Role of Pyruvate Kinase in the Regulation of Gluconeogenesis from L-Lactate*

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Glucagon and L-epinephrine stimulate gluconeogenesis from 20 mM L-lactate, the effect being about 3 times greater in liver cells from fed rats than in those from fasted rats. The rate of pyruvate kinase flux was estimated to be less than 10% of the rate of gluconeogenesis from lactate in hepatocytes from fasted rats, and neither glucagon nor epinephrine lowered the absolute rate significantly. In hepatocytes from fed rats, however, the rate of pyruvate kinase was nearly one-half that of gluconeogenesis. Glucagon caused a marked depression of pyruvate kinase flux, with 1 μM glucagon lowering the rate to nearly the level found in cells from fasted rats. Epinephrine at concentrations from 10^-9 to 10^-6 M actually increased pyruvate kinase flux during gluconeogenesis from lactate in cells from fed rats. These results are in accord with the view that the effects of glucagon and epinephrine on gluconeogenesis are not identical.

There is considerable evidence that, in isolated rat liver cells, the glycolytic enzymes glucokinase, phosphofructokinase, and pyruvate kinase may continue to function during active gluconeogenesis. (Whether futile cycling is as extensive in vivo as in vitro remains to be established.) The levels of these three enzymes are depressed upon fasting. Fasting of rats decreased liver pyruvate kinase activity from 27 units/g (normal diet) to 12 units/g after 24 h and to 10 units/g after 48 h (1). These results suggest that there may be an increased amount of futile or substrate cycling between pyruvate and P-enolpyruvate in the fed state as compared with the fasted state. In this paper we examine the extent of pyruvate kinase flux (P-enolpyruvate → pyruvate) during gluconeogenesis from L-lactate in liver cells from fed and fasted rats, and also describe the effects of glucagon and L-epinephrine on pyruvate kinase and on the overall rate of gluconeogenesis.

MATERIALS AND METHODS

Male Wistar rats were either fasted for 18 to 24 h, or fed ad libitum a normal chow diet. Isolated hepatocytes were prepared by the method of Berry and Friend (2) with the substitution of Krebs-Henseleit (3) buffer and the omission of hyaluronidase. The cells (0.15 to 0.55 ml of packed cells) were incubated, in duplicate with either 1L-[U-14C]lactate or NaH14CO3, with 20 mM L-lactate in 4 or 5 ml of Krebs-Henseleit buffer (containing 2.5 mM CaCl2) in 25 ml of Erlenmeyer flasks for 30 min at 38° in a rotary shaker bath. After addition of the cells, the flasks were gassed for 1 min with 5% CO2/95% O2, and then, in one set, NaH14CO3 (20 to 40 μCi) was injected through the rubber serum cap. A plastic well (Kontes, Vineland, N.J.) was suspended from the serum cap to collect 14CO2 at the end of the incubation, when 0.5 ml of 0.25% HClO4 was injected into the medium, and 0.25 ml of 4 N NaOH was injected into the hanging well. Shaking was continued for 2 to 3 h to collect 14CO2. The medium was washed out, made to 10 ml in conical tube, and centrifuged. Glucose was determined enzymically (4), with the substitution of NADP for NADP+ and the use of glucose-6-P dehydrogenase from Leuconostoc mesenteroides.

When liver cells from fasted rats were used, glucose formation was determined analytically and isotopically. An aliquot (9 ml) of the acidified medium was put on a column (1 × 4 cm) of Dowex 50 (H+ form, 100 to 200 mesh) on top of a column (1 × 8 cm) of Dowex 1-X8 (acetate form, 100 to 200 mesh), and the columns were washed with water until a 30-nl fraction was collected. An aliquot of this was counted to give the isotopic yield in glucose. Over a range of hormonal conditions, the average value of the ratio of the isotopic yield in glucose (from L-[U-14C]lactate) to analytical glucose formation was 0.66 (the range being from 0.63 to 0.71). In liver cells from fed rats, considerable glucose is formed from glycogen breakdown, and analytical glucose formation thus cannot be considered a measure of gluconeogenesis. In this case, gluconeogenesis is estimated by the isotopic yield in glucose from L-[U-14C]lactate, correcting for dilution by dividing by the factor 0.66. Whether this factor, derived from the use of cells from fasted rats, is applicable to cells from fed rats has not been established, but the errors involved are not likely to be sufficiently large to affect our conclusions.

Pyruvate kinase flux was estimated by the isotopic trapping method previously described (5), using NaH14CO3 as the labeled substrate. Lactate was eluted from the Dowex 1 column with 2 N acetic acid, and a fraction containing pyruvate was eluted with 3 N formic acid. The pyruvate yield was estimated by subtracting the yield obtained on drying this fraction from the total yield. Control experiments showed that more than 98% of authentic 14C-labeled pyruvate disappeared upon drying under these conditions. Since the 14C yields in pyruvate were small (<10% of those in lactate) and were difficult to estimate accurately by the difference procedure (the nonvolatile fraction being larger than the volatile fraction), determination of pyruvate kinase flux was based on comparison of the 14C yields in glucose and lactate alone. Also no attempts were made in this paper to correct the lactate yields for the amount of 14Cpyruvate, formed from 14C-labeled P-enolpyruvate, that is reutilized in the cell instead of being trapped in the large extracellular lactate pool (6). This may cause the pyruvate kinase fluxes to be underestimated by about 25%.

RESULTS AND DISCUSSION

It is well known that glucagon and epinephrine increase the rate of gluconeogenesis from L-lactate (6-11). From an extensive series of studies Exton et al. (see early review in Ref. 12) concluded that the site of hormone action was somewhere between pyruvate and P-enolpyruvate. However in spite of much further investigation the exact sites of action still have not been firmly established. In Table I we also show that both glucagon and epinephrine stimulate gluconeogenesis from L-
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Effects of glucagon and epinephrine on gluconeogenesis from L-lactate and on pyruvate kinase flux in isolated rat liver cells

Isolated rat liver cells were incubated with 20 mM L-lactate and either [U-14C]lactate or NaH14CO3 as described under "Materials and Methods." Rates of gluconeogenesis are the means of three experiments ± S. E. In the fasted condition, the small per cent changes in estimated pyruvate kinase flux are not significant and not presented.

<table>
<thead>
<tr>
<th>Dietary condition</th>
<th>Hormone addition (conc in parenth)</th>
<th>Average rate of gluconeogenesis (umoles gm wet wt/hr)</th>
<th>Average percent change</th>
<th>Ratio of 14C yields from NaH14CO3</th>
<th>Average percent change</th>
<th>Pyruvate kinase flux (umoles gm wet wt/hr)</th>
<th>Average percent change</th>
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<tr>
<td>Fasted</td>
<td>None</td>
<td>44.4 ± 1.8</td>
<td>-</td>
<td>0.073</td>
<td>-</td>
<td>0.4</td>
<td>-</td>
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<td></td>
<td>Glucagon (10^-7M)</td>
<td>45.9 ± 2.0</td>
<td>+3</td>
<td>0.073</td>
<td>0</td>
<td>6.7</td>
<td>-</td>
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<td></td>
<td>Glucagon (10^-6M)</td>
<td>49.4 ± 1.6</td>
<td>+11</td>
<td>0.064</td>
<td>-12</td>
<td>6.3</td>
<td>-</td>
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<td>Glucagon (10^-5M)</td>
<td>51.1 ± 1.2</td>
<td>+15</td>
<td>0.064</td>
<td>-12</td>
<td>6.6</td>
<td>-</td>
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<td></td>
<td>Glucagon (10^-4M)</td>
<td>55.1 ± 2.2</td>
<td>+24</td>
<td>0.056</td>
<td>-22</td>
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<td>L-Epinephrine (10^-6M)</td>
<td>40.5 ± 1.6</td>
<td>+5</td>
<td>0.073</td>
<td>0</td>
<td>6.8</td>
<td>-</td>
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<td>L-Epinephrine (10^-3M)</td>
<td>59.7 ± 5.0</td>
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<td>0.053</td>
<td>27</td>
<td>6.3</td>
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<tr>
<td>Fed</td>
<td>None</td>
<td>31.0 ± 1.0</td>
<td>-</td>
<td>0.460</td>
<td>-</td>
<td>28.5</td>
<td>-</td>
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<td>+85</td>
<td>0.460</td>
<td>0</td>
<td>33.6</td>
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<td>0.371</td>
<td>-19</td>
<td>35.6</td>
<td>+25</td>
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<td>L-Epinephrine (10^-3M)</td>
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<td>+89</td>
<td>0.243</td>
<td>-47</td>
<td>26.3</td>
<td>-1</td>
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</table>

Lactate (20 mM) in isolated liver cells from either fasted or fed rats. The stimulatory effect of the hormones is considerably greater in hepatocytes from fed rats, and the maximal effect of epinephrine we find to be slightly greater than that of glucagon in cells from both fasted and fed rats. We used the high concentration of lactate in order to estimate pyruvate kinase isotopically by a trapping method (5).

The main question which we have attempted to answer in this work is how much of the stimulatory action of the hormones on gluconeogenesis from lactate can be accounted for by an inhibition of a "leak back" reaction, namely the flow from P-enolpyruvate back to pyruvate, catalyzed by pyruvate kinase. In Table I, we show that the estimated absolute rate of pyruvate kinase flux, during gluconeogenesis from lactate in liver cells from either fasted or fed rats, is less than 10% of the carbon flux from P-enolpyruvate to glucose, and that neither glucagon nor epinephrine at concentrations tested had any significant effect on the absolute rate of pyruvate kinase. This shows that the stimulatory effect of the hormones on gluconeogenesis under these conditions is primarily at other sites than pyruvate kinase, presumably at one of the reactions (or transport steps) in the conversion of pyruvate to P-enolpyruvate (12). One might note that the hormones, while causing no net changes in absolute pyruvate kinase flux, did maintain apparent pyruvate kinase flux in the presence of an increased flux through P-enolpyruvate to glucose. One should also point out that the estimations of pyruvate kinase flux become less reliable when the isotopic fluxes are low, when isotopic exchange reactions could make larger contributions to the results (13, 14).

In liver cells from fed rats, the rate of pyruvate kinase during gluconeogenesis from lactate is much higher, with the rate of pyruvate kinase approaching one-half of that of the conversion of lactate to glucose (Table I). Here a difference in action between glucagon and L-epinephrine becomes very apparent. While both hormones cause a marked stimulation of gluconeogenesis from lactate in liver cells from fed rats, only glucagon causes a marked reduction in absolute pyruvate kinase flux. In fact, epinephrine at concentrations below 1 μM causes a slight increase in absolute pyruvate kinase flux, in spite of which the rate of glucose synthesis is increased. At 10 μM epinephrine, the absolute rate of pyruvate kinase flux is the same as that in the control (no hormone added) cells, but the proportion of P-enolpyruvate returned to pyruvate compared to that converted to glucose is depressed by nearly one-half. Thus the lack of effect on the absolute rate of pyruvate kinase does not necessarily mean that L-epinephrine at this high concentration has no effect on the pyruvate kinase system, although the effect is certainly much less than that produced by glucagon. At the lower range of concentrations, glucagon produces only small changes in pyruvate kinase flux while causing a substantial increase in gluconeogenesis. This indicates that glucagon also must act at a forward step in the gluconeogenic pathway. At higher concentrations, glucagon depresses pyruvate kinase flux to a level approaching that in liver cells from fasted rats, and causes an even more striking depression of the proportion of P-enolpyruvate reconverted to pyruvate compared to that converted to glucose. Comparing the decrease in pyruvate kinase flux (about 18 μmol/g/h) caused by 1 μM glucagon, to the net increase in gluconeogenesis (about 23 μmol of glucose/g/h or 46 μmol/g/h of P-enolpyruvate converted to glucose), it is again apparent that depression of pyruvate kinase flux cannot be the sole site of action of glucagon. Under these conditions, however, it is an important
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site of action. Considering that pyruvate kinase flux could be somewhat underestimated by the isotopic procedure (see "Materials and Methods"), nearly half of the stimulation of gluconeogenesis from lactate could be ascribed to depression of pyruvate kinase in cells from fed animals. On the other hand, the stimulatory effect at the "other site" appears to come into play at lower concentrations of glucagon.

Our results show that a marked effect of glucagon on pyruvate kinase flux during gluconeogenesis from lactate can only be seen in hepatocytes from fed rats. However, with other substrates such as pyruvate, fructose, and dihydroxyacetone, we and others have shown that glucagon can cause a definite depression of apparent pyruvate kinase flux even in livers from fasted rats (3, 14-16). In general, pyruvate kinase flux is lower when more reduced substrates are metabolized (14), suggesting that the NADH/NAD⁺ ratio may in some manner also regulate pyruvate kinase flux.

It seems highly likely that the glucagon effect on pyruvate kinase is mediated by cyclic AMP, since the actions of glucagon or added cyclic AMP produce very similar effects in the intact liver cell (5, 14). The mechanism of the effect is being investigated by several groups (17-22). Blair and co-workers (17, 18) have shown that glucagon treatment of the perfused rat liver decreases the affinity of pyruvate kinase for P-enolpyruvate. Engstrom and co-workers (19, 20) have shown that a cyclic AMP-dependent protein kinase caused phosphorylation and inhibition of rat liver pyruvate kinase, and that full activity could be obtained upon dephosphorylation. Epinephrine has much less effect than glucagon on pyruvate kinase flux in hepatocytes from fed rats. The effect of 10 μM epinephrine, on lowering the relative flux through P-enolpyruvate to pyruvate, presumably may be the result of the β-adrenergic effect of epinephrine at this rather high concentration. Our results are in general accord with the views of Fain and co-workers (23) that the mechanisms of action of glucagon and epinephrine on the liver cell are not identical.

REFERENCES

Role of pyruvate kinase in the regulation of gluconeogenesis from L-lactate.
R Rognstad and J Katz


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