Hepatic intralobular mapping of fructose metabolism in the rat liver

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Detailed mapping of glucose and lactate metabolism along the radius of the hepatic lobule was performed in situ in rat livers perfused with 1.5 mM lactate before and during the addition of 5 mM fructose. The majority of fructose uptake occurred in the periportal region; 45% of fructose taken up in the periportal half of the lobular volume being converted into glucose. Periportal lactate uptake was markedly decreased by addition of fructose. Basal perivenous lactate output, which was derived from glucose synthesized periportally, was increased in the presence of fructose. During fructose infusion there was a small decrease in cell pH periportally, but acidification of up to 0.5 pH units perivenously. The evidence suggests that in situ the apparent direct conversion of fructose into lactate represents, to a substantial extent, the result of periportal conversion of fructose into glucose and the subsequent uptake and glycolysis to lactate in the perivenous zone of some of that glucose. 31P NMR spectroscopy showed that the cellular concentration of phosphomonooesters changes very little periportally during fructose infusion, but there was an approximate twofold increase perivenously, presumably due to the accumulation of fructose 1-phosphate. It may be inferred that fructokinase activity is expressed throughout the hepatic lobule.

Key words: fructose 1-phosphate, hepatic glucose output, intracellular pH, lactate uptake.

INTRODUCTION

Addition of fructose to rat liver perfusions causes an immediate but temporary decrease in hepatic ATP and P_i, partly attributable to the accumulation of fructose 1-phosphate by the action of fructokinase (EC 2.7.1.4), degradation of adenine nucleotides to IMP and urate [1,2] and a simultaneous fall in hepatocyte intracellular pH (pH_i) [3]. Recovery of ATP is rapid but only partial, possibly because of irreversible degradation of adenine nucleotides. These phenomena have their counterparts in studies in man [4,5], and have had important implications for intravenous feeding regimes [2] and for the understanding of the metabolic disturbance in hereditary fructose intolerance [6]. The principal possible fates of fructose in the liver are: conversion into glucose or glycogen, fructolysis to pyruvate and lactate, and oxidation. This description of overall events does not take into account intralobular metabolic heterogeneity, and the sites within the lobule of fructose uptake and conversion into glucose and lactate are unclear or controversial [7,8]. The overall fall in pH_i measured by 31P NMR spectroscopy appears to be less than when determined using intracellular microelectrodes [3,9].

We have described a method [10] for detailed mapping of metabolic events and metabolite concentrations along the radius of the hepatic lobule in situ in the isolated perfused rat liver. The technique does not depend on the preparation of isolated hepatocytes from different zones of the lobule, thus avoiding changes of metabolite content and loss of polarity and vascular relationships during isolation. A further advantage is the facility with which reaction sequences which commence periportally and are completed perivenously can be investigated. In the present study, we have adapted this method to map fructose metabolism along the lobular radius to determine whether heterogeneity of metabolism could resolve the controversies and anomalies referred to above.

MATERIALS AND METHODS

Animals

Male Wistar rats (320 g) fed on standard rat chow were used after a 48 h fast. Isolated liver perfusions were set up at 37 °C as previously described [11] using erythrocyte- and albumin-free perfusate consisting of Krebs bicarbonate buffer [12] gassed with 95% O_2/5% CO_2 to give pH 7.4. Sodium t(+)-lactate (1.5 mM) was included as exogenous substrate for the liver throughout the protocol. The flow rate was 10 ml/min per 100 g of rat.

Mapping of intralobular fructose metabolism

In the original method [10], sharply defined lobular destruction, in amounts which varied between individual livers, was produced by retrograde perfusion of digitonin, followed by resumption of antegrade perfusion. The functional and microscopic effects of digitonin have been previously summarized [10,13–15]. Briefly, a sharply demarcated zone of destruction of hepatocytes, sinusoidal and Kupffer cells, centred on the central venules, is produced. The radius of these zones is dependent on the duration of retrograde digitonin perfusion. There are no effects on zones more periporal to the demarcation, since digitonin is avidly bound on a first-pass basis to membrane cholesterol. The metabolic activity or overall metabolite concentration of the remaining unaffected part of the lobule, was expressed as a fraction of the pre-digitonin value and plotted against the mean fractional lobular volume remaining (FVR) unaffected by digitonin, determined as described in [10]. Curves were fitted to the data and transformed to give plots of point-by-point metabolic

Abbreviations used: pH_i, intracellular pH; FVR, fractional volume remaining; HGO, hepatic glucose output; PME, phosphomonoesters.
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activity or metabolite concentration along the radius of the lobule. In the present study, livers were subjected to retrograde digitonin destruction of varying degree. Baseline observations of glucose and other metabolite output or uptake, hepatocyte cell pH, and ATP in the unaffected part of the lobule were then made and plotted against FVR. Infusion of fructose was then commenced and the observations repeated. Thus the modification consisted of comparisons made during fructose versus baseline on previously digitonin-treated livers, rather than before and after digitonin. In addition, substrate and metabolite uptake and output were expressed in absolute terms, rather than as ratios between different phases of the protocol; this modification was necessary for interpretation of the data but produces some scatter, since variation between livers is not completely compensated for.

The point of establishment of perfusion was taken as zero time. The perfusion was non-recirculating throughout. After 5 min of antegrade perfusion, flow was stopped and digitonin (4 mg/ml in Tris buffer, pH 7.4) perfused in the retrograde direction at approx. 30 ml/min for times that varied in the different livers between 10 s and 2 min. Antegrade perfusion was then re-established and the preparation placed in the bore of the NMR spectrometer. Baseline samples of influent and effluent perfusate were obtained at 20 min, 25 min and 30 min for measurement of pH, Pco₂, Po₂, glucose, lactate and fructose; a baseline spectrum was collected for estimation of pHᵢ, intracellular Pᵢ, ATP and phosphomonoesters (PMEs) over the period 29–33 min. d-Fructose was then infused to achieve a concentration in the portal influent of 5 mM over the period 33–45 min. Further spectra were collected over the period 33–45 min in 4 min blocks (i.e. three spectra), together with portal and hepatic venous samples at 38 min and 43 min. The liver was then fixed by perfusion of approx. 30 ml of 10% formalin. Tissue blocks were removed for determination of FVR by semi-automated quantitative histomorphometry, as previously described [9].

³¹P NMR studies

These were carried out in a Sisco 4.7T NMR spectrometer with a horizontal bore and a gradient insert of internal diameter 120 mm. A pulse repetition time of 0.33 s was used to saturate extracellular Pᵢ and thus eliminate Pᵢ signal from this source, as previously described [10]. Chemical shift of the Pᵢ resonance was referenced both to the α-ATP plus α-ADP peak, and then to an external standard of methylene diphosphonic acid placed under the liver, the chemical shift of which was used for subsequent calibration within each liver, before and during fructose infusion [10]. pHᵢ was calculated by reference to a standard curve. NMR spectra were interpreted by Lorentzian-line-shape analysis, using the FITPLA program [16]. Three singlet peaks were fitted to the partially overlapping PME, phosphodiester and Pᵢ resonances in the baseline spectrum. The analysis was then repeated in the spectrum recorded during fructose infusion, assuming the parameters width at half-height and chemical shift were unchanged for the phosphodiester peak from the values obtained in the baseline spectrum. The methylene diphosphonate and β-ATP resonances were analysed in the same way, the width at half-height and chemical shift being fixed to the pre-fructose values obtained by FITPLA.

Analytical procedures

Samples were stored at −20 °C until analysis. Glucose [15] and lactate [11] were determined as previously described. Fructose was measured enzymically [17] using a modification of a kit supplied by Boehringer Mannheim. Pco₂ and Po₂ were determined immediately in an Instrumentation Laboratory 1304 blood gas analyser.

Calculations

The balance of glucose, lactate and fructose across the liver was calculated using the Fick principle. Curves were fitted to the data plots of glucose output versus FVR before and during fructose infusion, using the Levenberg–Marquardt algorithm [18]. It should be noted that each point on these data plots represents the change of function or metabolite concentration induced by fructose in the whole of the remaining liver unaffected by digitonin. The curves fitted to the data plots were then transformed using the procedure described previously (see Appendix 1 in [10]) to give point-by-point plots of glucose uptake or output before and during fructose infusion against location, defined either by fractional lobular volume or fractional radial distance from the lobular centre, assuming the lobules to be on average spherical. This procedure does not include a scaling factor, which is immaterial when point function is described in relative terms (as in our previous use of this technique [10,15,21]); however, in the present context, in which absolute values of function are needed, it requires modification. Appendix 1 in [10] describes the analysis in terms of a single lobule. Scaling to liver weight per 100 g rat weight (eqn. 2 of that Appendix) becomes:

\[ y_i = -(1/4.2)(dF/dx) \]

where \( dF/dx \) is the slope of the curve fitted to plots of function (F) in µmol/min per 100 g rat against FVR (denoted as x), and \( y_i \), the point function in units of activity (µmol/min per g of tissue at a point where the fractional volume of the lobule periportal to it is x. The minus sign disappears when FVR is replaced by fractional volume from the centrilotubular vein (Figure 1, top panel). We have assumed a liver weight in starved rats of 4.2 g per 100 g rat weight and liver specific gravity of unity. The above equation has been used to derive point function per g of liver tissue for glucose before and during fructose (Figure 1, bottom panel). As in our previous publications using this technique [10,15,19], the transformation has been confined to data limited by the highest (FVR = 0.2) and lowest (FVR = 0.93) degrees of digitonin destruction achieved. Above the former limit, extrapolation of the curve is uncertain; below the latter limit, the very small volumes of tissue involved, and uncertainties caused by the variable size of the central venule, inevitably decrease accuracy. Confidence limits for the transformed curves were derived as in [10]. The variance/covariance matrices for this purpose were calculated using Genstat® [20].

It should be emphasized that the fitted curves do not represent any preconceived model of metabolic events, but are merely vehicles for the transformation procedure. The transformation was not attempted for substrates or metabolites other than glucose, since the scatter in other variables precluded unambiguous curve fitting. As previously explained [10], it is in any case not possible to perform the transformation on the pHᵢ data; notwithstanding this, it is possible to make some inferences about the distribution of pHᵢ and changes in pHᵢ from the data plots.

RESULTS AND DISCUSSION

Table 1 shows measurements in control livers (no digitonin) of the fractional change during fructose compared with baseline in Pᵢ, PMEs, and estimates of ATP from the resonances of the β-phosphorus resonance. It may be seen that a reasonable steady
Figure 1 (top panel) plots hepatic glucose output (HGO) against the fractional volume of the hepatic lobule remaining unaffected after digitonin, before and during fructose infusion. In interpreting diagrams of this format, it should be borne in mind that the data at an FVR of, for example, 0.75 is generated by the whole of the lobular volume perportal to the limit of the digitonin destruction, which has involved the 25% of the lobular volume more perivenous to that limit. Curves have been fitted to the data as described in the Materials and methods section. Since hepatic glucose output before and during fructose is measured on the same livers, a paired t-test can be done without making assumptions about the fitted curves. This establishes that separate curves are needed ($P < 0.001$).

In whole perfused liver, mean glucose production during fructose is 3.1 times greater than baseline (i.e. data at FVR = 1.0). It may be seen that HGO is greater during fructose at FVR = 0.75 than at FVR = 1.0, indicating that some of the glucose (approx. 1 μmol/min per 100 g of rat) generated more peripherally may be taken up by the more perrivenous regions; therefore when those regions are destroyed, there is an increase in net glucose output [15]. In contrast, larger degrees of destruction (leaving smaller FVRs) produce a rapid decline of HGO, indicating that the glucose synthesized was derived from the more perivenous 75% of the lobular volume.

To obtain a point-by-point map of glucose handling, the equations of best fit curves have been used to transform the data in Figure 1 (top panel) into a plot (Figure 1, bottom panel) of HGO in cells according to their location within the lobule, before and during fructose. Thus during fructose, point HGO in the extreme periregional region was approximately five times that before fructose. Point HGO declines in the periportal direction until it reaches zero both before and during fructose. The 95% confidence limits in Figure 1 (bottom panel) in the periregional region encompass zero glucose flux before fructose.

We have previously shown that perivenous glucose uptake may occur with lactate as the sole substrate [15]. We have postulated that the relatively high concentration of perivenous glucokinase activity remaining in starved livers might be responsible for perivenous glucose uptake [15]. If glucokinase was responsible for this phenomenon then the high $S_{p}$ with respect
to glucose (approx. 8 mM), might be expected to result in increased perivenous glucose uptake during fructose, when the sinusoidal glucose concentration is highest. In this study, sinusoidal glucose concentration reaching the last 30% of the lobular volume was approx. 0.2 mM before and 0.5 mM during fructose.

Data on fructose uptake using the same format (Figure 2) shows considerable scatter, but is most compatible with the major uptake of fructose being in the perportal half of the lobular volume, since there is no clear change in fructose uptake over a range of destructions over the FVR range of 0.5–1.0. By comparing the glucose and fructose data it may be calculated that the perportal region converts approx. 45% of fructose taken up into glucose. The data below suggest that in the perportal region the fate of that fraction of fructose which is taken up, but not converted into glucose, is glycogen formation and/or oxidation, rather than conversion into lactate.

Figure 3 shows lactate uptake plotted against FVR, before and during fructose infusion. Before fructose, in the intact liver (FVR = 1.0) mean lactate uptake was 2.4 μmol/min per 100 g of rat; small destructions (FVR 0.7–1.0) increase net lactate uptake, so that at FVR = 0.75, the best estimate of lactate uptake is approx. 3.0 μmol/min per 100 g of rat. This suggests that perivenous cells located in the centrilobular 20% of the lobule may produce lactate. Larger destructions (FVR range 0.2–0.8) produce a rapid decline in lactate uptake (Figure 3), consistent with the perportal site of lactate uptake and gluconeogenesis from lactate previously shown [10,15].

During fructose infusion in the continued presence of 1.5 mM lactate (Figure 3), mean lactate balance across the intact liver (FVR = 1.0) becomes slightly negative, indicating net lactate production in most cases (i.e. peripoortal lactate uptake is exceeded by perivenous lactate output). There is a clear separation of the data from that obtained before fructose in the FVR range 0.5–1.0, with lactate uptake much decreased in the FVR range 0.4–1.0 compared with that before fructose. A paired t-test establishes that lactate uptake during fructose is significantly different from that before fructose (P < 0.001). The positive slope in the FVR range 0.2–0.5 indicates that lactate uptake is still occurring, but since that slope is clearly less than in the same region before fructose, lactate uptake must be decreased by fructose. Linear-regression analysis of the data before and during fructose in the FVR range 0.8–1.0, indicates that a stronger trend toward a negative slope exists for the data during fructose (P = 0.07) than for the pre-fructose data (P = 0.23). This data suggests production of lactate by perivenous hepatocytes especially during fructose infusion, which is consistent with the net production of lactate during fructose infusion in the intact liver, despite continuing peripoortal lactate uptake.

Supporting this interpretation are the observations on hepatic cell pHi. Figure 4 (top panel) shows pHi derived from the 31P NMR P, peak before and during fructose. It may be seen that when FVR is low (i.e. a relatively thin peripoortal shell of viable cells remains), pHi is high (7.6–7.8). This is consistent with our previous observations [9], and we have attributed this phenomenon to the high rate of gluconeogenesis from lactate (a proton-consuming process [11,21]) in the peripoortal cells, with lactate as sole substrate. pHi in the peripoortal region must be lower than the values in the region of high FVR in Figure 4 (top panel), because the latter values are contributed to by the high pHi of the peripoortal region. It may be noted that the points obtained before fructose lie above those during fructose infusion. Figure 4 (bottom panel) shows the change in recorded pHi (∆pHi) induced by fructose infusion. At high FVRs there is marked lowering of pHi during fructose, whereas at low FVR the change in pHi is small, resulting in a significant linear regression of ∆pHi versus FVR (P < 0.01). There must therefore be considerable peripoortal acidification during fructose, accounting for the major part of the fall in pHi during fructose in the intact liver. The substantial fall in pHi peripoorly during fructose strongly suggests fructolysis and/or glycolysis to lactate in this region, as inferred from the lactate data above. The lesser fall in pHi periportally may be due to the inhibition of gluconeogenesis from lactate observed during fructose infusion and its replacement by gluconeogenesis from fructose, a proton-neutral process.
The above data suggests that generation of lactate plus protons is taking place perivenously, in contrast to the situation peripor tally. We conclude that under the present conditions the more periporal zones convert almost half of the fructose taken up into glucose, with the fate of the remainder of the periporal fructose uptake being either glycogen synthesis or oxidation, but not lactate. The perivenous zones generate lactate plus protons from fructose and/or peripor tally synthesized glucose. Since, as indicated above, the perivenous uptake of fructose is likely to be small, it seems possible that most of the perivenous lactate output is derived from glucose originating peripor tally from fructose. Comparison of Figure 1 (top panel) and Figure 3 indicates that the perivenous uptake of glucose during fructose is more than sufficient to account for the lactate output in this region. Although fructose uptake is probably less peripor tally than peripor tally, some at least must occur since fructose 1-phosphate accumulates predominantly in the perivenous zone (see below). Direct fructolysis within the same hepatocytes which originally take up fructose is also a potential source of perivenous lactate production. Though this doubtless occurs to some extent, its extent might be greatly overestimated by studies in mixed suspensions of isolated hepatocytes containing both periporal and perivenous cells, or in balance studies across whole liver perfusions, in which pathways commence peripor tally (e.g. fructose conversion into glucose) and continue peripor tally (glucose conversion into lactate), might appear only as direct fructose conversion into lactate.

Figure 5 (top panel) shows the fractional change in PME during fructose infusion. It may be calculated from the data of Woods et al. [1] that approx. 85% of the increase in PME in whole perfused rat liver during fructose is attributable to the accumulation of fructose 1-phosphate, so the great majority of the PME increase shown in Figure 5 (top panel) is likely to arise from fructose 1-phosphate. When FVR is less than 0.5, there is very little change in PME, despite the major conversion of fructose into glucose in the periporal region. However, at FVR 0.5–1.0, and in controls (no digitonin), there are substantial increases in PME. Since fructose uptake may be less peripor tally than peripor tally (Figure 2), these data suggest a relative impediment to the onward metabolism of fructose 1-phosphate in the perivenous region. Previous workers have identified smaller increments in PME and little change in pH, using 31P NMR following fructose administration in rat models of hepatic centrilobular necrosis [22]. These observations are consistent with our findings that accumulation of PME and decreased pH arise from glucose and fructose metabolism in the perivenous zone.

It can be concluded that fructokinase is expressed throughout the liver lobule, since periporal cells convert fructose into glucose and PME accumulates peripor tally during fructose infusion. ‘Fructolysis’ is, however, limited peripor tally, perhaps because of the intrinsically low expression of fructose 1-phosphate aldolase. Alternatively, the rate of provision of ATP for the fructokinase reaction may be limited peripor tally by the low capacity of that zone for aerobic oxidation [19], leaving glycolysis as the main mechanism of ATP provision. Though periporal falls in ATP during fructose might be expected to stimulate glycolysis by release of ATP inhibition of phosphofructokinase-1, that effect would be offset by the marked fall in pH, in this region (Figure 4) [23].

Fructose infusion at low portal concentrations (approx. 0.4 mM) has been shown to stimulate hepatic glycogen formation from glucose in dogs [24], possibly due to the accumulation of intracellular fructose 1-phosphate, which is known to release glucokinase from its regulatory protein at very low concentrations [25,26]. Fructose 1-phosphate may also contribute to glycogen accumulation by inhibition of glycogen phosphorylase [27]. Since in livers from 48-h starved rats glucokinase activity (and enzyme protein concentration [28]) are markedly concentrated in the most periporal cells [15], where the accumulation of fructose 1-phosphate is greatest (Figure 5), glucokinase activation would occur primarily in those cells and present...
glucose 6-phosphate for glycolysis to lactate, as well as for glycogen synthesis.

Finally, the present study sheds some light on the relatively small changes in pH during fructose infusion, previously detected by 31P NMR in whole liver [3]. The NMR-measured changes in the present study were rather greater than previously described; however, in whole livers, perivenous acidification would have been partially obscured by the lack of change in pH periportal. The larger falls in pH observed using pH-sensitive micro-electrodes [9] might be purely due to change impalements of perivenous cells, in which marked falls in perivenous pH during fructose infusion are suggested by the present data, the apparent discrepancy being thus a manifestation of heterogeneity within the hepatic lobule.

Figure 5 Fractional change in PME during fructose infusion, and the relationship to ∆ATP

Top panel: fractional change in PMEs (predominantly fructose 1-phosphate) concentration during fructose infusion (compared with pre-fructose). Plotted against FVR. Means ± S.E.M. of the fractional change seen in control studies (n = 6), i.e. with no digitonin destruction, is shown at FVR = 1.0 (□). Bottom panel: 1/ΔATP plotted against ∆PME. The regression line is 1/ΔATP = 0.60 + 0.54(ΔPME).

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