Lactate supply as a determinant of the distribution of intracellular pH within the hepatic lobule

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When isolated livers from starved rats are perfused with lactate at constant perfusate pH and P_{co_2}, there is a marked gradient of cell pH (pH_i) along the length of the lobular radius, with periportal cells being substantially more alkaline than perivenous cells. In the present studies, the perivenous 21 % of the lobular volume was destroyed by retrograde digitonin perfusion, and antegrade perfusion restored. pH_i was determined by ^{31}P-NMR. The remaining periportal cells, the site of gluconeogenesis from lactate, had a substantially higher mean pH_i (7.42) than did the intact liver (7.23). When lactate was removed from the perfusate, mean pH_i decreased to 7.25. The corresponding concentration of cell bicarbonate fell with a half-time of approximately 5 min. When lactate was re-introduced mean pH_i rose to 7.34. We conclude that a major contributor to periportal alkalinity under these conditions is proton consumption during gluconeogenesis from lactate ions.

Key words: hepatic intracellular pH, hepatic intralobular heterogeneity, gluconeogenesis, digitonin.

INTRODUCTION

We have recently shown [1] that, when isolated livers from starved rats are perfused with sodium-L-(+)-lactate (1.5 mM) as the sole substrate, there is a marked gradient of intracellular pH from the extreme periportal cells down the radius of the hepatic lobule to the most perivenous cells, with pH_i being highest periportally. pH_i was as high as 7.7–7.9 in the extreme periportal region, falling to less than 7.3 in the most perivenous region. In the present study we examined the mechanism of this novel observation.

Using the metabolic mapping procedure described in that study, we demonstrated that gluconeogenesis was most active in the periportal zone and diminished to zero by approximately 40 % down the lobular radius. We have subsequently shown [2] that lactate uptake has a similar distribution to gluconeogenesis. It has been demonstrated that a substantial fraction of entry of lactate from physiological concentrations into the hepatocyte is as the lactate ion [3,4], rather than as the undissociated acid, with entry being mediated by the hepatocyte monocarboxylate transporter (apparent K_m for lactate 1.9 mM); at high concentration, entry appears to be largely by pH-dependent non-ionic diffusion [5]. When ions of weak acids are converted into neutral products (in the case of lactate; glucose, glycogen, CO_2 and water or triacylglycerols), protons are consumed stoichiometrically [6]. We therefore hypothesized that the relative alkalinity of the more periportal cells under these conditions is due to their function as the site of lactate disposal. The purpose of the studies reported here was to test this hypothesis.

In the work demonstrating periportal alkalinity, hepatic intracellular pH was determined by ^{31}P-NMR spectroscopy, using the chemical shift of the peak of the inorganic phosphate (P_i) resonance. When this is performed on intact perfused liver, the P_i resonance is rather broad, due to contributions from cells at different sites in the lobule with respect to their distance from the central venule [1]. For this purpose, the liver lobule may be regarded as consisting of a series of concentric shells of cells, centred on the central venule; if all cells in a single shell have the same pH_i, then the contribution of that shell to the overall P_i resonance is a function of that pH_i, the volume of cells in that shell and their P_i concentration. In our previous work [1,2,7,8], we studied the distribution of variables of metabolic interest, including glucose and lactate uptake and output and pH_i, by destroying varying numbers of these shells by retrograde perfusion of digitonin, reverting to antegrade perfusion and observing the properties of the unaffected more periportal cells. The validity of this model was supported by the observation that the P_i resonance became narrower and sharper as the extent of destruction increased [1]. Thus when only a thin periportal rim of unaffected cells remains, the value of pH_i, determined by the chemical shift of the P_i resonance, approaches a value that is unaffected by contamination by signal from more perivenously located shells. In the present study, therefore, we aimed to destroy approximately 20 % of the volume of the lobule from the perivenous direction, in order to permit more specific studies of pH_i control in that part of the lobule where lactate disposal and glucose synthesis have been shown to occur.

EXPERIMENTAL

Isolated perfused liver preparations were set up as previously described [9,10] from 48 h starved male Wistar rats of weight 280–320 g. The perfusate was erythrocyte- and albumin-free Krebs buffer (120 mM NaCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, $7H_2O$, 4.8 mM KCl, 1.72 mM $CaCl_2$, $2H_2O$, 29 mM $NaHCO_3$) at 37 °C, gassed with 95 % O_2 and 5 % CO_2, with resultant mean pH 7.38 and P_{co_2} 5.0 kPa. The perfusate flow rate was 10 ml/min per 100 g rat weight. To ensure that the proportion of lactate

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entry as the ion was high, the concentration of the sole substrate, sodium L-(+)-lactate was chosen as 1.5 mM, which is below the apparent K_m for lactate of the hepatocyte monocarboxylate transporter (1.9 mM) [3]. The preparation was set up in a 4.7T Sisco NMR spectrometer (Varion Associates, Palo Alto, CA, U.S.A.). The NMR sequences, repetition time and referencing for determination of pH_i from the chemical shift of the peak of the P_i resonance were as previously described [1]. With that repetition time, signal from extracellular P_i and P_i in the portion of the liver affected by digitonin is suppressed, and NMR measurements of pH refer to the intracellular compartment of the unaffected portion of the lobules.

After a period of stabilization of the preparation of 25 min, a spectrum of 1800 repetitions was recorded over 10 min. Digitonin (10–15 ml of a 4 g/l solution in Tris/HCl buffer, pH 7.40) was then perfused retrogradely to achieve approximately 20% destruction of lobular volume. Antegrade perfusion with the original medium was then resumed, and after 20 min a further 10 min spectrum recorded. The perfusate was then replaced by lactate-free perfusate and after a further 25 min spectra recorded again. Lactate-containing perfusate was then restored as before, and after a further 25 min a final spectrum obtained. In the final 10 min of each period, two pairs of portal and hepatic venous samples were taken for measurement of glucose, lactate and pH, P_{co_2} and P_{o_2}, and flow rate recorded. In two studies spectra were recorded at 4 min intervals after removal of lactate to establish the time course of cell pH and bicarbonate changes.

Glucose and lactate were measured by standard enzymic assays, and pH, P_{co_2} and P_{o_2} using an IL 1304 blood gas analyser (Instrumentation Laboratory, Warrington, Cheshire, U.K.). In each period the mean lactate uptake and glucose output was calculated using the Fick principle. The degree of destruction achieved by digitonin was determined as previously described [1] by automated histomorphometry on haematoxylin and eosin sections prepared from perfused-fixed (10% formalin) tissue obtained at the end of each experiment. Paired *t* tests were used to test the significance of mean differences between measurements obtained at the different stages of the protocol.

RESULTS

Figure 1 shows the results of 13 studies of pH_i, in ten of which lactate was reintroduced after the stage of lactate removal. The data is summarized in Table 1. Retrograde digitonin perfusion achieved a mean destruction of 21% by lobular volume, but there was no significant change in lactate uptake (2.86 ± 0.18 μmol/min per 100 g body weight). Every liver showed a marked rise in estimated pH_i (mean rise 0.19 ± 0.03 unit, $P < 0.01$) after digitonin perfusion to a mean value of 7.42 ± 0.03. Switching to lactate-free perfusate resulted in a significant mean decrease of pH_i of 0.17 ± 0.03 units ($P < 0.01$), to a level (7.25 ± 0.04) not significantly different from that in the liver prior to digitonin perfusion. Net lactate uptake after substitution with lactate-free perfusate (-0.05 μmol min per 100 g body weight) was not significantly different from zero. Re-introduction of lactate ($n = 10$) caused a significant intracellular re-alkalinization of 0.11 ± 0.04 unit ($P < 0.05$) to pH_i 7.34 ± 0.06 ($n = 10$), slightly, but not significantly, below the pH obtained before removal of lactate. Lactate uptake rose to 2.67 ± 0.30 μmol/min per 100 g body weight, not significantly different from that before the removal of lactate.

Mean glucose output during the four stages of the protocol are summarized in Table 1. During perfusion with lactate-free medium, glucose output was 0.26 ± 0.03 μmol/min per 100 g body weight, 18% of that during the previous period of lactate perfusion, and presumably derived from endogenous substrates.

In the two studies to investigate the kinetics of bicarbonate changes after lactate was removed, calculated cell bicarbonate levels (using $pK_a = 6.1$) fell in an exponential manner, with half-lives of 4.7 and 6.3 min respectively.

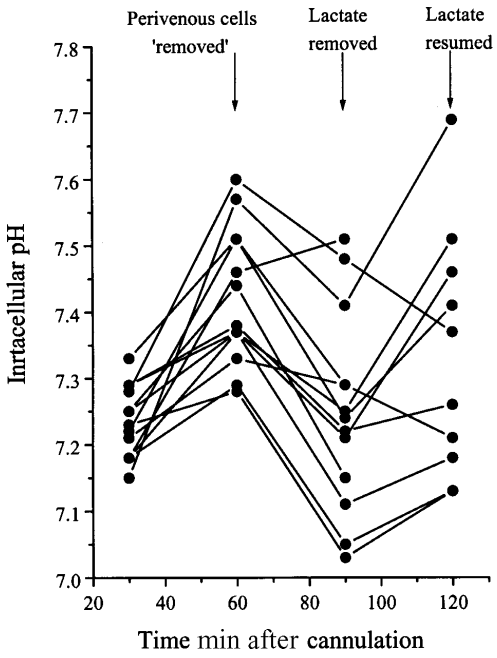

Figure 1 Changes in ^{31}P-NMR-determined pH_i in liver from starved rats, perfused with lactate (1.5 mmol/l) at successive stages of the study

Observations from a single liver are joined by lines. From left to right, the stages are; (1) intact liver after stabilization, (2) 25 min after digitonin-mediated destruction of the perivenous 21% of lobular volume, (3) 25 min after removal of lactate from perfusate and (4) 25 min after restoration of lactate.

Table 1 Changes in pH_i, lactate uptake and hepatic glucose output during stages of the protocol

The timings of the measurements are described in the Experimental section.

Description of stage	pH_i	Lactate uptake (μmol/min per 100 g body weight)	Hepatic glucose output (μmol/min per 100 g body weight)
Perfused (1.5 mM lactate)	7.23 ± 0.02	2.80 ± 0.24	1.67 ± 0.16
Perivenous cells destroyed	7.42 ± 0.03	2.86 ± 0.18	1.43 ± 0.09
Lactate removed	7.25 ± 0.04	-0.06 ± 0.02	0.26 ± 0.03
Lactate re-introduced	7.34 ± 0.06	2.67 ± 0.29	1.23 ± 0.13

DISCUSSION

We have previously shown [1], in livers of starved rats perfused with lactate as the sole substrate, that gluconeogenesis occurs in the outer (periportal) 75% of the lobular volume, assuming each lobule on average to be spherical. Approximately 25% of the glucose synthesized periportally is taken up by the perivenous 20–25% of the lobular volume [7]. At least part of this fraction

of glucose is converted into lactate in the perivenous cells [2]. Whereas in the steady state, gluconeogenesis from lactate ions consumes protons [2,9], glycolysis produces protons and thus, tends to acidify cells in which this is a significant pathway [2]. We interpret the mean rise of 0.19 units in pH_i after digitonin perfusion in the present study, as determined by ^{31}P-NMR to be due to the removal of the acidifying contribution to pH_i.

The remainder of the studies, comprising observations after digitonin perfusion followed by omission of lactate and then its re-introduction to the perfusate, were made in the periportal part of the lobule, to which gluconeogenesis is confined. It should be noted that, because of spherical geometry, the width of the periportal zone studied is only 40% of the lobular radius, although it contains 79% of the lobular volume. The results demonstrate that intracellular alkalinity in the periportal zone of the hepatic lobule under the present conditions is at least partially dependent on the presence of lactate. Since the pK of lactic acid is 3.8, it can have no appreciable buffering capacity. The concentration of lactate employed (1.5 mM) ensures that a major fraction of lactate entry is in the ionized form (see Introduction). The most likely explanation is that the alkalinization is caused by lactate ion conversion to neutral products [6,9], e.g. glucose or CO_2 and water. The present results provide clear support for this principle, as suggested by earlier work from this laboratory [9], in which pH_i was estimated by the laborious technique of determining the partition of DMO (dimethyloxazolidine 2,4-dione) between intra- and extracellular compartments. That study was on whole perfused liver whereas the present work sharpens that result by confining the pH_i measurement to the periportal zone where lactate uptake occurs, accompanied by a marked elevation of pH_i. However, an unknown effect of lactate on the cell pH regulatory mechanisms cannot be entirely excluded.

The proton consumption during lactate conversion to electroneutral products results in intracellular bicarbonate generation [6]. The amount of intracellular bicarbonate accumulation during the re-introduction of lactate can be calculated from the pH_i after 25 min of re-introduction and the fixed P_{CO_2}; it can thus be shown that in that period the intracellular bicarbonate concentration rises from 16.8 mM at the end of lactate omission to 20.5 mM. The amount of bicarbonate generation during that 25 min period can be calculated from the total lactate uptake, assuming, as is likely under the present conditions, that the great majority of lactate entry is as the ion [3–5]. Such calculations show that the amount of bicarbonate generation is about 3.3 times greater than is needed to provide the observed increment in cell bicarbonate concentration. This implies that about two-thirds of the generated bicarbonate is either titrated by protons dissociating from cellular buffers or produced metabolically, or is exported from the cell, through several bicarbonate transport systems known to be present in the hepatocyte plasma membrane; the probable major mechanism being electrogenic Na^+-coupled HCO_3^- transport [11,12]. The observations of time course of cell bicarbonate decline after removal of lactate from the perfusate provide measures of the overall activity of these various mechanisms *in situ*.

The bicarbonate concentration in the most extreme periportal cells, which appear to have a pH in the range 7.7–7.9 [1], must be remarkably high (40–70 mM). It must be emphasized that such high pH is likely to be a consequence of the present experimental conditions, where lactate is the sole substrate; however, it is also seen, albeit to a lesser extent, if palmitate is included in the perfusate in addition to lactate [8]. We have shown that livers from rats with severe diabetic ketoacidosis show a complete reversal of pH_i distribution to that described above when perfused with medium containing lactate (1.3 mM) and palmitate (0.8 mM), so that the perivenous cells are more alkaline than cells from the periportal zone. We believe this to be due to generation of ketoacids periportally and to acidotic suppression of gluconeogenesis from lactate in that zone specifically [8], the now more alkaline perivenous zone becoming gluconeogenic, and maintaining its alkalinity because of the consequent proton consumption, by a mechanism similar to that suggested by the present study.

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