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Effects of Glucagon on Gluconeogenesis from Lactate and Propionate in the Perfused Rat Liver (39213)

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Materials and methods. Male Sprague–Dawley rats, weighing 100–160 g, were fed Purina Chow pellets, then starved 48 hr prior to use. Procedures for *in situ* liver perfusion have been described (7). Unless otherwise stated, livers were perfused without substrate for the first 37 min. Addition of quinolinate, butyrate, glucagon, or their combinations, were made 2 min prior to the addition of lactate. Glucagon solutions were prepared as described by Exton and Park (9). In some experiments, portions of the liver were frozen with aluminum tongs prechilled in liquid nitrogen, 40 min after perfusion with substrate plus other additions. Extraction of tissues and assays for glucose and other metabolites either in tissue extracts or in perfusate have been described elsewhere (7). Statistical significance and P values were determined by the use of the Student's t test (8).

Results and discussion. The effects of glucose production under various conditions are shown in Table I. One potential hazard in studying glucagon stimulation of gluconeogenesis without the use of radioactive-labeled substrates is the well documented glycogenolytic effect of glucagon. The slow rate of glucose production, in the absence of added substrate, suggests that under these conditions the main source of glucose was endogenous substrate(s), which was not influenced by glucagon. The salient points of potential control of gluconeogenesis from pyruvate and propionate are shown in Fig. 1. Figure 1 also indicates some of the potential control points as possibly affected by glucagon, as well as the site of action of many of the inhibitors used in this study. The rate of glucose production from either lactate or propionate was increased by the addition of glucagon to approximately the same extent. Although the pathways of gluconeogenesis from lactate and propionate converge at the point of malate-oxaloacetate, their similar responses to glucagon does not necessarily imply that the hormone acts above the point of oxaloacetate formation. With propionate as substrate, glucagon could have stimulated steps that are not in common with pyruvate gluconeogenesis. Exton *et al.* (4) observed a possible stimulation, between α-ketoglutarate and succinate in the tricarboxylic acid cycle, by glucagon in the perfused rat liver.

In addition to the pyruvate carboxylase and PEP carboxykinase reaction, transport of dicarboxylic acids across the mitochondrial membrane has also been suggested as a possible process affected by glucagon, and leading to stimulation of glucose production (ref. 4; Fig. 1). It is therefore still possible that the glucagon effects are identical with both lactate and propionate. This possibility was examined by the use of butylmalonate, which has been shown to inhibit both malate transport across the mitochondrial membrane and gluconeogenesis from lactate in the perfused rat liver (10). Results in Table I show that butylmalonate inhibited glucose production from lactate more drastically than from propionate. The addition of glucagon did not significantly alter the rate of glucose production from lactate plus butylmalonate, while with propionate the butylmalonate inhibition was almost completely reversed by the hormone, indicating that glucagon action is not likely exerted on points common to both propionate and lactate. Since the directions of flow of carbon from lactate and propionate to the dicarboxylic acids are opposite to each other, glucagon could have stimulated the formation of a dicarboxylic acid from propionate, whose transport across the mitochondrial membrane was not inhibited by butylmalonate. A stimulation of some reactions resulting in elevated fumarate formation from propion-

TABLE I. EFFECT OF GLUCAGON ON RATES OF GLUCONEOGENESIS.[a]

Substrate (m*M*)	Rate (μmol/min/g liver) −Glucagon	+Glucagon	*P*
None	0.20 ± 0.08 (4)	0.23 ± 0.10 (4)	>0.10
Lactate (10)	1.05 ± 0.07 (8)	1.36 ± 0.12 (4)	<0.05
Lactate + butyrate (5)	1.63 ± 0.04 (4)	–	–
Lactate + Q.A. (4.8)	0.51 ± 0.06 (4)	0.52 ± 0.05 (4)	>0.10
Lactate + Q.A. + Butyrate	0.55 ± 0.04 (4)	–	–
Propionate (10)	0.70 ± 0.13 (8)	0.95 ± 0.05 (4)	0.05
Lactate (10) + Butylmalonate (2)	0.54 ± 0.08 (4)	0.68 ± 0.04 (4)	<0.10
Propionate (10) + Butylmalonate	0.49 ± 0.02 (4)	0.81 ± 0.09 (4)	<0.01

[a] The rates of gluconeogenesis represent the linear rate of glucose output after substrate addition ± SEM. Numbers in parentheses after − and + glucagon data represent the number of animals used. Glucagon was added to the perfusion medium to give a final concentration of 1×10^{-9} *M*. *P* values indicate the significance between the absence and addition of glucagon.

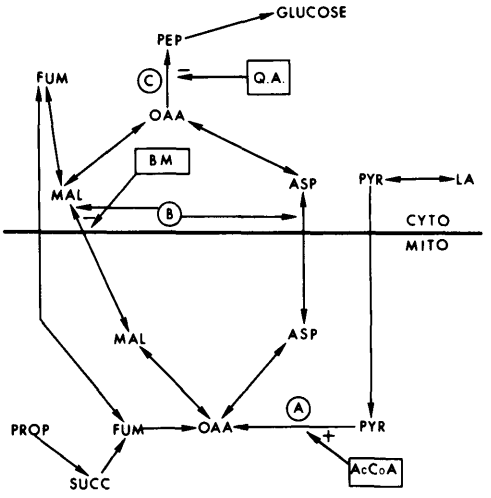

FIG. 1. Metabolic flow chart. Potential control points by glucagon indicated by ○: i.e., A, pyruvate carboxylase; B, dicarboxylic acid transport; and C, phosphoenol pyruvate carboxykinase. Inhibitors and activators are indicated by ☐ with a − indicating inhibition and a + indicating activation: i.e., Q.A. −, quinilinic acid (inhibitor of PEP carboxykinase); B.M. −, butylmalonate (inhibitor of malate transport across the mitochondrial membrane); and Ac CoA +, an activator of pyruvate carboxylase.

ate could circumvent the butylmalonate inhibition, provided that transport of fumarate is not sensitive to butylmalonate inhibition.

To further examine the glucagon effect on gluconeogenesis from lactate, particularly at points between pyruvate and PEP, we designed experiments to impose a limitation at the point of PEP synthesis. Quinolinate, an inhibitor of PEP carboxykinase, has been shown to inhibit gluconeogenesis in the perfused rat liver from L-alanine (11), L-serine, and L-lactate (7), with accumulations of intermediates between pyruvate and PEP (see Fig. 1). It has also been shown that caprylate caused further increases in malate and aspartate (11). One of the possible explanations was an activation of pyruvate carboxylase by acetyl CoA, a postulate often advanced to explain fatty acid stimulation of gluconeogenesis (5, 12). Therefore, an activation of pyruvate carboxylase by glucagon in the presence of quinolinate should further elevate malate and aspartate levels, while either stimulation of dicarboxylic acid transport of PEP carboxykinase activity should not affect levels of these metabolites.

In agreement with our previous observation (7), quinolinate inhibited gluconeogenesis from lactate by about 50% (Table I). This inhibition was not reversed by either butyrate or glucagon, both of which elevated the rate of gluconeogenesis in the absence of the inhibitor.

An examination of some metabolic intermediates in these perfused livers showed that quinolinate did not affect total ketone body production, but elevated the β-hydroxybutyrate: acetoacetate ratio ($P < 0.01$), indicating a more reduced mitochondrial compartment, while lowering the lactate: pyruvate ratio ($P < 0.01$), representing a more oxidized cytosolic environment (Table II). Ketone body production was dramatically increased by the addition of butyr-

TABLE II. EFFECTS OF GLUCAGON ON THE REDOX STATE OF PERFUSED RAT LIVER.

Additions (m*M*)	β-OH-butyrate (nmol/g liver)	Acetoacetate (nmol/g liver)	Lactate[a] / Pyruvate	β-OH-butyrate / Acetoacetate
Lactate (10)	90 ± 8	786 ± 70	14.4 ± 1.9	0.12 ± 0.02
Lactate (10) + Q.A. (4.8)	253 ± 35	475 ± 68	8.5 ± 0.5	0.52 ± 0.11
Lactate (10) + Q.A. (4.8) + Butyrate (5)	769 ± 63	564 ± 40	43 ± 3	1.39 ± 0.13
Lactate (10) + Q.A. (4.8) + Glucagon	255 ± 27	434 ± 39	9.4 ± 1.3	0.59 ± 0.06

[a] Lactate and pyruvate concentrations are taken from data in Fig. 1.

ate which also made the liver more reduced ($P < 0.01$). Glucagon did not produce further changes in these parameters over those caused by quinolinate.

Results in Fig. 2 show elevated levels of lactate and pyruvate in the presence of quinolinate, indicating a diminished utilization. The PEP level was drastically reduced while aspartate level was significantly elevated. The addition of either butyrate or glucagon dramatically elevated malate and aspartate levels over those caused by quinolinate alone. This suggests that butyrate and glucagon possibly affected the same reaction, namely, pyruvate carboxylation. These results, however, do not necessarily imply that glucagon's effect was mediated by fatty acid oxidation, a theory which has been convincingly disproven (13, 14). It therefore appears from our results that glucagon may act on at least two locations in the perfused rat liver prior to the site of PEP synthesis (see Fig. 1). Butylmalonate inhibition on glucose production from propionate suggested that a significant portion of propionate carbon is transported across the mitochondrial membrane as malate. On comparing the different glucagon effects on butylmalonate inhibition with lactate and propionate, one may visualize glucagon acting to stimulate increased formation of dicarboxylic acid(s) from propionate, whose transport was not affected by butylmalonate. An attractive speculation would be an increase of fumarate formation and transport by glucagon. It therefore appears to us that glucagon may activate the part of the propionate pathway between propionate and fumarate. Possible stimulation by glucagon of the portion of tricarboxylic acid cycle between α-ketoglutarate and succinate (4) or the overall activity of the cycle (15) has been suggested. Exton *et al.* (4) observed a stimulation of

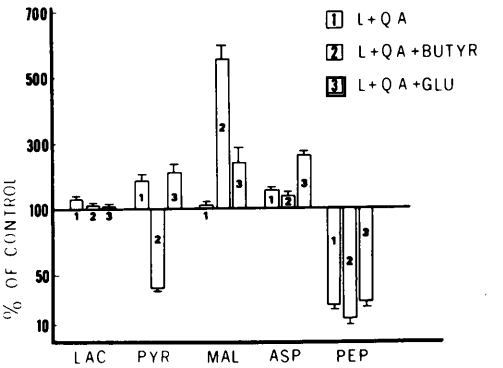

FIG. 2. Effects of glucagon and butyrate on metabolite concentrations in livers perfused with 10 m*M* L-lactate plus 4.8 m*M* quinolinate. The bar on top of each column represents one SEM. Abbreviations: LAC, lactate (control levels in nanomoles per gram of liver = 2435 ± 203); PYR, pyruvate (176 ± 12); MAL, malate (113 ± 9); ASP, aspartate (618 ± 13); PEP, phosphoenolpyruvate (202 ± 12); Q.A., quinolinic acid; Butyr, butyrate; GLU, glucagon.

incorporation of label from [^{14}C]glutamate into glucose by glucagon, due probably to an increase in α-ketoglutarate utilization through the TCA cycle. These results, together with our observations with propionate, suggest that the succinate thiokinase reaction is a possible site of glucagon action. Stimulation of this reaction will also increase GTP synthesis, which, although not shown to be a factor of limitation, is required for PEP synthesis. However, any stimulation by glucagon of propionyl CoA formation or its carboxylation cannot be ruled out.

The second site of action of glucagon was suggested by results from experiments with quinolinic acid. The additional increases in malate and aspartate caused by glucagon and butyrate, with PEP synthesis blocked by quinolinate, suggested that there was stimulation of dicarboxylic acid formation result-

ing from a possible activation of pyruvate carboxylation. Although the glucagon and butyrate effects are similar, their mechanism of stimulation may not be the same. The butyrate effect is probably mediated by increased mitochondrial acetylCoA concentration, thereby activating pyruvate carboxylase, whereas glucagon could stimulate pyruvate entry into the mitochondria (4) or have direct effects on enzymes that are yet to be discovered. A recent study using mitochondria from glucagon-treated isolated hepatocytes has also indicated that pyruvate carboxylation is an important regulatory site for the action of this hormone (16).

Summary. Quinolinic acid (Q.A.), which inhibits gluconeogenesis at the site of phosphoenolpyruvate (PEP) synthesis, reduced the content of PEP while elevating that of aspartate and malate in rat livers perfused with a medium containing 10 m*M* L-lactate. Glucagon at 10^{-9} *M* did not affect Q.A. inhibition of lactate gluconeogenesis nor the depression of PEP level, but further elevated malate and aspartate accumulation. Exogenous butyrate had the same effect as glucagon on these parameters. Butylmalonate (BM), an inhibitor of mitochondrial malate transport, inhibited lactate and propionate gluconeogenesis to similar extents. The addition of 10^{-9} *M* glucagon had no effect on BM inhibition of lactate gluconeogenesis, but almost completely reversed BM inhibition of propionate gluconeogenesis. These results suggest that glucagon may act on at least two sites, resulting in elevated hepatic gluconeogenesis. First, it may stimulate dicarboxylic acid synthesis (malate and oxaloacetate, specifically) through activation of pyruvate carboxylation. Secondly, it may stimulate synthesis of other dicarboxylic acids (fumarate, for example) by activating certain steps of the tricarboxylic acid cycle.

The stimulatory effect of glucagon on gluconeogenesis in the perfused rat liver is well documented (1, 2). Exton *et al.*, who earlier located the site of stimulation between pyruvate and PEP synthesis (3), proposed that glucagon stimulated PEP synthesis in the perfused rat liver (4), while reports from Williamson *et al.* (5) suggested the pyruvate-carboxylase reaction as the site of glucagon action. Stimulation at sites above PEP formation and of portions of the tricarboxylic acid cycle (4) by glucagon have also been suggested (6). In the present experiments, we have used substrates entering at different parts of the gluconeogenic pathway, and specific inhibitors to further resolve the action of glucagon.

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