Effect of varying $PCO_2$ on intracellular pH and lactate consumption in the isolated perfused rat liver

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Summary

1. The effects of varying $PCO_2$ on lactate uptake and intracellular pH ($pH_i$) were studied in the isolated rat liver perfused with differing concentrations of lactate.

2. In general, $pH_i$ and lactate uptake are inversely related to $PCO_2$, and $pH_i$ and lactate uptake are directly related to each other, but the quantitative aspects and significance of these relationships vary with the availability of lactate. A model of hepatic lactate metabolism is proposed which may account for the quantitative variation.

3. The metabolism of lactate within the hepatocyte exerts a destabilizing effect on hepatocyte cell pH, in contrast to the buffering effect seen in predominantly glycolytic tissues.

4. An attempt is made to relate the findings to the disturbances of lactate metabolism in clinical respiratory failure.

Key words: hypercapnia, hypocapnia, intracellular pH, lactate, lactic acidosis.

Abbreviations: $pH_e$, perfusate pH; $pH_i$, intracellular pH.

Introduction

It has been previously shown that acidification of the perfusate entering the isolated rat liver (simulating metabolic acidosis) results in a fall of intracellular pH ($pH_i$) and an inhibition of lactate consumption (Lloyd, Iles, Simpson, Strunin, Layton & Cohen, 1973; Iles, Cohen, Rist & Baron, 1977). In the present paper, the effects of respiratory, rather than metabolic, changes in the acid–base status of the perfusate are reported; in addition, we have explored the effect of varying the lactate concentration in the perfusate. The bearing of the results on lactate homeostasis in clinical respiratory acidosis and on the mechanisms of control of cell pH are discussed.

Methods

Inbred male Glaxo Wistar rats, of weight 120–200 g, were starved for 48 h before the experiments. Isolated perfused liver preparations were set up as previously described (Cohen, Iles & Lloyd, 1973), the perfusate containing expired (4–5 weeks after donation) human erythrocytes at a packed cell volume of approximately 0.17 and fraction V bovine serum albumin (Sigma, 40 g/l) in Krebs bicarbonate buffer. The albumin was dialysed for 48 h before use against two changes of 2 litres of buffer and sufficient sodium bicarbonate was added to the medium before the experiment to achieve a nominal concentration of 24 mmol/l at $PCO_2$ 5.33 kPa; the addition of bicarbonate was made in a diluent designed to maintain the concentration of other constituents of the plasma unchanged: The $PCO_2$ was then adjusted to the desired value, and no further adjustment of the bicarbonate concentration made, in order to simulate uncompensated respiratory acidosis and alkalosis. The ‘standard bicarbonate’ was between 22 and 27 mmol/l in all experiments. Within any one experiment, during the period of non-recirculation (see below) perfusate pH varied by no more than 0.05, $PCO_2$ by no
more than 10% of the initial value, and perfusate bicarbonate by 2.5 mmol/l.

Sodium L(+)-lactate was added to the medium at the start of the perfusion to give nominal initial lactate concentrations of 1, 2 or 3 mmol/l. The perfusion rate was 7 ml/min per 100 g body weight (liver weight in starved rats is approximately 3.7% of body weight). The temperature of perfusion was 35–36°C. The duration of each perfusion was 45 min; in the first 25 min the perfusate recirculated, but in the final 20 min the perfusate was not returned to the reservoir after passage through the liver. During this final period, therefore, the perfusate entering the liver was of constant composition. The reservoir was gassed with O₂/CO₂ mixtures to give a constant Pco₂ and Po₂ throughout the experiment; perfusate Pco₂, measured on the portal venous side of the liver, varied in different experiments between 1.33 kPa (10 mmHg) and 19.3 kPa (145 mmHg). Perfusate pH (pHᵢ) consequently varied from 6.92 to 8.01. At the beginning of the experiment, 5,5'-[2-¹⁴C]dimethyloxaloidine-2,4'-dione (DMO), hydroxy[¹⁴C]methylinulin and ³H₂O were added to the perfusate to measure intracellular pH (pHᵢ). The amounts added, together with the quantities of carrier DMO and inulin, have been previously described (Cohen, Iles, Barnett, Howell & Strunin, 1971).

At the end of the perfusion, duplicate samples of portal and hepatic venous perfusate were obtained and aliquots added to tubes containing 10% perchloric acid for lactate estimation or fluoride/oxalate tubes for glucose estimation and further aliquots were processed for isotope counting (Cohen et al., 1971; Iles et al., 1977). The liver was then rapidly excised and clamped between tongs cooled in liquid nitrogen, ground under liquid nitrogen and the frozen powder divided into portions before deproteinization in perchloric acid (0.6 mol/l) and subsequent preparation for isotope counting or estimation of intermediary metabolites of gluconeogenesis. The results of measurements of glucose output and the hepatocyte content of intermediary metabolites are described in the following paper (Iles, Baron & Cohen, 1978).

The methods for pHᵢ, Pco₂ and Po₂, perfusate lactate, and the calculation of pHᵢ and lactate uptake and glucose output (by using the Fick principle) were as previously described (Cohen et al., 1971).

Regression and correlation analysis were performed by standard parametric methods. Slopes and, when appropriate, intercepts of regression lines, were compared by using the method described in Documenta Geigy (1970), or, when indicated in the text, analysis of covariances (Snedecor & Cochran, 1967). Comparison of correlation coefficients was performed with Fisher's z transformation (Snedecor & Cochran, 1967). Two-tailed tests of significance were used throughout.

**Results**

For the purpose of analysis, the experiments have been divided into three groups according to the concentration of lactate in the portal venous perfusate at the end of the experiment. The groups were defined as follows: group 1 (n = 22), lactate concentration 0.75-1.5 mmol/l (mean 1.17 mmol/l); group 2 (n = 32), 1.5-2.5 mmol/l (mean 1.93 mmol/l); group 3 (n = 27), 2.5-4.0 mmol/l (mean 3.49 mmol/l). To examine whether unintentional bias had occurred, the regressions of portal venous perfusate lactate concentration against Pco₂ were calculated in the three groups; the regression lines had slopes which were not significantly different from zero in groups 1 and 2, but there was a significant (P < 0.05) negative regression in group 3. However, since in group 3 there was a positive regression between intracellular lactate concentration and Pco₂ (Iles et al., 1978), the relationships described below in group 3 could not have been due to a fortuitous association between Pco₂ and the availability of lactate to metabolic pathways within the cell.

**Effect of changes of Pco₂ on pHᵢ and lactate consumption**

Fig. 1 shows the highly significant (P < 0.001) linear relationship between log Pco₂ and pHᵢ in

![Fig. 1. Relationship between pHᵢ and log Pco₂ (kPa) in group 3. Each point represents the result from a single perfusion. The correlation coefficient was 0.84 (P < 0.001). See Table 1(a) for regression data.](image-url)
PCO₂, cell pH and lactate in liver

### Table 1. Linear regression equations and correlation coefficients

A and B refer to the coefficients in equations of the type $y = Bx + A$. The standard errors of $A$ and $B$ are shown in parentheses. The variables taken as independent are indicated by asterisks. $r$, Pearson's correlation coefficient; $P$, probability of $B$ or $r$ being zero. In all sections $n$ was 22 for group 1, 32 for group 2 and 27 for group 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>$B$ (slope)</th>
<th>$A$ (intercept)</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) $\text{pH}$ vs log $\text{PCO}_2$ (kPa)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-0.455 (±0.096)</td>
<td>7.56 (±0.082)</td>
<td>-0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>-0.450 (±0.083)</td>
<td>7.57 (±0.07)</td>
<td>-0.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>-0.515 (±0.066)</td>
<td>7.71 (±0.056)</td>
<td>-0.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(b) Lactate uptake (μmol/min per 100 g rat) vs log $\text{PCO}_2$ (kPa)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-1.20 (±0.701)</td>
<td>4.86 (±1.19)</td>
<td>-0.36</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>2</td>
<td>-1.91 (±0.994)</td>
<td>6.78 (±1.70)</td>
<td>-0.33</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>3</td>
<td>-3.39 (±0.394)</td>
<td>8.29 (±1.56)</td>
<td>-0.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(c) Lactate uptake (μmol/min per 100 g rat) vs pH*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.40 (±0.82)</td>
<td>6.37 (±6.04)</td>
<td>0.36</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>2</td>
<td>2.13 (±1.28)</td>
<td>10.42 (±9.37)</td>
<td>0.29</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>3</td>
<td>3.62 (±1.12)</td>
<td>21.03 (±8.27)</td>
<td>0.54</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>(d) $\text{pH}$ vs lactate uptake (μmol/min per 100 g rat)*</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.033 (±0.041)</td>
<td>7.07 (±0.16)</td>
<td>0.18</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>2</td>
<td>0.032 (±0.019)</td>
<td>7.04 (±0.11)</td>
<td>0.30</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>3</td>
<td>0.080 (±0.014)</td>
<td>6.86 (±0.09)</td>
<td>0.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(e) $\text{pH}$ vs $\text{pH}_e$*</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.52 (±0.12)</td>
<td>3.42 (±0.86)</td>
<td>0.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.52 (±0.11)</td>
<td>3.35 (±0.82)</td>
<td>0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.59 (±0.07)</td>
<td>2.96 (±0.58)</td>
<td>0.83</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Group 3; similar relationships were also found in groups 1 and 2 ($P < 0.001$, Table 1(a)). There was no significant difference in the slopes of the regression lines between any pair of groups. However, the intercept (or elevation) of the regression line differed significantly between groups 2 and 3 ($0.01 < P < 0.02$) and between groups 1 and 3 ($P < 0.01$). It may be calculated from the regression equations that, at $\text{PCO}_2$ 2-66 kPa (20 mmHg), $\text{pH}$ in group 1 was 7.38, compared with 7.49 in group 3; the corresponding intracellular bicarbonate concentrations were 11.3 and 14.9 mmol/l. At $\text{PCO}_2$ 10.64 kPa (80 mmHg), the values were: group 1, $\text{pH}_e$ 7.10, intracellular bicarbonate 24.1 mmol/l; group 3, $\text{pH}_e$ 7.18, bicarbonate 29.1 mmol/l.

Fig. 2 shows the relationship between $\text{PCO}_2$ and lactate uptake in group 3. Also shown in this Figure are the calculated regression lines in the three groups, details of which are given in Table 1(b). It may be seen that both the negative slope and the intercept on the lactate uptake axis (at $\text{PCO}_2 = 1$ kPa) increase progressively through the groups. Analysis of covariance shows that the variance of slopes between groups is only of borderline significance ($0.05 < P < 0.01$). However, it should be noted that such an analysis

![Fig. 2. Relationship between lactate uptake and log $\text{PCO}_2$ (kPa) in group 3. Each point represents the result from a single perfusion. The lines are the regressions of lactate uptake in $\text{PCO}_2$ in group 1 (-----), group 2 (---) and group 3 (-----). For statistical analysis see Table 1(b) and text.](image-url)
does not take into account the rank order of the slopes. Though there was a wide variation of lactate uptake from 2.5 to 10.2 μmol/min per 100 g rat throughout the groups, lactate uptake was never completely inhibited by high $P_{CO_2}$.

**Relationship between lactate uptake and $pH_e$ and $pH_i$**

In Table 1(c) the relationship between lactate uptake and $pH_e$ is shown. Whereas the slope of the regression line is barely significant in group 1 and not significant in group 2, it is quite highly significant in group 3 ($P < 0.005$). Fig. 3 shows the relationship between $pH_i$ and lactate uptake in group 3, and the regression lines for group 3 and group 2 are also included. The relationship between $pH_i$ and lactate uptake shows an increase of slope ($P = 0.05$) between groups 2 and 3 and a pronounced increase in the association between the two variables with increasing group number (as indicated by the correlation coefficients) (Table 1d). Application of Fisher's $z$ transformation shows that the correlation coefficient in group 3 was significantly different from that in either group 2 or group 1 ($P < 0.02$).

**Relationship between $pH_e$ and $pH_i$**

This is shown in Fig. 4 for group 2. The regression equations are given in Table 1(e). Superimposed on Fig. 4 is the regression line of $pH_i$ on $pH_e$ found by Lloyd et al. (1973) in simulated metabolic acidosis in the perfused rat liver. Group 2 is chosen for comparison since the lactate concentrations in the perfusate were similar to those used by Lloyd et al. The regression lines in the present group 2 and in the experiments of Lloyd et al. do not differ significantly in slope ($0.1 < P < 0.25$) or intercept.

**Discussion**

These studies have demonstrated a complex series of interrelationships between $pH_e$, $pH_i$, $P_{CO_2}$ and lactate uptake in livers perfused under conditions simulating pure 'respiratory' disturbances of acid-base status. There is the expected dependence of $pH_i$ on $log P_{CO_2}$, but the first point to note is that this regression line is higher in the experiments with the highest perfusate lactate concentration, than in the other two groups. This means that at any $P_{CO_2}$ value within the range studied, $pH_i$ and calculated intracellular bicarbonate are higher when more lactate is available.

We have also shown an inverse relationship between $log P_{CO_2}$ and lactate uptake, and positive relationships between both $pH_e$ and $pH_i$ and lactate uptake. These relationships appear linear throughout the whole $pH$ range studied, with the exception of group 1. They contrast somewhat with previous findings in metabolic acidosis in the perfused liver. Hems, Ross, Berry & Krebs (1966), using 10 mmol/l lactate, found that gluconeogenesis from lactate was not depressed until $pH_i$ fell below 7.1; Lloyd et al. (1973), using 2.5 mmol/l lactate and measuring lactate uptake, which is not necessarily proportional to the rate of gluconeogenesis, found that lactate uptake was de-
pressed only when pH fell below 7.05. The reason for the difference in behaviour between respiratory and metabolic acidosis is uncertain; it could simply be related to the fact that the greater number of experiments performed by Lloyd et al. (1973) in the acidotic range permitted more precise definition of the relationship. It should be noted that in those experiments Pco₂ was held constant and normal, so that cell bicarbonate fell in acidosis, whereas in the acidosis experiments in the present work, Pco₂ and cell bicarbonate were both high. Though the common factor in both studies was a fall in pH, specific effects of CO₂ and HCO₃⁻ on lactate metabolism can by no means be excluded.

An important feature of the present studies is that the relationships between lactate uptake on the one hand, and pH, pH, and log Pco₂ on the other, change with increasing availability of lactate in the perfusate. For example, a primary change in lactate uptake would be associated with a much greater change in pH, in group 3 (highest lactate concentration) than in group 2. Similar trends are seen in the slopes of the regression lines of lactate uptake on pH, and log Pco₂. It should be noted that the higher pH/log Pco₂ regression line in the group with the highest lactate concentration is equivalent to a greater slope of the H⁺/Pco₂ relationship at any given Pco₂. The pHₜ/lactate uptake relationship, in particular, demonstrates a marked increase in the positive association of the two variables [as assessed by increasing correlation coefficients; see Table 1(d), penultimate column] with progressive increase in the availability of lactate.

Fig. 5(a), which presents a model of hepatic lactate metabolism, provides a possible explanation of these findings. Considering the circular system at the top of the diagram, evidence for its left-hand limb is provided by Lloyd et al. (1973) and by the present work. The right-hand limb indicates that a fall in the rate of metabolism of the lactate within the hepatocyte acidifies the cell (and vice versa), as demonstrated by Cohen et al. (1971). This cycle, which has been discussed in detail by Cohen & Simpson (1975) and Cohen & Iles (1975), constitutes a positive feedback system by which a change in pH, caused by, for instance, an alteration in Pco₂, is amplified. One might expect this system to play a considerable role in determining pH when lactate is readily available (e.g. in group 3), but when lactate is less available (group 1) pHₜ would be dominated by the direct effect of Pco₂. This mechanism could account for the quantitative alteration with lactate availability of the relationships summarized in the previous paragraph. As depicted in Fig. 5(a), the model refers to the situation when pHₜ is decreased; however, Fig. 5(a) is also intended to apply (with those arrows indicating the direction of changes reversed) to circumstances where pHₜ is raised, e.g. by lowering Pco₂.

Siesjö & Messeter (1971) have summarized the mechanisms by which cells maintain their internal pH: direct physicochemical buffering, consumption or production of H⁺ by the metabolic activity of the cell, and transmembrane fluxes of H⁺. An important contribution to homeostasis of cell pH was felt to be related to the consumption or production of H⁺ associated with changes in the intracellular concentrations of lactate, pyruvate, glutamate and other intermediate metabolites of carbohydrate metabolism induced by alteration in pHₜ. Folbergrova, MacMillan & Siesjö (1975) suggested that this mechanism (Fig. 5b) might be due to the effect of pHₜ on the activity of phosphofructokinase, this being the rate-limiting enzyme of glycolysis. Changes in organic acid production and consumption in Siesjö’s system constitute a defence against pHₜ change, in other words a biological buffering system. This concept could apply to organs which normally produce lactate and other organic acids, e.g. skeletal muscle, brain. However, in the liver, which has a major role in extracting lactate from circulation and converting it into neutral products (glucose, CO₂ and water), the scheme that we propose in Fig. 5(a) applies. In contrast to the mechanism shown in Fig. 5(b) this constitutes a negative or anti-buffering system. A possible advantage to the organism of such a system is that it would accelerate the consumption of lactate, with regeneration of the bicarbonate titrated by lactic acid production, in exercise. We have discussed elsewhere the operation of this positive feedback
system in the direction of falling pH and lactate consumption, its possible relevance to the pathogenesis of lactic acidosis and the normal safeguards against its operation in this direction (Cohen & Iles, 1975; Cohen & Woods, 1976).

If lactate uptake is so markedly inhibited by hypercapnia in the perfused liver, why does lactic acidosis not arise frequently in clinical acute respiratory failure? In fact, Penman (1962) showed that moderate elevations of blood lactate (1–5 mmol/l) were not uncommon in this condition. However, the administration of oxygen to Penman’s patients lowered blood lactate concentrations even though $P_{a,CO_2}$ rose; this suggests that hypoxia was the prime factor in blood lactate elevation, either causing increased peripheral production of lactate, or inhibiting removal by the liver and kidney. Table 2 shows the values for hepatic lactate uptake at $PCO_2$ values of 5·3 and 10·6 kPa, at two different blood lactate concentrations, calculated from our regression equations. The Table shows that the inhibitory effect of hypercapnia is more than compensated for by a rise in blood lactate concentration, so, if the liver in vivo behaves similarly to the perfused organ, the blood lactate concentration would tend to stabilize. Two other stabilizing factors might also be significant in clinical hypercapnia. The first is inhibition of peripheral glycolysis by the intracellular acidosis associated with hypercapnia; secondly, the kidney may react to respiratory acidosis as it does to metabolic acidosis, by increasing its uptake of lactate (Yudkin & Cohen, 1975). Conversely, in hypocapnia, lactate uptake is stimulated in the perfused liver; Fig. 5(a) shows that the operation of the positive feedback in this simple form would be limited only by the supply of lactate. In our non-recirculating perfusions the portal venous lactate concentration remains constant. In vivo, however, a fall in blood lactate concentration would tend to occur as lactate uptake increased and the rate of lactate consumption would therefore eventually fall. Hypocapnia is accompanied by increased concentrations of blood lactate in vivo (Zborowska-Sluis & Dossetor, 1967; Eldridge & Salzer, 1967), possibly as a result of increased peripheral glycolysis. The rather small rise in blood lactate (<1 mmol/l) that is seen in voluntarily hyperventilating healthy men (Eldridge & Salzer, 1967) could conceal a substantial rise in lactate turnover.

Poole-Wilson & Cameron (1975) have reviewed the evidence that the cell pH of cardiac and skeletal muscle is better defended against a metabolic than a respiratory alkalosis. In the present work, we have compared the relationships between hepatic pH, and $P_{H^+}$ in respiratory acid–base disturbance with that previously obtained under similar conditions by Lloyd et al. (1973) in metabolic acidosis. The slope of the pH/$P_{H^+}$ line was not significantly different in metabolic acidosis. The reason for this apparently different behaviour of liver is not clear; it is possible that a more detailed study in the respiratory acidotic range might reach a different conclusion.

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**References**


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