Enhanced Carrier-mediated Lactate Entry into Isolated Hepatocytes from Starved and Diabetic Rats*

Hilary K. Metcalfe, John P. Monson†, Robert D. Cohen, and Charles Padgham

From the Medical Unit, The London Hospital Medical College, Whitechapel, London E1 2AD, United Kingdom

Hepatic plasma membrane lactate transport was studied in isolated hepatocytes prepared from fed, starved, and streptozotocin diabetic rats. Carrier-mediated lactate entry was determined using the lactate transport inhibitors α-cyano-3-hydroxycinnamate and D-3-hydroxybutyrate and was significantly greater in hepatocytes from starved compared to fed rats and in hepatocytes from diabetic fed compared to fed rats. The saturable component of lactate entry which corresponds to carrier-mediated transport was higher in the starved than in the fed state with results from diabetic fed being intermediate between the two. Insulin treatment prevented the increment in carrier-mediated lactate transport observed in hepatocytes from diabetic fed rats. The data indicate that hepatic plasma membrane lactate transport is increased under conditions of starvation and diabetes mellitus. This may partly explain the increased gluconeogenic flux under these conditions.

Carrier-mediated hepatic plasma membrane lactate transport is a well-documented phenomenon (1-3), and we have previously shown, in isolated rat hepatocytes, that inhibitors of hepatic plasma membrane lactate transport may be used to quantify that component of the lactate entry process which is attributable to carrier-mediated transport and to distinguish it from the residual component due to passive diffusion (1, 4). Suitable inhibitors for this purpose are α-cyano-3-hydroxycinnamate and D-3-hydroxybutyrate. We have used both inhibitors in the present studies and have also compared the saturation characteristics of lactate entry into hepatocytes under the various conditions.

EXPERIMENTAL PROCEDURES

Materials—Sodium L-[14C]lactate and tritiated water (specific radioactivities, 161 mCi/mmol and 100 mCi/ml, respectively) were obtained from Amersham International, Bucks, United Kingdom; 5,5′-dimethyl-[2,4,6-14C]oxazolidine-2,4,6-trione (DMO) (specific radioactivity, 50 mCi/mmol) from New England Nuclear Corp., Dreieich, West Germany; L(+)-lactic acid from Sigma Chemical Co., Poole, Dorset, U. K.; α-cyano-3-hydroxycinnamonic acid from BDH Chemicals Ltd., Poole, Dorset, U. K.; 5,5′-dimethylafoxazolidinedione (DMO) from Baxter Laboratories, Morton Grove, IL; and sodium D-3-hydroxybutyrate from Marlborough Biopolymers Ltd., Stockton-on-Tees, Cleveland, U. K. All other chemicals were of the highest grade available commercially.

Experimental Animals—Male normal and diabetic Sprague-Dawley rats (200-250 g) were used for all experiments and were either fed ad libitum on standard rat cake or starved for 48 h. Diabetes mellitus was induced 2 days prior to experiments by a single intraperitoneal injection of streptozotocin (75-100 mg/kg) and glucose intolerance confirmed by demonstrating blood glucose values in excess of 10 mm (mean blood glucose 19.66 ± 1.72 mM, n = 30) and weight loss. Mean blood ketones (3-hydroxybutyrate plus acetoacetate) concentrations in these animals were 1.88 ± 0.48 mm (n = 30). Insulin-treated animals were administered streptozotocin as described above and then treated intraperitoneally with 1 unit/100 g of rat weight of isophane insulin (Lilly) per day at 0 and 24 h. Insulin was administered subcutaneously at approximately 1630 h, shortly before the onset of the dark phase of their light-dark cycle, when the rats normally start their most active feeding. The mean blood glucose and ketone body concentrations in the insulin-treated animals at the time of the experiment were 5.5 ± 0.97 mm (n = 6) and 0.13 ± 0.02 mm (n = 6), respectively. Isolated hepatocytes were prepared by collagenase perfusion as previously described (1) and suspended in Krebs bicarbonate buffer (5) (wet weight, 30-60 mg/ml) at pH 7.4 in an atmosphere of 95% O2, 5% CO2. Viability was assessed by trypan blue exclusion (>90%).

Inhibitor Studies—Aliquots of hepatocyte suspension were preincubated at 37 °C for 20 min ("standard preincubation protocol") prior to the addition of either 5 mM α-cyano-3-hydroxycinnamonic acid (CHC) or 15 mM D-3-hydroxybutyrate or appropriate osmolar control solutions (5 and 16 mM sodium chloride, respectively) together with 0.17 μCi of L-[1,14C]lactate. The concentrations of CHC and D-3-hydroxybutyrate employed have been previously shown to produce maximal inhibition of transporter-mediated [U,14C]lactate entry (1, 4). 15 min after the additions, the incubations were terminated by rapid centrifugation of the cells through silicone oil (specific gravity, 1.04) into 1.0 m perchloric acid. H2O was used as a cell water marker. 1H and 14C activity in samples of supernatant above the oil layer and the acid cell extract were determined in a Packard scintillation counter using an external standard for quench correction. Intracellular 14C activity, corrected for extracellular contamination, was calculated as previously described (1). We have previously shown that under these conditions in hepatocytes from starved rats less than 12% of intracellular 14C activity after 15 min is in compounds other than lactate (1); similar results have been obtained in hepatocytes from fed rats by others (2), and we have assumed that approximately similar conditions apply in hepatocytes from diabetic rats. The component of [11,14C]lactate entry due to carrier-mediated transport was determined by subtraction of entry in the presence of inhibitors from total entry in parallel control incubations (1).

Saturation Studies—Aliquots of hepatocyte suspension prepared from fed or starved rats, both normal and diabetic, and from insulin-treated diabetic fed rats were preincubated at 37 °C for 30 min, centrifuged, and washed at room temperature in 9 ml of fresh buffer

† To whom correspondence should be addressed.

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1 The abbreviations used are: DMO, 5,5′-dimethyl-[2,4,6-14C]oxazolidine-2,4,6-trione; CHC, α-cyano-3-hydroxycinnamonic acid.
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Inhibitor Studies—Total and carrier-mediated lactate entry into isolated rat hepatocytes from normal (control) and diabetic rats, both fed and starved. Aliquots of hepatocyte suspension were incubated in the presence or absence of CHC (5 mM) and [14C]lactate for 15 s prior to rapid centrifugation through silicone oil into perchloric acid. Extracellular [14C] was normalized to 900 dpm/μl cell water. Total intracellular [14C] was measured in the absence of inhibitor, and carrier-mediated lactate entry is that component of entry which was inhibited by CHC. Mean intracellular [14C] entry ± S.E. is shown with the number of cell preparations in parentheses, with three individual observations for each cell preparation. In addition to the statistical comparisons shown, there was no significant difference between mean carrier-mediated entry in cells from diabetic starved and control starved animals.

RESULTS

Inhibitor Studies—Total and carrier-mediated lactate entries into isolated hepatocytes prepared from fed and starved rats, both normal and diabetic, are shown in Figs. 1 and 2. Comparison of these figures shows that estimation of carrier-mediated lactate entry using either CHC or D-3-hydroxybutyrate as inhibitors gave a similar pattern of [U-14C]lactate uptake. In cells from normal animals, carrier-mediated entry was significantly greater when the donors were starved as compared to fed. Thus in the series in which CHC was used as the inhibitor, the mean entry of [U-14C]lactate on the transporter was 1204 ± 93 dpm/μl of cell water in cells from starved normal rats compared with 318 ± 36 dpm/μl of cell water in cells from fed normal animals. There was no significant difference between diabetic fed and diabetic starved rats but significantly greater plasma membrane lactate transport in cells from diabetic fed compared to normal fed rats. The component of entry due to passive diffusion (represented by the difference between the bars for total and carrier-mediated entry) was approximately equal under all conditions studied with a single inhibitor. However, the quantitative effect of the inhibitor varied somewhat, being greater with CHC than D-3-hydroxybutyrate. This was reflected in an apparently higher carrier-mediated component and lower diffusion component in the CHC experiments. The extracellular unlabeled lactate concentration was predictably higher in those incubations of cells from fed and diabetic fed rats than in starved rats due to glycogenolysis and glycolysis (0.864 ± 0.194 mM (n = 11) for normal fed; 0.420 ± 0.119 mM (n = 5) for diabetic fed; <0.1 mM for starved rats). The saturation studies described below were designed partly to examine the possible effects of differing lactate concentrations on the interpretation of the above results.

Saturation Studies—Lactate concentrations in the medium were adjusted as described under “Experimental Procedures”
Fig. 3. Effect of unlabeled lactate on the entry of [14C]lactate into isolated rat hepatocytes prepared from normal starved (O), normal fed (C), and diabetic fed (x) rats. Cells were incubated in the presence of [14C]lactate and different concentrations of unlabeled lactate for 15 s prior to rapid centrifugation through silicone oil into perchloric acid. Results are expressed as intracellular [14C] with extracellular [14C] normalized to 900 dpm/µl cell water. The number of cell preparations for 0–4 mM lactate was 12, 9, and 15 for normal starved, normal fed, and diabetic fed, respectively, and the whole range of concentrations from 0 to 4 mM was used for cells from each rat. The results for 10 mM lactate are mean ± S.E. of 4 separate cell preparations for each condition. Data for each cell preparation were derived from three separate incubations.
transport, of a similar order to those described here, were being 973 150 (n individual observations in each category for each cell preparation). The similarity of the data suggests the transporter-mediated component of lactate transport and by examining differences in the saturation behavior of lactate entry into hepatocytes. In normal rats, both methods demonstrate a significantly greater carrier-mediated lactate entry in the starved compared to the fed state. There was also greater carrier-mediated entry in the diabetic fed compared to the normal fed state. Extracellular lactate concentrations in the inhibitor studies were higher in the fed (0.86 mM) and diabetic fed (0.4 mM) incubations compared to corresponding starved incubations, and these differences could, by partial saturation of the transporter, possibly account for the differences in apparently transporter-mediated uptake. It can be estimated from the saturation curves (Fig. 3) that if in the inhibitor studies the external lactate concentrations in the fed cells had been zero instead of 0.86 mM, there would have been an extra increment of [1-14C]lactate entry of 210 dpm/μl cell water; however, this would account for only a small part of the difference of inhibitable [U-14C]lactate entry observed in fed and starved cells (886 and 664 dpm/μl cell water in the CHC and D-3-hydroxybutyrate studies, respectively). Similarly, only about 25–30% of the differences in inhibitable 14C uptake between the fed and fed diabetic cells could have been accounted for by the different extracellular lactate concentration. The saturation effects thus do not substantially alter the conclusions reached in comparing starved with fed and fed diabetic incubations.

The saturation studies provide independent evidence of the effect of the nutritional state and diabetes on transporter activity. Cells from starved normal rats show a considerably higher [U-14C]lactate uptake at zero (nominally) external lactate concentration than did those from normal fed animals. Furthermore, the depression of uptake by high concentrations (4 mM) of external lactate was greater in starved than in fed cells; the extent of this saturation by high external lactate concentration is the transporter-mediated component of lactate uptake (1). The saturation curve obtained from cells derived from diabetic fed animals lies between the fed and starved curves from normal animals. Application of our previously described model for hepatocyte lactate entry (1), which assumes unidirectional carrier-mediated transport and bidirectional diffusion, to the curves in Fig. 3 suggests that the changes observed are due to alterations in maximal transport capacity rather than alteration in transporter affinity for

**TABLE I**

<table>
<thead>
<tr>
<th>Lactate (mmol/liter)</th>
<th>Standard protocol</th>
<th>Additional wash protocol</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>dpm/μl cell water</td>
<td>dpm/μl cell water</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2732 ± 318</td>
<td>2132 ± 155</td>
<td>NS</td>
</tr>
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<td>0.25</td>
<td>2393 ± 334</td>
<td>2019 ± 192</td>
<td>NS</td>
</tr>
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<td>0.50</td>
<td>2247 ± 273</td>
<td>1944 ± 197</td>
<td>NS</td>
</tr>
<tr>
<td>0.75</td>
<td>2179 ± 234</td>
<td>1821 ± 199</td>
<td>NS</td>
</tr>
<tr>
<td>1.00</td>
<td>2043 ± 226</td>
<td>1730 ± 191</td>
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<tr>
<td>4.00</td>
<td>1588 ± 152</td>
<td>1342 ± 106</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS, not significant.

(n is the number of preparations). The similarity of the data under the various conditions argues against the differences in uptake seen in the saturation curves being due to changes in the pH gradient; we have previously shown that alterations in the pH gradient sufficient to produce changes in lactate transport, of a similar order to those described here, were readily detectable by the DMO method (1).

**DNA Content per Milliliter of Intracellular Water**—There were no substantial differences in this ratio between normal starved or fed and diabetic fed rats, the respective mean values being 373 ± 150 (n = 2); 1068 ± 150 (n = 3); and 947 ± 77 (n = 4) g of DNA/ml. The figures in parentheses indicate the number of rats. Each measurement was performed in duplicate.

**DISCUSSION**

In the present studies carrier-mediated hepatic lactate entry has been measured by using inhibitors of plasma membrane
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lactate. The experiments comparing saturation curves from cells obtained from diabetic fed and streptozotocin-injected but insulin-treated fed animals strengthen the evidence that the diabetic state itself alters the behavior of cells from fed animals toward that of the starved state.

In these experiments the lactate entry is expressed per unit volume of intracellular water. It is possible that cells obtained under the various experimental conditions used differed in the amount of intracellular water/cell and that this could have given rise to some of the changes which we have attributed to differences in carrier-mediated lactate entry. To exclude this possibility the ratio of DNA to intracellular water was measured in some preparations from the different groups of rats. No significant differences were found, suggesting that the intracellular water is a valid index of cell number under the different conditions.

We considered the possibility that the known enhancing effect of increased extracellular/intracellular hydrogen ion gradient on lactate transport into hepatocytes (1–3) might have been responsible for the differences in transport observed in the present studies. The measurements made in steady state incubations of hepatocytes from starved and fed control and fed diabetic rats render this explanation unlikely. A potential reason for enhanced lactate transport in diabetes might be decreased food intake rather than a primary effect of the diabetic state on transport. Careful measurements of food intake and previously published data (11) suggested that this was not the case, the decrease in food intake during the first 24 h not exceeding 25%. However, the animals were in a state of negative energy balance, and it is thus not possible to state with certainty whether diabetes per se has an effect distinct from that of starvation, bearing in mind the qualitative similarity of hormone profiles in the two states.

The effects of starvation on lactate transport demonstrated in these experiments could contribute to the stimulation of gluconeogenesis under these conditions in vivo. The mechanism of the changes in lactate transport reported here is unknown but may be due to hormonal induction of carrier synthesis or mobilization; further elucidation must await studies in hepatocyte cultures with defined hormonal environments.

Nutritional alterations in the A system for transport of neutral amino acids into hepatocytes under conditions of starvation and high protein diets have been previously described (12), and the effects of starvation on both alanine and lactate transport may be of considerable physiological importance, bearing in mind the fact that both alanine and lactate metabolism are limited by the rate of plasma membrane transport (4, 13, 14) under these conditions. Nutritional effects on other transport systems such as blood brain barrier ketone transport are also well described (15, 16). The influence of diabetes on lactate transport is analogous to the previously documented enhancement of neutral amino acid transport into liver plasma membrane vesicles and hepatocytes prepared from diabetic rats (17–19). These processes thus constitute a possible mechanism for enhanced gluconeogenic flux in diabetes mellitus in addition to the known increased activity of several gluconeogenic enzymes (for recent review, see Ref. 20).

In this context some comment is indicated on the apparent paradox that conditions of increased gluconeogenic flux in vivo are normally associated with increased ketogenesis, whereas in this and previous work (4) we have used hydroxybutyrate and acetocetate as inhibitors of hepatic plasma membrane lactate transport. Furthermore, other workers have documented enhancement of gluconeogenesis from lactate by hydroxybutyrate (21–23). The explanation for this apparent discrepancy lies in the fact that the effects of hydroxybutyrate on lactate uptake critically depend on the lactate and hydroxybutyrate concentrations employed. In studies using extracellular lactate concentrations in excess of the $K_m$ for lactate transport (21, 22) the predominant mode of lactate entry into cells is by simple passive diffusion so that the net effect of hydroxybutyrate is to enhance intracellular pyruvate metabolism for example by increasing mitochondrial pyruvate transport in exchange for acetocetate (21, 23). The same mechanism may explain the previous observations by Arinze et al. (22) that concentrations of hydroxybutyrate which are too low to inhibit plasma membrane lactate transport may increase gluconeogenesis in perfused livers. In contrast, in the present studies using inhibitors, the lactate concentrations employed were well below the apparent $K_m$ for the transporter, so that transporter-mediated mechanisms accounted for a substantial fraction of entry; in addition, maximally inhibitory concentrations of hydroxybutyrate were used.

The present experimental findings provide a basis for the hypothesis that changes in hepatic plasma membrane lactate transport may partly account for the increased gluconeogenic flux in starvation and diabetes mellitus. However, because of the artificial increase in passive diffusion which occurs in the isolated hepatocyte model it is difficult to draw firm conclusions about rate determination of lactate metabolism in intact liver by modulation of plasma membrane transport. We have previously addressed this question using D-3-hydroxybutyrate and a rapid sampling technique in perfused livers from starved rats and provided evidence for rate determination of lactate metabolism by plasma membrane transport (4). Preliminary data from similar studies in perfused livers from fed and diabetic rats are consistent with the conclusions from the present work (24).

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REFERENCES