THE EFFECT OF SIMULATED METABOLIC ACIDOSIS ON INTRACELLULAR pH AND LACTATE METABOLISM IN THE ISOLATED PERFUSED RAT LIVER


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SUMMARY

1. The relationship between extracellular pH (pH_e) and intracellular pH (pH_i) and lactate uptake was studied in the isolated perfused rat liver during simulated metabolic acidosis.

2. pH_i fell to a considerably less extent than pH_e when the latter was decreased from pH 7.4 to 6.7.

3. The liver took up lactate when pH_i was greater than 7.0; at lower values of pH_i, lactate output occurred.

4. The relevance of these observations to the control of hepatic pH_i and lactate metabolism is discussed.

Key words: lactate, acidosis, intracellular pH, hepatic metabolism, pyruvate carboxylase, lactic acidosis.

It has been shown in previous work that the intracellular pH (pH_i) of the isolated perfused rat liver may be altered by inducing changes in lactate uptake, an increase in the latter being accompanied by a rise in pH_i (Cohen, Iles, Barnett, Howell & Strunin, 1971). In those experiments, the pH (pH_e) and Pco_2 of the perfusate were kept constant and within normal limits, and the changes in lactate uptake were achieved by altering the lactate concentration of the perfusate. In the present experiments an attempt was made to determine whether, at constant lactate concentration, changes in pH_e and pH_i affected lactate uptake. pH_e was varied in a manner simulating uncompensated metabolic acidosis by employing low bicarbonate concentrations at constant and normal Pco_2.

METHODS

Inbred Wistar rats (weight 100–150 g), previously starved for 20–24 h were used. The technique of isolated liver perfusion was as described previously (Cohen et al., 1971; Cohen, Iles &
Lloyd, 1973). The perfusing medium contained equine erythrocytes; these were used 5 days after the blood had been taken into standard acid–citrate–dextrose solution. The packed cell volume ranged from 0·15 to 0·20. The medium contained bovine serum albumin (Armour, fraction V), 4 g/100 ml. L(+)-Lactic acid (Sigma) was added to the perfusate to produce an initial concentration of approximately 2·5 mmol/l. At the beginning of the experiment, after equilibration in the circuit at a $P_{CO_2}$ of approximately 40 mmHg, the medium was usually acid (pH 6·6–7·0). The pH was then adjusted to the required level by the addition of Krebs bicarbonate buffer (Krebs & Henseleit, 1932) in which chloride had been replaced by bicarbonate. In each perfusion, portal venous pH remained steady (within 0·05 pH units) throughout its predetermined level. Mean portal venous $P_{CO_2}$ at the end of the perfusion was 38·3 mmHg (SD 4·70, $n=42$) and did not vary in any single experiment by more than 9 mmHg. The perfusion rate was approximately 7 ml min⁻¹ 100 g rat⁻¹ and was held constant. A large volume of medium was used (approx. 500 ml) so that changes in medium composition during the perfusion were as small as possible. Each batch of medium was used for two or three successive perfusions at the same external pH.

At the start of the perfusion, [¹⁴C]5,5-dimethylloxazolidine-2,4-dione ([¹⁴C]DMO), ³H₂O and hydroxy[¹⁴C]methyl inulin were added to the medium for measurement of intracellular pH; the quantities, specific radioactivities and amounts of carrier used were as previously described (Cohen et al., 1971).

The perfusions were run for 50 min; for the first 20 min the medium was recirculated. The system was then changed to 'non-recirculation' for the last 30 min, to ensure further that conditions were constant during the sampling period. Two pairs of specimens of portal and hepatic venous blood were taken for measurement of lactate, pH and $P_{CO_2}$ at 40 and 50 min; pH, $P_{CO_2}$ were also measured at 15 and 30 min. The pH of hepatic venous blood was taken as pHₑ, for this was considered to be closer to the pH of interstitial fluid than that of the arterial inflow. Flow rate was determined at each sampling time. Just before the taking of the last pair of samples, hepatic venous blood was taken for measurement of isotope concentrations. At the end of the perfusion the right lobe of the liver was removed, lightly blotted and placed in 5 ml of distilled water in a stoppered tube.

The methods for measurement of the pH, $P_{CO_2}$ and lactate concentrations in the medium have been previously described (Cohen et al., 1971) as has the subsequent treatment of the specimens for the calculation of intracellular pH and lactate uptake. The lactate uptakes calculated from the pairs of samples taken at 50 min are reported; however, the relationships between pHₑ and lactate uptake were not appreciably different if the mean of lactate uptakes at 40 and 50 min was employed.

The significance of differences between means and of the difference of a mean from zero were assessed using a two-tailed t-test (Snedecor & Cochran, 1967). When the distribution or variance ratio in the groups under comparison was not appropriate for the t-test, the difference between the groups was assessed by the Mann-Whitney U-test (Siegel, 1956).

RESULTS

Lactate concentration of perfusate

The mean lactate concentrations in the perfusate at the time of pHₑ measurement were 2·13±SEM 0·219, 2·02±0·140, 2·19±0·290, 2·36±0·124 and 1·98±0·241 mmol/l for the
experiments in which pH$_i$ lay within the ranges 7.4–7.2, 7.2–7.1, 7.1–7.0, 7.0–6.9 and 6.9–6.8 respectively. None of these mean values differed significantly from each other.

**Relationship between pH$_e$ and pH$_i$**

Fig. 1 shows that as pH$_e$ decreased from 7.4 to 6.85, mean pH$_i$ fell relatively little (from 7.2 to 7.07). Further lowering of pH$_e$ from 6.85 to 6.7 resulted in almost as great a fall of mean pH$_i$; this decrease was significant ($P<0.05$) whereas the changes between other adjacent groups were not significant. The linear regression of the individual values of pH$_i$ on pH$_e$ was pH$_i$ = 0.366 (±SEM 0.058) pH$_e$ + 4.506. The regression coefficient is significantly different from zero ($P<0.001$)

![Graph of pH$_e$ vs pH$_i$](image)

**Fig. 1.** The relationship between pH$_e$ and pH$_i$. The pH values represent the mid-points of the pH ranges 7.4–7.3, 7.3–7.1, 7.1–6.9, 6.9–6.8 and 6.8–6.6. pH$_i$ is represented as mean ± SEM. The numbers in each group are given in parentheses.

**Relationship between pH$_e$ and lactate uptake (Fig. 2)**

Mean lactate uptake was between 0.9 and 2 μmol min$^{-1}$ 100 g rat$^{-1}$ when pH$_e$ lay between 7.4 and 7.1. Lower values of pH$_e$ were associated with progressively lower lactate uptakes and when pH$_e$ fell below 7.1–7.0, mean lactate uptake was negative, although the mean value of the lactate outputs for the two groups of perfusions with the two lowest pH$_e$ levels (Fig. 2), were not significantly less than zero ($0.1 < P < 0.2$) when combined.
FIG. 2. The relationship between pH_e and lactate uptake. The pH_e values represent the mid-points of the pH ranges defined in Fig. 1. Lactate output is represented as mean ± SEM. The numbers in each group are given in parentheses.

FIG. 3. The relationship between pH_i and lactate uptake. The pH_i values represent the mid points of the pH ranges 7·4–7·2, 7·2–7·1, 7·1–7·0, 7·0–6·9 and 6·9–6·8. The numbers in each group are given in parentheses.
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**pH\(_i\) and lactate uptake (Fig. 3)**

Mean lactate uptake did not vary appreciably over the pH\(_i\) range 7.4–7.0. At lower levels of pH\(_i\) an abrupt change over from uptake to output of lactate occurred. The mean lactate uptake, \(-1.86 (SEM 0.864) \mu\text{mol min}^{-1} 100 \text{ g rat}^{-1}\) for experiments with pH\(_i\)<7.0 was significantly less than zero (\(P<0.05\)) and also significantly less (\(P<0.02, <0.05\) and <0.01 respectively) than the individual mean values in those groups with pH\(_i\)>7.0 (1.73, 0.62 and 1.46 \mu\text{mol min}^{-1} 100 \text{ g rat}^{-1}\) respectively).

Tables giving numerically the results expressed in Figs. 1–3 have been deposited with the Librarian, the Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE, from whom copies may be obtained (Clinical Science and Molecular Medicine Table 73/19).

**DISCUSSION**

The relationship between extracellular and intracellular pH has been studied in a number of tissues. Adler, Roy & Relman (1965) showed that in the isolated rat diaphragm the pH of the external medium could be decreased from 7.39 to 6.92 by varying the concentration of bicarbonate without significant fall in pH\(_i\). Above and below these values, pH\(_i\) rose and fell respectively. This resistance of cell pH to external metabolic acidosis has also been demonstrated in dog brain (Kibler, O'Neill & Robin, 1964), human platelets (Zieve & Solomon, 1966) and in man in vivo by using measurements of mean whole body pH\(_i\) (Lambie, Anderton, Cowie, Simpson, Tothill & Robson, 1965). The effect of uncompensated metabolic acidosis on hepatic pH\(_i\) has not previously been studied. The present results show that as in other tissues, the pH of liver cells is protected against external acidosis except when this reaches a degree which would be very severe in clinical practice. Nevertheless, a range of pH\(_e\) over which pH\(_i\) remains constant is not seen and it is of interest that Adler (1972) in repeating the observations of Adler et al. (1965) found that the ‘plateau’ was much less definite than in the earlier experiments.

All the studies quoted above have used DMO for the measurement of pH\(_i\). However, Adler (1972) found rather similar changes in pH\(_i\) in metabolic acidosis with \[^{14}\text{C} \}\text{nicotine as the pH indicator in the rat diaphragm. Qualitatively similar results were also obtained by using pH-sensitive intracellular microelectrodes by Kostyuk & Sorokina (1961) in frog sartorius and by Paillard (1972) in rat and crab muscle.}

The mechanisms of defence of intracellular pH against change have recently been studied by Siesjö and his colleagues in rat brain, exposed in vivo to high concentrations of CO\(_2\) (Siesjö, Folbergrova & MacMillan, 1972; Folbergrova, MacMillan & Siesjö, 1972). Three mechanisms of intracellular pH control appear to occur: physicochemical buffering, consumption of intracellular organic acids in excess of their production (thereby generating bicarbonate), and transmembrane fluxes of H\(^+\) or HCO\(_3\)\(^-\). In the liver an extra dimension is added to the contribution made by changes in organic acids, since one of the roles of the liver is to take up lactate ions from the circulation and convert it into glucose (mainly) or CO\(_2\) and water. It has been shown that this process removes hydrogen ions and raises intracellular pH (Cohen et al., 1971), increases and decreases in lactate uptake resulting in similar directional changes in pH\(_i\). The present study shows little effect on lactate uptake as pH\(_i\) is decreased to 7.1; thereafter a rapid fall in lactate uptake occurs and pH\(_i\) values below 7.0, lactate output occurs. This is not due to differences in lactate concentration in the perfusate at the time of measure-
ment of pH. The fall in pH values resulting from a decrease in lactate uptake, and the decrease in lactate uptake caused by that fall in pH might create a vicious circle whereby a cessation of lactate uptake eventually occurs. This effect would only occur at pH values below 7.0; at higher values the situation is stable.

We also have shown that the relationship between pH and lactate uptake is somewhat similar to that between pH and lactate uptake, though less striking. The cessation of uptake as pH falls to 7.0 is similar to the observations of Hems, Ross, Berry & Krebs (1966), who measured glucose output rather than lactate uptake and employed a higher lactate concentration (10 mmol/l) in rats subjected to more prolonged starvation (48 h); they did not make observations at pH values below 7.0, nor was pH measured.

The capacity of the isolated perfused liver to produce lactate by glycolysis has been studied by Woods & Krebs (1971). Gevers & Dowdle (1963) showed that rat liver slices produced more lactate from glucose when the medium was alkaline and less under acid conditions; their observations made it probable that the rate-limiting step at which pH control was exercised was that catalysed by phosphofructokinase. The lactate production at low pH in the present experiments could be explained if the effect of intracellular acidosis in suppressing glycolysis from residual glycogen was not yet complete at values of pH when the rate-limiting enzyme concerned with the pH effect on lactate uptake is completely inactive; alternatively lactate could have been derived from other sources such as alanine. We have previously suggested that the enzyme of gluconeogenesis whose activity might be suppressed in acidosis may well be pyruvate carboxylase, which demonstrates in vitro a marked pH dependence for its obligatory activation by acetyl-coenzyme A (Scrutton & Utter, 1967; Kleineke & Söling, 1971); direct evidence for this suggestion has yet to be obtained.

The effect of metabolic acidosis in converting hepatic lactate uptake to output could be relevant to clinical lactic acidosis, whether due to poor tissue perfusion or to other mechanisms. It is possible that the success of therapeutic measures in either type of lactic acidosis may be dependent on raising hepatic intracellular pH and thereby breaking the vicious circle referred to above.

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REFERENCES


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