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Palmitate inhibits liver glycolysis

Involvement of fructose 2,6-bisphosphate in the glucose/fatty acid cycle

Louis HUE, Liliane MAISIN and Mark H. RIDER
Hormone and Metabolic Research Unit, Louvain University Medical School, and International Institute of Cellular and Molecular Pathology, UCL 7529, Avenue Hippocrate 75, B-1200 Brussels, Belgium

In hepatocytes from overnight-fasted rats incubated with glucose, palmitate decreased the production of lactate, the detritiation of [2-^{3}H]- and [3-^{3}H]-glucose, and the concentration of fructose 2,6-bisphosphate. Similarly, perfusion of hearts from fed rats with β-hydroxybutyrate resulted in an inhibition of the detritiation of [3-^{3}H]glucose and a fall in fructose 2,6-bisphosphate concentration. This fall could result from an increase in citrate (hepatocytes and heart) and *sn*-glycerol 3-bisphosphate concentration. It is suggested that a fall in fructose 2,6-bisphosphate concentration participates in the inhibition of glycolysis by fatty acids and ketone bodies.

INTRODUCTION

In general, changes in glycolytic flux in liver are related to, and probably caused by, corresponding changes in fructose 2,6-bisphosphate (Fru-2,6-P_2) concentration, except during anoxia (for recent reviews, see [1] and [2]). For example, in isolated rat hepatocytes, the stimulation of glycolysis by glucose concentrations greater than 10 mM can be explained by an increase in Fru-2,6-P_2, which itself results from a glucose-induced rise in fructose 6-phosphate concentration. Conversely, a decrease in Fru-2,6-P_2 concentration is observed when hepatocytes are incubated in the presence of glucagon or when large concentrations of gluconeogenic precursors such as lactate, pyruvate, alanine, glycerol or fructose are added. The effect of glucagon results from the cyclic AMP-dependent phosphorylation of 6-phosphofructo-2-kinase (PFK-2)/fructose-2,6-bisphosphatase (FBPase-2). Phosphorylation inactivates PFK-2 and activates FBPase-2. The effect of gluconeogenic precursors might be explained by an inhibition of PFK-2 activity and/or stimulation of FBPase-2 activity by intermediates such as phosphoenolpyruvate or *sn*-glycerol 3-phosphate, which accumulate under these conditions.

Fatty acids and ketone bodies are known preferred substrates for heart and skeletal muscle, where their oxidation inhibits glucose utilization and thus allows for glucose sparing [3–5]. On the other hand, fatty acids are known to stimulate gluconeogenesis in liver [6,7], and one may wonder whether they inhibit liver glycolysis. Our results show that this is the case and that a decrease in Fru-2,6-P_2 is involved in this mechanism. The effect of palmitate in hepatocytes was compared with that of β-hydroxybutyrate in perfused hearts.

MATERIALS AND METHODS

Biochemicals

Glucose, D-β-hydroxybutyrate, sodium palmitate, bovine serum albumin fraction V (all from Sigma) and other biochemical reagents (Sigma or Boehringer) were purchased as indicated. Albumin was defatted [8] and complexed with palmitate as described [9].

Preparation and incubation of hepatocytes

Hepatocytes were prepared from overnight-fasted male Wistar rats as described previously [10]. The cells (about 100 mg wet wt. in 2 ml) were shaken (120 strokes/min) in stoppered 20 ml flasks at 37 °C for 45 min with glucose and albumin-bound palmitate at the concentrations indicated, and in the presence of O_2/CO_2 (19:1). the measurement of enzyme activities and of Fru-2,6-P_2, samples (0.1 ml) were taken after 30 min of incubation, addition of 0.2 ml of ice-cold concentrated $HClO_4$. For the measurement of enzyme activities and of Fru-2,6-P_2 samples (0.1 ml) were taken after 30 min of incubation, frozen in acetone/solid CO_2 and further processed as indicated below. For the measurement of ^3H_2O release from [2-^{3}H]- or [3-^{3}H]-glucose (0.5 μCi/ml), ^3H_2O was separated from the radioactive glucose in neutralized $HClO_4$ extracts [11]. The release of ^3H_2O from [2-^{3}H]- and [3-^{3}H]-glucose gives an indirect estimation of the glucose flux through glucokinase and 6-phosphofructo-1-kinase (PFK-1) respectively [12]. To measure the intracellular concentration of hexose 6-phosphate and *sn*-glycerol 3-phosphate [13], the cells were separated from the incubation medium and deproteinized as described previously [14].

Perfusion of hearts

Hearts from fed or overnight-fasted Wistar rats (200–250 g) were weighed, wash-out preperfused for 10 min and then perfused with recirculation for 30 min by the Langendorff technique as described by Mowbray & Ottaway [15]. The perfusion medium contained, in addition to 5 mM-glucose, various concentrations of β-hydroxybutyrate as indicated, and in some experiments 0.2 μCi of [3-^{3}H]glucose/ml. For the measurement of ^3H_2O release, samples (2 ml) were taken from the main reservoir at 0, 10, 20 and 30 min, deproteinized with

Abbreviations used: Fru-2,6-P_2, fructose 2,6-bisphosphate; FBPase-1, fructose-1,6-bisphosphatase; FBPase-2, fructose-2,6-bisphosphatase; PFK-1, 6-phosphofructo-1-kinase; PFK-2, 6-phosphofructo-2-kinase.

$HClO_4$ and processed as indicated below. After the perfusion period, the hearts were clamped between liquid-N_2-cooled aluminium plates and processed as indicated below for the measurement of metabolites.

Assay of metabolites and enzyme activity

Glucose 6-phosphate [16], fructose 6-phosphate [16], lactate [17], citrate [18], *sn*-glycerol 3-phosphate [19] and ATP [20] were determined enzymically in neutralized $HClO_4$ extracts, and Fru-2,6-P_2 [21] was determined in alkaline extracts as described in the references. The activation state of PFK-2 from hepatocytes was determined as described previously [22]. For the study of PFK-2 inhibition by citrate, the liver and the heart enzyme were purified as described [23], and the activity was assayed [24] at pH 7.1 in the presence of 0.3 mM-glucose 6-phosphate, 0.1 mM-fructose 6-phosphate, 5 mM-MgATP and 5 mM-potassium phosphate.

RESULTS

Inhibition of liver glycolysis by palmitate

Palmitate decreased the production of lactate, the flux through PFK-1 as measured by detritiation of [3-^{3}H]glucose, and the concentration of Fru-2,6-P_2 in hepatocytes incubated with various glucose concentrations (Fig. 1). Both the sensitivity towards glucose and the maximal rate of lactate release were decreased by palmitate. Thus, it appears that, like in muscle [5], fatty acids are capable of inhibiting glycolysis. The inhibitory concentrations of palmitate (Fig. 2) correspond to fatty acids/albumin molar ratios of 0.9–4.5, which overlap the range of values *in vivo* (0.2–3). Therefore, the inhibitory effect reported is probably of physiological relevance. The effect of palmitate in liver glycolysis and Fru-2,6-P_2 contrasts sharply with its effect in isolated adipocytes. In these cells, palmitate increased glucose detritiation without changing Fru-2,6-P_2 concentration and lactate release [25].

To understand the mechanism(s) involved in the inhibition of liver glycolysis by palmitate, metabolic fluxes and the concentrations of several effectors of PFK-1 were measured (Table 1). Palmitate was found to inhibit glucose phosphorylation, as measured by the detritriation of [2-^{3}H]glucose. The inhibition was reflected by the small but significant fall in the intracellular concentration of hexose 6-phosphate. This effect of palmitate on glucose phosphorylation in intact cells is consistent with the inhibition of glucokinase *in vitro* by long-chain acyl-CoA [26]. However, glucokinase inhibition in intact cells cannot be solely responsible for the inhibition of glycolysis (Table 1, Fig. 1), since the decreases in lactate release and in PFK-1 flux were greater than the inhibition of glucose phosphorylation. The combined effect of decreased concentration of positive effector (Fru-2,6-P_2) and increased concentration of negative effectors (citrate and *sn*-glycerol 3 phosphate) of PFK-1 is likely to explain inhibition of PFK-1 and of glycolysis. However, at 20 mM-glucose, the inhibition of lactate release by palmitate was greater than the effect on [3-^{3}H]glucose detritiation. The difference does not necessarily imply that steps downstream from PFK-1 should be regulated by palmitate. Indeed, it may be explained by the occurrence of metabolite cycling through PFK-1 and fructose-1,6-bisphosphatase (FBPase-1). The cycling rate could be as large as the difference between the rates of [3-^{3}H]glucose detritiation, which gives an estimation of PFK-1 flux, and lactate release (expressed in the same units). The difference between [3-^{3}H]glucose detritiation and lactate release was equal to about 9 μmol of glucose/45 min per g of hepatocytes, and was not influenced by palmitate. In the absence of palmitate, the difference was roughly equal to lactate release, whereas with palmitate it was at least 5 times larger. Thus the latter condition favours gluconeogenesis. Alternatively, the difference between detritiation and lactate release might have been caused by the utilization of *sn*-glycerol 3-phosphate for the esterification of palmitate. The latter condition is characterized by an elevated concentration of *sn*-glycerol 3-phosphate [27] that was also observed under our conditions (Table 1).

Regarding the mechanisms involved in decreasing Fru-2,6-P_2 concentrations, changes in the activation of PFK-2/FBPase-2 can be ruled out, since palmitate did not induce changes in the activity of the bifunctional enzyme (results not shown). On the other hand, the data of Table 1 suggest that the increased concentrations of citrate and *sn*-glycerol 3-phosphate (both inhibit PFK-2, and the latter also stimulates FBPase-2 [1,2]) can account for the fall in Fru-2,6-P_2 concentration. Inhibition by

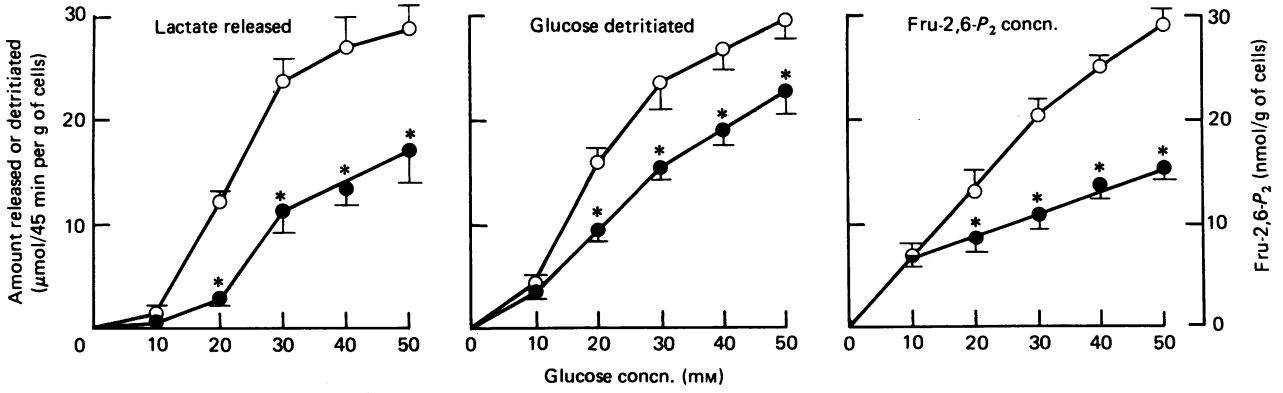

Fig. 1. Effect of 1 mM-palmitate on the production of lactate, the detritiation of [3-^{3}H]glucose and the concentration of Fru-2,6-P_2 in hepatocytes incubated with various concentrations of glucose

The values shown are means ± S.E.M. for five different cell preparations. ○, controls; ●, with 1 mM-palmitate; * refers to values that are significantly different ($P < 0.01$) from controls.

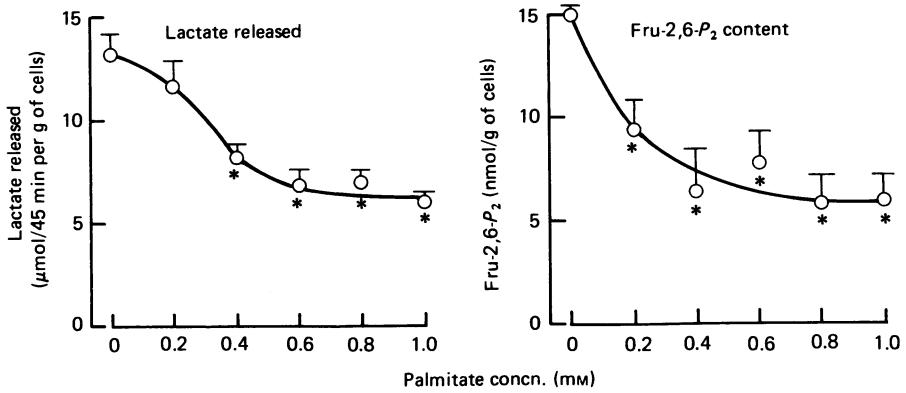

Fig. 2. Effect of various concentrations of palmitate on the production of lactate and the concentration of Fru-2,6-P_2 in hepatocytes incubated with 20 mM-glucose

The values are means ± S.E.M. for four different cell preparations; * refers to values that are significantly different ($P < 0.01$) from controls.

Table 1. Effect of palmitate on the concentration of several metabolites and on metabolix fluxes in hepatocytes

The values shown are means ± S.E.M. for three different preparations.

Metabolite concn. and fluxes	Hepatocytes incubated with 20 mM-glucose	Hepatocytes incubated with 20 mM-glucose + 1 mM-palmitate	P
ATP (μmol/g)	2.02±0.04	2.01±0.03	> 0.1
Glc-6-P + Fru-6-P (nmol/g)	66±2	60±1	< 0.05
Citrate (μmol/g)	0.54±0.02	0.75±0.02	< 0.001
sn-Glycerol 3-phosphate (μmol/g)	0.21±0.02	0.51±0.03	< 0.001
[2-^{3}H]Glucose detritiated (μmol/45 min per g)	22.6±1.9	18.4±1.3	< 0.01
[3-^{3}H]Glucose detritiated (μmol/45 min per g)	15.8±0.9	10.2±0.9	< 0.001
Lactate released (μmol/45 min per g)	14.2±0.1	2.7±0.3	< 0.001

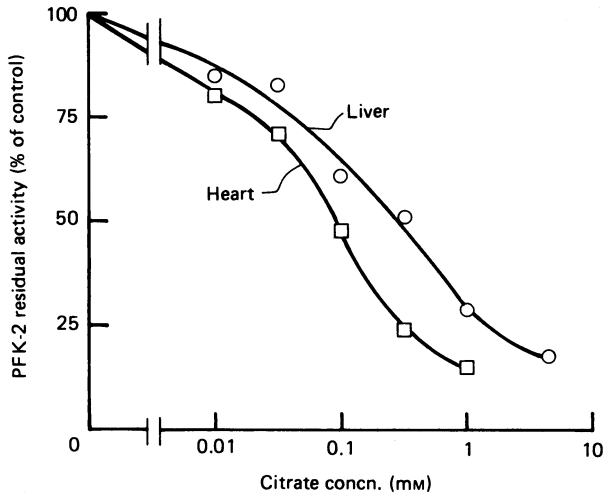

Fig. 3. Inhibition by citrate of PFK-2 purified from rat liver and bovine heart

The activity was measured at pH 7.1 in the presence of 0.1 mM-fructose 6-phosphate, 0.3 mM-glucose 6-phosphate, 5 mM-MgATP and 5 mM-potassium phosphate, and was equal to 33 (liver; ○) and 250 (heart; □) nmol/min per mg of protein under control conditions.

citrate of PFK-2 purified from rat liver and heart is illustrated in Fig. 3. The enzyme from both sources is inhibited by concentrations of citrate that are within the physiological range. The heart enzyme ($K_{0.5}$ about 90 μM) appears more sensitive towards citrate than the liver enzyme ($K_{0.5}$ about 250 μM). These values are similar to those reported in the literature for the rat liver and the pigeon muscle enzyme [23,28]. They should be compared with the cytosolic concentrations of citrate. These are difficult to evaluate, because of compartmentation and possible binding to proteins. In hepatocytes, the cytosolic content represents about 50% of the total citrate with a mitochondrial/cytosolic gradient of 10 or more [29].

Inhibition of heart glycolysis by ketone bodies

Fatty acid and ketone bodies are known to inhibit glucose utilization in heart *in vitro* [3,4]. This led to the concept of the glucose/fatty acid cycle, which explains the glucose-sparing effect of fatty acids and ketone bodies [5]. The inhibition of glucose utilization by cardiac muscle is explained, at least in part, by an inhibition of PFK-1 by citrate, whose concentration in increases during fatty acid oxidation [30].

We have confirmed these observations (Table 2), namely that perfusion of hearts from fed (Table 2) or overnight-fasted rats (results not shown), in the presence

Table 2. Effect of various concentrations of β-hydroxybutyrate on the detritiation of [3-^{3}H]glucose, and the concentrations of Fru-2,6-P_2 and citrate in perfused hearts from fed rats

The values are means ± S.E.M. for the numbers of observations given in parentheses: * values from controls ($P < 0.05$).

β-Hydroxybutyrate added	[3-^{3}H]Glucose detritiated (μmol/min per heart)	Fru-2,6-P_2 concn. (nmol/g)	Citrate concn. (μmol/g)
0	0.28 ± 0.02 (3)	2.7 ± 0.3 (9)	0.17 ± 0.02 (3)
0.25 mM	–	2.4 ± 0.6 (3)	0.15 ± 0.02 (3)
0.50 mM	–	2.5 ± 0.3 (3)	0.24 ± 0.02 (3)
1.0 mM	–	2.0 ± 0.1 (3)*	0.29 ± 0.08 (3)
2.5 mM	–	1.3 ± 0.1 (3)*	0.44 ± 0.04 (3)*
5.0 mM	0.11 ± 0.02 (3)*	1.4 ± 0.1 (3)*	0.32 ± 0.03 (9)*

of β-hydroxybutyrate decreased the detritiation of [3-^{3}H]glucose, which gives an estimation of glucose utilization through PFK-1. The data also show that β-hydroxybutyrate caused opposite and dose-dependent changes in the concentrations of Fru-2,6-P_2 and citrate; however, the concentration of *sn*-glycerol 3-phosphate was unchanged (results not shown). The fall in Fru-2,6-P_2 concentration might result from an inhibition of PFK-2 by citrate, since the concentrations required to inhibit the enzyme *in vitro* (Fig. 3) are similar to those measured in perfused hearts (Table 2). As for hepatocytes, the concentration of cytosolic citrate is difficult to evaluate. It is proposed that, as in liver, citrate inhibits both PFK-1 and PFK-2. Inhibition of the latter lowers Fru-2,6-P_2 concentration, and so reinforces the inhibition of PFK-1 by citrate.

Conclusions

The demonstration that fatty acids inhibit glycolysis not only in muscle, but also in liver, extends to this organ the concept of the glucose/fatty acid cycle. Whether this contributes much to glucose sparing for the whole animal is unlikely, since the proportion of body glucose that is glycolysed in the liver is relatively small. However, by inhibiting glucokinase and, mainly, PFK-1, fatty acids set the liver in gluconeogenic conditions. Therefore, the inhibition of glycolysis further adds to the well-known stimulation of pyruvate carboxylase by acetyl-CoA derived from fatty acids [31], and so works towards a concerted mechanism for the stimulation of gluconeogenesis.

Fatty acids, like relatively large concentrations of gluconeogenic precursors such as lactate, pyruvate and alanine, decrease the concentration of Fru-2,6-P_2 in the liver. During aerobiosis, Fru-2,6-P_2 could be regarded as a glycolytic signal which is turned on by glucose availability and switched off by alternative fuels. Moreover, in heart, as in the muscles of flying locusts [32], a fall in Fru-2,6-P_2 concentration could signal the presence of non-glycolytic substrates and, by doing so, it can contribute to the glucose-sparing effect of these substrates. In these tissues, the increased citrate concentration probably plays a crucial role in inhibiting PFK-1 and PFK-2.

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