeogenic action is due to a failure to generate energy. Additional studies using phenylpropionic acid revealed no inhibition of glucose or palmitate oxidation in rat heart cells or rat brain synaptosomes (data not shown).

Studies on the mechanism of action of the antigluconeogenic activity of the phenylalkanoic acids focused on whether these compounds or metabolites of them were the active inhibitory species. Phenylacetyl CoA was isolated and identified by HPLC analysis (Fig. 1) from a rat liver mitochondrial preparation incubated with 2 mmol/L phenylacetic acid. HPLC analysis showed that the mitochondria had made a product easily separable from the parent phenylacetic acid and of identical retention time as commercially available phenylacetyl CoA. Phenylacetic acid and phenylacetyl CoA have different HPLC retention times. Phenylpropionic acid and synthetically prepared phenylpropionyl CoA also had different retention times.

Purified rat liver pyruvate carboxylase was found to be inhibited by phenylacetyl CoA but not by phenylacetic acid. Concentrations of phenylacetyl CoA above 0.1 mmol/L caused a graded inhibition of PC activity. The K_i was dependent on the concentration of acetyl CoA used. The studies shown in Fig. 2 used a concentration of acetyl CoA (3.0×10^{-5} mol/L) that stimulated PC to 50% of maximal activity. Acetyl CoA concentrations could be raised in this *in vitro* experiment high enough (low mmol/L range) to overcome the inhibitory action of the phenylalkanoyl CoA esters on PC. At low concentrations of acetyl CoA, substantial inhibition of PC activity could be obtained with 5–50 μmol/L phenylacetyl CoA.

Oxaloacetic acid is the product of the reaction of PC on pyruvate. L-Glutamine enters the metabolic pathway as α-ketoglutarate and is converted in the tricarboxylic acid cycle to oxaloacetic acid. Aspartate is transaminated to

TABLE 1. Oxidation of [^{14}C]palmitate and [^{14}C]glucose with or without 4 mM phenylacetic acid (PAA) by isolated hepatocytes

	$^{14}CO_2$ production (dpm)	
Labeled substrate	**Control**	**4 mM PAA**
[1-^{14}C]Palmitate	2640 ± 30	2620 ± 240
[U-^{14}C]Glucose	470 ± 70	490 ± 30

$^{14}CO_2$ production in 1 hr for 4×10^6 hepatocytes at 37°. Values are means ± SEM, N = 4.

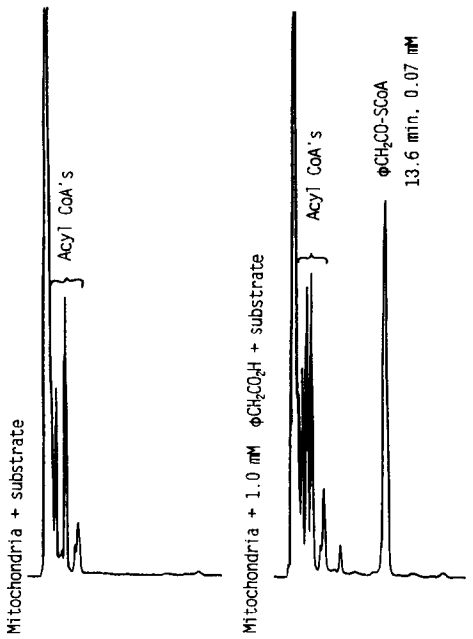

FIG. 1. HPLC identification of phenylacetyl CoA produced by rat liver mitochondria. Mitochondria incubated with or without 1 mM phenylacetic acid for 10 min were extracted, and CoA esters were separated by reverse phase chromatography. The retention time of the peak at 13.6 min that appeared following incubation with phenylacetic acid was identical to authentic phenylacetyl CoA.

oxaloacetic acid, and neither substrate is dependent upon the actions of pyruvate carboxylase in their conversion to glucose. To ascertain whether the phenylalkanoic acids inhibit gluconeogenesis from lactate/pyruvate at a site or sites other than PC, we studied the capacity of phenylacetic acid to inhibit gluconeogenesis from several substrates that do not have to be acted on by PC in isolated hepatic cells from 24-h fasted rats. The effect of phenylacetic acid on gluconeogenesis from lactate/pyruvate or L-glutamine, with ^{14}C-labeled aspartate was studied in isolated liver cells (Table 2). Phenylacetic acid significantly inhibited gluconeogenesis from lactate/pyruvate, but did not significantly inhibit gluconeogenesis from glutamine. Gluconeogenesis from [U-^{14}C]aspartate was not inhibited significantly when the primary substrate was either lactate/pyruvate or glutamine.

A number of *in vivo* studies on the inhibition of glucose appearance in 250 g 24-h fasted rats by phenylpropionic acid were carried out. Phenylpropionic acid is a phenylalkanoic acid which, like phenylacetic acid, inhibits gluconeogenesis [21] without interfering with the metabolism of glucose or palmitate (data not shown).

A study utilizing two different boluses of 3-phenylpropionic acid followed by a continuous infusion of phenylpropionic acid in normal rats resulted in significant falls in plasma glucose concentration. Figure 3 shows the decreases in plasma glucose at 120 min using a 20 mg bolus and 1 mg/min continuous infusion, and a 50 mg bolus and a 2.5 mg/min continuous infusion. Plasma glucose concentrations decreased from 110 ± 12 to 66 ± 11 mg/dL ($P < 0.05$) and 108 ± 8 to 50 ± 15 mg/dL ($P < 0.05$) for the two groups, respectively, following phenylpropionic acid infusion.

The effect of phenylpropionic acid (20 mg bolus and 1 mg/min continuous infusion) on hepatic glucose production in 24-h fasted rats is shown in Fig. 4. This was a non-maximal, but effective phenylpropionic acid bolus and infusion dose. Following a lag period of approximately 60 min, phenylpropionic acid caused a progressive decline in hepatic glucose production in the treated group compared with their time zero values from 6.6 ± 0.7 to 4.5 ± 0.4 mg/kg/min ($P < 0.05$). The decline in plasma glucose concentration was from 110 ± 12 to 66 ± 11 mg/dL ($P < 0.05$). Hepatic glucose production remained unchanged in normal fasted rats receiving only saline infusion. In separate studies, the complete absence of liver glycogen in the fasted rats was documented (0.01 ± 0.01 mg/g wet weight, N = 9). Therefore, it can be concluded that the reduction of hepatic glucose production effected by phenylpropionic acid was due to a decrease in gluconeogenesis.

Streptozotocin-induced diabetic rats were studied to assess the effect of an infusion of phenylpropionic acid on basal hepatic glucose production. The 24-h fasted diabetic rat livers had only small amounts of residual glycogen. The phenylpropionic acid infusion used in the treated rats was a 20 mg bolus followed by an infusion of 1 mg/min over 120 min. The untreated diabetic rats were infused with normal saline. The fasting blood glucose of the diabetic rats was 281 ± 16 mg/dL (N = 12). The blood glucose of the phenylpropionic acid-treated diabetic group declined from 295 ± 14 to 225 ± 12 mg/dL (N = 7, $P < 0.01$) after 2 h of

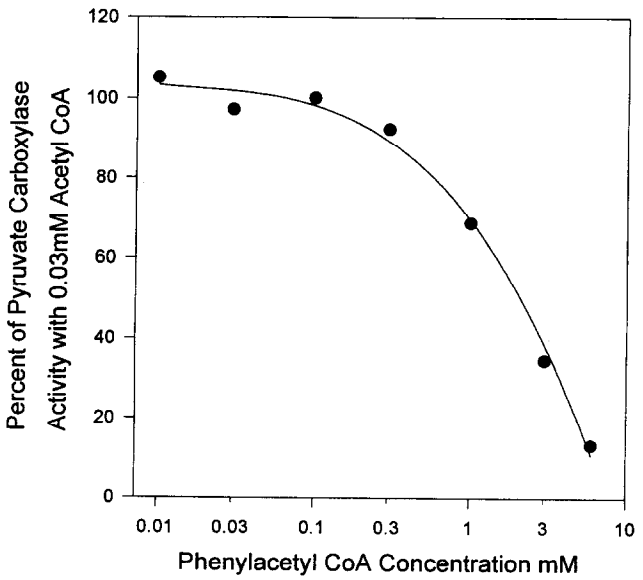

FIG. 2. Purified PC activity assayed with increasing concentrations of phenylacetyl CoA. The concentration of acetyl CoA (0.03 mM) was calculated to provide 50% of maximal PC activity. The assay measured oxaloacetic acid produced in the presence of NADH and malic dehydrogenase by measuring the appearance of oxidized NAD spectrophotometrically at 340 nm.

TABLE 2. Unlabeled and labeled glucose production from different substrates

Substrate	Unlabeled glucose (nmol)		Labeled glucose (dpm)	
	Control	4 mM PAA	Control	4 mM PAA
10 mM Lactate/1 mM pyruvate + 2.25 μM [^{14}C]aspartate	54.9 ± 3.9	34.7 ± 6.9*	57,860 ± 430	60,830 ± 450
10 mM Glutamine + 2.25 μM [^{14}C]aspartate	49.1 ± 6.9	42.4 ± 3.8	35,610 ± 380	31,160 ± 410

Glucose production in 1 hr for 4 × 10^6 hepatocytes. PAA = phenylacetic acid. Values are means ± SEM, N = 4.
* $P < 0.05$.

infusion, whereas the blood glucose concentration in the untreated diabetic rats remained unchanged (262 ± 19 vs 251 ± 21 mg/dL, N = 5).

After a lag period of approximately 45 min, phenylpropionic acid infusion (20 mg bolus followed by infusion of 1 mg/min) caused a progressive decline in hepatic glucose production from 9.5 ± 1.5 to 7.9 ± 1 mg/kg/min, $P < 0.05$. Hepatic glucose production decreased slightly in the diabetic rats infused with saline (Fig. 5).

To eliminate neuroendocrine responses to a falling plasma glucose concentration, a euglycemic clamp technique was employed. The effect of a 20 mg bolus of phenylpropionic acid followed by a 1 mg/min infusion on hepatic glucose production in normal fasted rats is shown in Fig. 6. When phenylpropionic acid was infused and the plasma glucose concentration was maintained at the basal level, suppression of hepatic glucose production was similar to that of normal fasted rats in which no glucose was infused (see Fig. 4). Hepatic glucose production began to decrease after 30 min, and showed a significant decline from basal by 120 min (6.5 ± 1.0 to 4.5 ± 1.1 mg/kg/min, $P < 0.05$). Figure 7 shows the data on the suppression of glucose production and total body glucose uptake over the 120-min period. The uptake of glucose was unchanged during the basal period (6.4 ± 1.2 mg/kg/min) and was not changed significantly by the phenylpropionic acid infusion (Figs. 6 and 7). Plasma insulin concentration was unchanged by the phenylpropionic acid infusion (237 ± 18 vs 220 ± 21 pmol/L), as was the plasma glucagon concentration (98 ± 12 vs 92 ± 13 pg/mL). Plasma lactate concentration increased slightly with the 2-h infusion from 0.37 ± 0.05 to 0.84 ± 0.13 mEq/L.

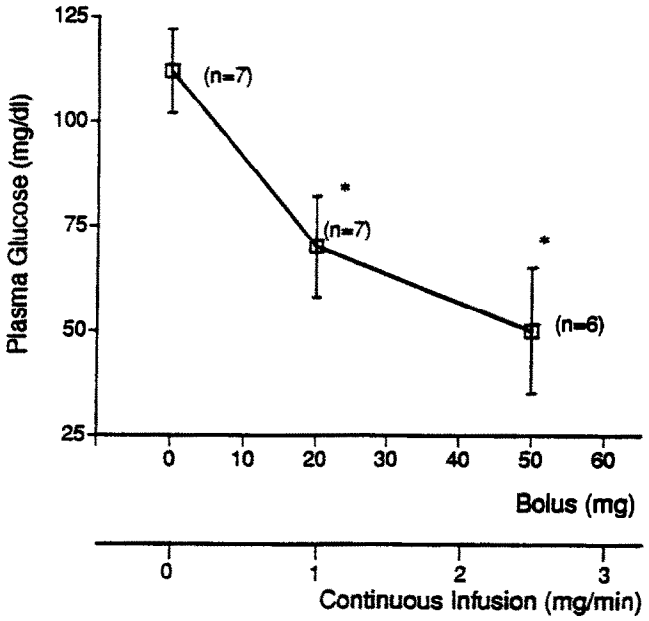

FIG. 3. Effect of phenylpropionic acid on plasma glucose levels in fasted non-diabetic rats. Rats received a bolus of 0, 20, or 50 mg of phenylpropionic acid at time zero and were infused with 0, 1, or 2.5 mg/min, respectively, of phenylpropionic acid for 120 min. Blood samples were collected five times between 100 and 120 min, and the measured values for each animal were averaged. The values from all animals in a group were averaged, and the mean with SEM bars is shown. Key: (*) $P < 0.05$ control vs treated.

DISCUSSION

The importance of the contributory role of hepatic gluconeogenesis in the fasting and postabsorptive hyperglycemia of diabetes mellitus in experimental animals is well established [1, 3, 6, 13, 14]. The increased production of glucose by the liver of the diabetic results from hepatic resistance to the suppressive effects of both hyperinsulinemia and hyperglycemia on hepatic glucose release. Later in the natural

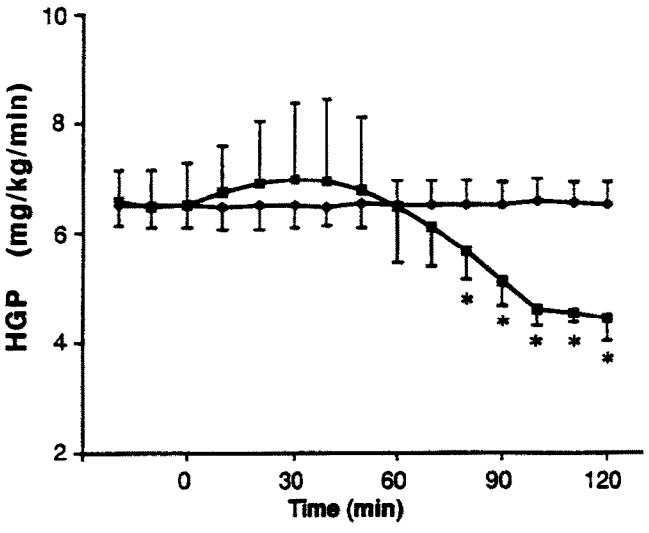

FIG. 4. Time course of hepatic glucose production in fasted normal rats treated with either saline (♦) (N = 7) or phenylpropionic acid (20 mg bolus plus 1 mg/min infusion) (■) (N = 7). Values are means with SEM bars. Key: (*) $P < 0.05$ comparing each animal with its own value at time zero.

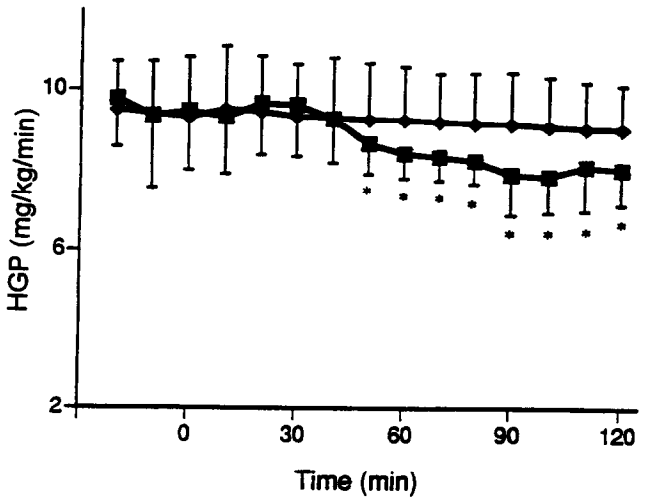

FIG. 5. Effect of phenylpropionic acid on hepatic glucose production in fasted streptozotocin-induced diabetic rats (group III, N = 5) treated with saline (♦) or with a 20 mg bolus plus 1 mg/min infusion of phenylpropionic acid (■). Data are means with SEM bars. Key: (*) $P < 0.05$ comparing each animal with its own value at time zero.

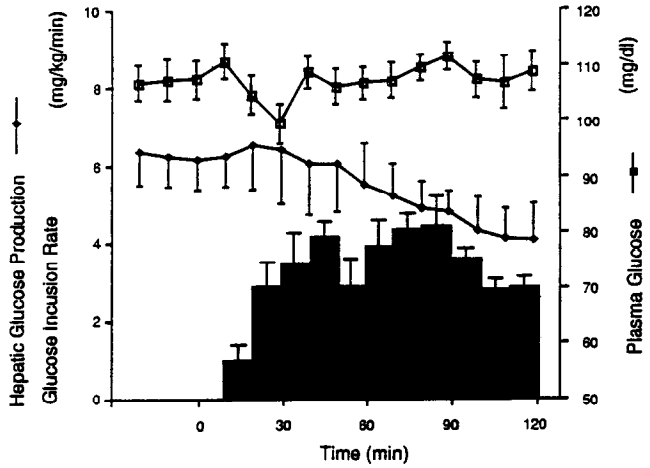

FIG. 7. Effect of phenylpropionic acid on hepatic glucose production and plasma glucose concentration in normal fasted rats (group II, N = 7). When phenylpropionic acid (20 mg bolus plus 1 mg/min infusion) treated rats were clamped at the basal glucose level (□) by an exogenous infusion of unlabeled glucose (solid bars), the suppression of hepatic glucose production (♦) was similar to that observed in normal rats without glucose infusion (group I, N = 9). Data are means with SEM bars.

history of type II diabetes, insulin-deficiency develops and contributes to the increased rate of hepatic gluconeogenesis [1, 2, 4, 5]. Additional factors that play a role in the increased hepatic gluconeogenesis in diabetic models include increased plasma glucagon concentrations, increased hepatic sensitivity to glucagon, and accelerated substrate fluxes [1, 38–41]. The increased hepatic glucose output in diabetes is associated with a decrease in glycolysis and an increase in the four unidirectional enzymes of gluconeogenesis (pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose, 1,6-diphosphatase, and glucose-6-phosphatase) [1, 39, 40, 42, 43]. These enzymes are increased in amount and activity in states of insulin insufficiency [39, 40, 42–44].

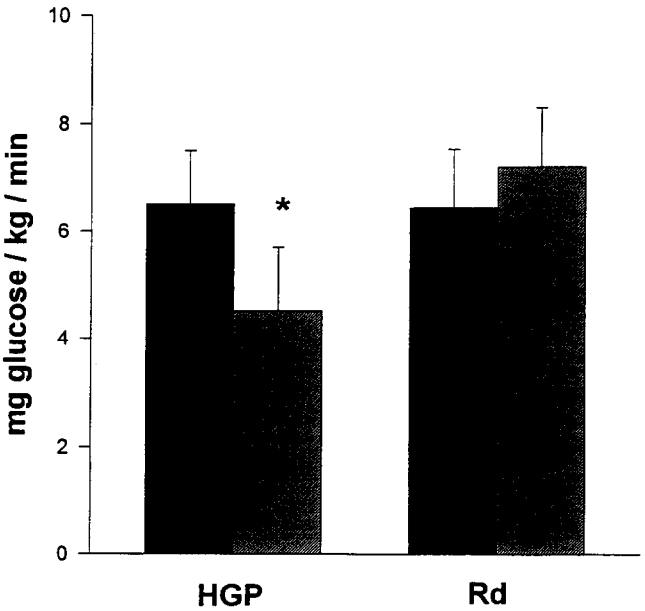

FIG. 6. Hepatic glucose production (HGP) and total glucose disposal (Rd) in normal rats under basal (solid bars) and euglycemic (stippled bars) conditions (group II). Normal fasted rats received phenylpropionic acid (20 mg bolus plus 1 mg/min infusion) and an exogenous infusion of unlabeled glucose to maintain euglycemia. Values are means with SEM bars. Key: (*) $P < 0.05$ compared with the basal value for the same rat.

PC is a mitochondrial enzyme that may be rate-limiting in gluconeogenesis [2, 43, 44]. Its activation depends on allosteric activation by acetyl CoA derived from fatty acid oxidation. In the absence of basal fatty acid oxidation, gluconeogenesis is decreased and blood glucose falls in both normal and diabetic animals [40, 43, 45–47]. Decreased fatty acid oxidation also is detrimental to gluconeogenesis because it provides energy for glucose production, which is an energy-consuming process.

Because of the interest of our laboratory in the regulation of gluconeogenesis, we focused on the PC enzyme. Our efforts were directed at potential competitive inhibition of the activation of the enzyme since the endogenous allosteric activator acetyl CoA was known [15, 16, 41–43].

We assessed a number of phenylalkanoic acids for their capacity to be activated to an acyl CoA derivative, to inhibit gluconeogenesis from lactate/pyruvate significantly (over 40%), but not to inhibit the conversion of glucose or palmitate to carbon dioxide. These screening studies were done on liver mitochondrial and homogenate preparations and on isolated rat liver cells. Phenylacetic and phenylpropionic acids fulfilled our screening criteria for the *in vivo* studies, which have been presented.

The inhibition of gluconeogenesis in our studies was probably at the PC site. The purified rat liver PC enzyme

was inhibited by phenylacetic CoA, but not by phenylacetic acid. The phenylacetic acid inhibition of gluconeogenesis was obtained only when gluconeogenic precursors, which had to be converted to glucose by passage through PC, were used [21]. Substrates that do not require PC in their conversion to glucose (glutamate, aspartate) [48] were not inhibited.

In recent years, several compounds that depend on an inhibition of long-chain fatty acid oxidation for their activity [45–47, 49, 50] have been studied for the treatment of the hyperglycemia of diabetes mellitus. Decreasing fatty acid oxidation lowers the production of acetyl CoA and causes a decrease in PC activity and gluconeogenesis [47, 49, 50]. The phenylalkanoic acids differ from these compounds by directly acting on the PC enzyme, and inhibiting it and gluconeogenesis.

The studies carried out *in vitro* and *in vivo* were acute, lasting for 2 hr. During these short-term *in vivo* studies, no overt toxicity was noted. Because the site of the inhibition involves PC, the plasma levels of lactate were assessed in the *in vivo* studies. Plasma lactate levels were elevated insignificantly in these studies (0.4 mEq/L). These levels of lactate elevation are similar to those found with pharmacologic doses of metformin [51].

Phenylacetic acid has been studied in several diverse clinical conditions *in vitro* and *in vivo*. It has been used as an inducer of fetal hemoglobin in patients with urea cycle disorders [52], and has decreased excessive blood ammonia levels by acylation. It has been studied in a number of human malignant and premalignant cells *in vitro* and found to cause nontoxic growth arrest and cellular maturation [53–56].

The acute studies presented in this paper suggest that inhibition of PC in a competitive manner is possible, and that such inhibition can lower the blood glucose concentration in a dose-dependent fashion. The studies were carried out to test the hypothesis that PC plays an important role in the regulation of blood glucose levels in normal and diabetic rats and not to develop a specific pharmacological agent. However, the lack of significant toxicity in the *in vitro* or *in vivo* uses of phenylacetic acid is encouraging, and suggests that the phenylalkanoic acid class of agents may represent a potential new pharmacological approach to the therapy of hyperglycemia.

We wish to thank K. G. Thampy and S. J. Wakil for their gift of purified PC. This work was funded, in part, by a grant from the University Heart Center, The University of Arizona Health Sciences Center, Tucson, AZ.

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