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The influence of renal function on lactate and glucose metabolism

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1. The relationship of lactate metabolism to renal function was studied in the isolated perfused rat kidney. A new radioisotopic method has been developed that enables the simultaneous measurement of lactate production and consumption in the presence of physiological concentrations of both lactate and glucose. 2. In kidneys from fed rats, when glucose was absent, lactate production was only 12 μmol/h per g dry wt, and in kidneys from starved rats there was no lactate production, indicating that neither the phosphoenolpyruvate/pyruvate substrate cycle nor other analogous cycles for the recycling of lactate carbon are operating in the intact kidney cortex. 3. Lactate production from glucose occurred at a high rate, at the same time as lactate consumption, demonstrating that lactate recycling between renal cortex and medulla can occur in the intact kidney. 4. Lactate production from glucose correlated with glomerular filtration rate ($P<0.001$), urine flow rate ($P<0.01$) and sodium reabsorption ($P<0.05$). There was significant basal lactate production at zero glomerular filtration rate. Lactate consumption was not correlated with any renal function. 5. When Na^+ reabsorption was inhibited with the diuretic frusemide, or when filtration was entirely prevented (the 'non'-filtering kidney'), lactate production was decreased by 39% and 50% respectively. Basal lactate production determined in this way was the same as that calculated above by linear regression. Prevention of filtration, but not the addition of frusemide, significantly inhibited lactate consumption. 6. It is concluded that glycolysis is required for medullary Na^+ transport, and that some different transport function(s) require lactate oxidation.

A prime problem in renal biochemistry is the correlation of metabolic processes with known transport events. Kidney has an exceptionally high rate of O_2 consumption, most of which is dedicated to supporting Na^+ transport (Deetjen & Kramer, 1960). However, the best fuels of respiration in intact kidney are not necessarily most effective in supporting Na^+ reabsorption (Nishiitsutsuji-Uwo *et al.*, 1967). A special role for glucose in renal Na^+ transport has long been recognized, and it has been suggested that the production of a dilute urine requires non-oxidative glucose metabolism in the cortical distal tubule (Baines & Ross, 1982). However, glucose is a relatively poor fuel of respiration in intact kidney (Ross *et al.*, 1973) and in renal cortex (Weidemann & Krebs, 1969).

The kidney is a heterogeneous tissue capable of carrying out both glycolysis and gluconeogenesis. A major precursor for renal gluconeogenesis is lactate, which is also the final product of medullary glycolysis, but the anatomical distribution of blood vessels across the kidney is such that the products of the medulla do not reach the cortex (Kriz, 1967). Similarly, glucose produced in the cortex cannot directly provide substrate for medullary metabolism, although it could possibly be used elsewhere in the renal cortex (Baines & Ross, 1982).

The use of the isolated perfused kidney preparation (Ross, 1978) allows delivery of the products of metabolism of one area to the other by the recirculation of perfusion medium. Metabolic rates determined in this preparation are therefore the result of simultaneous and synchronous gluconeogenesis and glycolysis. The rates of both pathways will be seriously underestimated if only measurements of the concentrations of substrates and products are made. We have developed new methods for the study of glucose metabolism in the functioning kidney involving the use of

Abbreviation used: GFR, glomerular filtration rate.

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radioisotope dilution. These methods exploit the recirculation of substrates and products in the perfusion system so that changes in the specific radioactivities of lactate reflect the rates of glycolysis and lactate consumption.

In this paper we report studies to determine the rates of lactate production and consumption in relation to variations in renal transport function.

Methods

Isolated perfused rat kidney

The kidney was perfused by the method of Ross *et al.* (1973). The Ca^{2+} content of the perfusion medium is half that of Krebs–Henseleit (1932) medium, to allow for the increase in protein-bound Ca^{2+} that otherwise occurs during dialysis. Lactate (2mM) and/or glucose (5mM) was added as indicated to achieve near-physiological concentrations of both substrates for the duration of the perfusion experiment. In radioisotopic experiments, 30μCi of [U-^{14}C]lactate was added to the perfusion medium together with 5mM-glucose and 2mM-lactate. Radiolabels were added after 5–10min of equilibration of the kidney with the perfusion medium.

During the course of the perfusion (normally 90min) samples were taken at 10–15min intervals for calculation of metabolic rates.

Separation of glucose and lactate and determination of specific radioactivities

Two methods were used to separate radioactive lactate, glucose and other metabolites, before determination of the specific radioactivity of both metabolites. The first has been described previously in detail (Janssens *et al.*, 1980). In a second method, which proved to be more rapid and equally accurate, glucose and lactate were separated in perfusate samples by a modification of the method of Hammerstadt (1980) as described by Challiss (1983). Dowex columns of 2ml bed volume were arranged in tandem manner, with the formate column on top of the borate column.

Measurement of lactate and glucose

Lactate was measured by the method of Gutmann & Wahlefeld (1974), but with additional EDTA and Tris/hydrazine buffer, pH9.5. Glucose was measured by the method of Bergmeyer *et al.* (1974). GFR was estimated by addition of 5mM-creatinine to the perfusion medium.

Determination of the rate of lactate production

To determine the rate of lactate production, a novel method has been applied, originally presented by Janssens *et al.* (1980) for the estimation of lactate recycling in kidney tubules. The method involves incubation or perfusion with 2mM-lactate plus [U-^{14}C]lactate in the presence or absence of 5mM-glucose. Isolated kidneys were perfused as described above with 100ml of modified Krebs–Henseleit buffer, pH7.4, containing 6.7 (w/v) of bovine serum albumin, 5mM-glucose, 2mM-lactate and [U-^{14}C]lactate to give an initial specific radioactivity of approx. 100000d.p.m./μmol.

The specific radioactivity of lactate will fall in proportion to the amount of unlabelled lactate that is being produced from glucose from endogenous sources or by lactate 'cycling' during the course of the incubation or perfusion. Since both labelled and unlabelled lactate will be continuously removed at the same time as they are being formed (the result of glucose synthesis and lactate oxidation to CO_2), a mathematical treatment is required to calculate the true rate of lactate production. Janssens *et al.* (1980) showed that, in the absence of glucose, lactate continued to be formed, probably owing to lactate recycling. Control perfusions without added glucose were carried out for this reason in the intact kidney.

The rate of lactate production (i.e. the rate of entry of unlabelled lactate into the labelled lactate pool) may be calculated from eqns. (1) and (2) below, knowing the initial (Y_0) and final (Y_t) amounts of radioactive lactate present and the final concentration of unlabelled lactate (Z_t):

$$\alpha = \ln\left(\frac{Y_0}{Y_t}\right) \quad (1)$$

$$Z_t = \frac{\beta}{\alpha}\left(1-\frac{Y_t}{Y_0}\right) \quad (2)$$

where β is the rate of formation of unlabelled lactate, α is the rate constant for the removal of lactate from the pool, Y_0 is lactate (μmol) at initial specific radioactivity, Y_t is lactate (μmol) at the same specific radioactivity at time t, and Z_t is the concentration of unlabelled lactate present at the end of the incubation. Thus:

$$Z_t = X_t - Y_t \quad (3)$$

where X_t = measured lactate at time t (X_0 = measured lactate at time 0). For the derivation of eqns. (1) and (2), please see Janssens *et al.* (1980). $(X_0 - Y_t)$ represents the true rate of lactate consumption (i.e. net removal minus the rate of synchronous lactate production).

Incorporation of ^{14}C into glucose: glucose synthesis from lactate

Perfusion of kidney or incubation of tubules with [U-^{14}C]lactate, and 2mM-lactate in the pres-

ence or absence of glucose, also allows for calculation of the rate of incorporation of ^{14}C into glucose, and hence an estimate of the relative rate of glucose synthesis in the presence of a physiological glucose concentration. Inevitably, since, as outlined above, the specific radioactivity of lactate (the precursor) falls progressively as a consequence of glycolysis, this apparent rate falls too. To correct for the falling specific radioactivity, eqn. (4) has been applied:

$$\text{Glucose formed at time } t = \frac{\text{Total radioactivity (d.p.m.) in glucose at time } t}{\text{Sp. radioactivity of lactate at time } t} \times \frac{1}{2} \times \frac{1}{\text{dry wt. of kidney}} \quad (4)$$

This results in a linear time course accurately reflecting the net rate of lactate incorporation into glucose.

In practice, eqn. (4) has been applied to each timed sample, and the rate established from a graph of glucose formed at time t versus time.

Glucose formation has intentionally not been designated rate of glucoeneogenesis, because of uncertainty concerning the recycling of glucose or isotope exchange known to occur in kidney cortex (Vinay *et al.*, 1978).

Expression of results

Results are expressed in terms of kidney dry weight, to take into account the extra interstitial water present in kidney after perfusion. Means ± S.E.M., as well as linear regressions, have been compared by Student's t test (abbreviation: N.S., not significant).

Results

Effect of glucose on renal lactate production

When kidneys from well-fed rats were perfused with 2mM-[U-^{14}C]lactate alone, lactate was removed at a mean rate of 179.9 μmol/h/per g dry wt. The specific radioactivity of lactate altered very little, so that the rate of lactate production was less than 12 μmol/h per g dry wt. (Table 1). This is in marked contrast with earlier findings in isolated cortex tubules (Janssens *et al.*, 1980) and suggests that cycling of lactate carbon in the intact renal cortex is insignificant.

Addition of 5mM-glucose promoted renal tubular Na^+ transport and had a marked effect on both lactate consumption and production. Lactate consumption (300 ± 43 μmol/h per g dry wt.) was approximately twice that observed when lactate was the sole substrate.

The fall in specific radioactivity of lactate is most readily accounted for by the formation of lactate from unlabelled glucose. The rate of lactate production can be calculated to be 176 μmol/h per dry wt. The overall rate of lactate consumption observed in the presence of glucose was close to the sum of lactate consumption measured when lactate was the sole substrate and lactate production from glucose. The small discrepancy [(180 + 176) − 300 = 56 μmol/h per g dry wt.] is in-

Table 1. *Effects of diet, frusemide and filtration on the rates of lactate production and consumption and glucose formation*
Kidneys from male Wistar rats (280–350g) were isolated and perfused as described in the Methods section. Frusemide was included at a concentration of 0.1 μM. Results are the means ± S.E.M. for the numbers of perfusions shown in parentheses. Statistical significance is indicated by $*P<0.05$, $**P<0.01$, $***P<0.001$, compared with fed controls (top line).

		Rates (μmol/h per g dry wt.)		
	Substrates	Lactate production	Lactate consumption	Glucose formation
Fed	5mM-Glucose, 2mM-lactate	176.2 ± 23.9 (7)	300.6 ± 43.1 (7)	36.2 ± 2.6 (7)
Fed	2mM-Lactate	11.7 ± 3.0 (5)***	179.9 ± 18.7 (5)**	18.8 ± 4.4 (5)**
Starved	5mM-Glucose, 2mM-lactate	172.5 ± 39.3 (3)	298.8 ± 40.8 (4)	
Starved	2mM-Lactate	5	235.9 ± 74.8 (3)	
Fed + frusemide	5mM-Glucose, 2mM-lactate	97.9 ± 14.7 (4)*	286.3 ± 17.5 (4)	57.5 ± 9.3 (4)***
Starved + frusemide	5mM-Glucose, 2mM-lactate	108.7 ± 18.4 (4)*	210.8 ± 18.7 (4)	
Starved, non-filtering	5mM-Glucose, 2mM-lactate	87.5 ± 10.9 (4)*	171.2 ± 17.0 (4)*	

dicative of the preferential oxidation of glucose by some renal cells when both glucose and lactate are presented to the kidney at the same time.

Formation of glucose

[^{14}C]Glucose 'production rate' was calculated to be 36.2 μmol/h per g dry wt. This value is significantly different from zero, so that it is likely that some gluconeogenesis occurred in the presence of added glucose. The rate is surprisingly low, however, when compared with that in cortical tubules (Janssens *et al.*, 1980). In the absence of glucose the rate of incorporation of lactate into glucose was only about half of that in the presence of glucose; however, the rate of glucose production determined by spectrophotometric measurement of glucose produced from lactate was almost three times the value obtained with the isotopic determination (18.8 ± 4.4 versus 46.6 ± 6.5 μmol/h per g dry wt; $P < 0.01$). These results suggest that it is unlikely that ^{14}C incorporation into glucose in these experiments represents the true rate of gluconeogenesis. Thus although frusemide increased the rate of lactate incorporation into glucose (from 36.2 to 57.5 μmol/h per g dry wt.; $P < 0.01$), thus suggesting that it stimulates glucose production, it is more likely that the extra glucose accumulated on account of the inhibition of glycolysis described below.

Relationship between renal function and metabolism

There was considerable variation in renal tubular function in the individual animals used in these experiments, which is reflected in variations in both lactate consumption and production, as indicated by a relatively large S.E.M. for the values in Table 1.

A significant correlation between lactate production and urine flow rate ($r = 0.907$; $P < 0.001$) was observed (Fig. 1*b*), but there was no correlation between lactate consumption and urine flow rate ($r = 0.135$; N.S.).

Renal work is more closely correlated with the tubular filtered load or GFR than with urine flow rate itself. Lactate production was significantly correlated with GFR ($r = 0.770$; $P < 0.01$), but again there was no correlation between lactate consumption and GFR ($r = 0.452$; N.S.). Na^+-reabsorption data were available in a limited proportion of the experiments. However, when these data were pooled there was a significant and inverse relationship ($r = 0.880$; $P < 0.01$; $n = 5$) between lactate production and fractional Na^+ excretion (Fig. 2*a*), and a direct relationship ($r = 0.815$; $P < 0.02$; $n = 5$) between lactate production and total Na^+ reabsorption (T_{Na}) (Fig. 2*b*). No such correlations existed for lactate consumption.

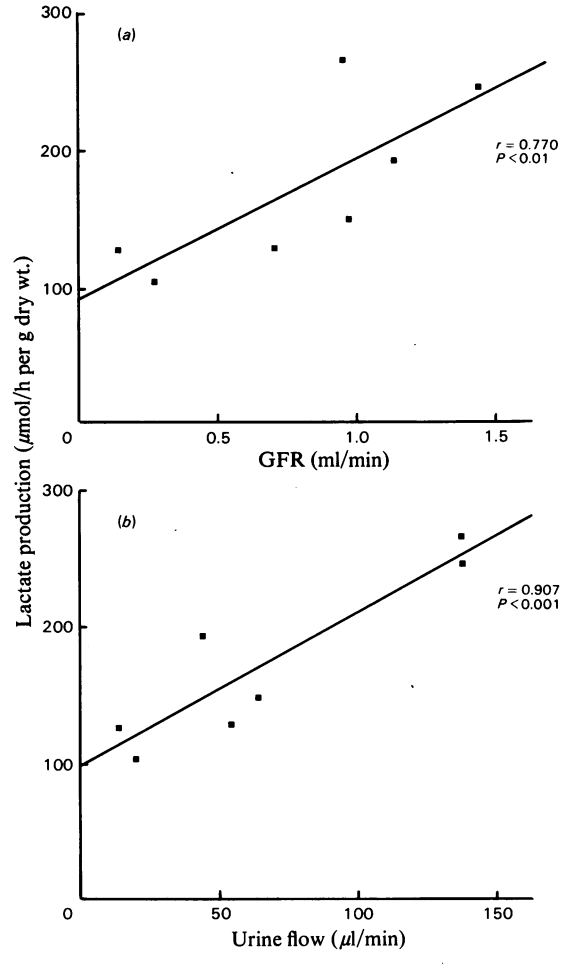

Fig. 1. *Correlation between lactate production and GFR (a) and urine flow (b)*
Kidneys of well-fed rats were perfused with lactate (2mM) and glucose (5mM) for 1h. Mean GFR (ml/min) was determined from at least two urine-collection periods, by creatinine clearance. Lactate production from glucose was measured from the change in specific radioactivity of radioactive lactate. Each point represents a single perfusion. The intercept of the regression lines on the ordinate is equivalent to the basal rate of lactate production, in the absence of renal tubular transport, when urine flow or GFR was zero.

Effects of inhibition of filtration and Na^+ reabsorption on renal lactate metabolism

In the non-filtering kidney, lactate production in the presence of 5mM-glucose and 2mM-lactate was only half that in the filtering kidney. Furthermore, the rate of lactate production of the non-filtering

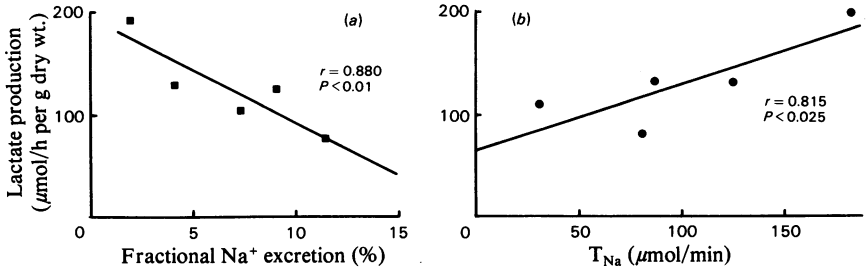

Fig. 2. *Correlation between lactate production and fractional and total Na^+ reabsorption*
For experimental details, see Fig. 1. Each point represents a single perfusion, but both fed and starved rats have been included. T_{Na} (total Na^+ transport) is defined as GFR × plasma $[Na^+]$ × % reabsorption of filtered load. As in Fig. 1, the intercept on the ordinate of the regression of Fig. 2(*b*) is equivalent to the rate of lactate production at zero Na^+ transport. It was not significantly different from the rate of lactate production observed in the non-filtering kidney.

kidney (87.5 μmol/h per g dry wt.) was remarkably close to that obtained by extrapolation in Fig. 1(*b*) to zero GFR (intercept = 90 μmol/h per g dry wt.) (Table 1).

Frusemide (0.1 mM) effectively inhibited Na^+ reabsorption in the isolated kidney; fractional reabsorption fell from 96% to approx. 82%, and urine flow was more than double (60 to 160 μl/min). Lactate production was inhibited markedly by frusemide, from 174 to 98 μmol/h per g dry wt. ($P < 0.05$) in kidney from fed rats and 109 μmol/h per g in those of starved rats (this latter value achieved statistical significance when pooled with the results from fed rats; $P < 0.05$). The rate of lactate production observed in the presence of frusemide was somewhat greater than that observed in the kidney when no urine was formed at all, 'non-filtering kidney', so that some lactate may be produced in renal cells which are insensitive to frusemide.

Non-filtering kidneys in which the flow rate of perfusion medium was also decreased had a significantly lower rate of lactate consumption than normal fed controls (171.2 versus 298.8 μmol/h per g dry wt.). There were no significant differences in lactate consumption between controls and frusemide-treated kidneys from fed rats or 24 h-starved rats (Table 1).

Results are consistent with a site of lactate consumption in the functioning kidney which is not influenced by frusemide.

Discussion

Lactate recycling in the intact kidney

We have reported previously that the rate of lactate production in the absence of glucose in cortical tubules from fed rats was 120 μmol/h per dry wt., and was negligible in tubules from starved rats (Janssens *et al.*, 1980). At the time we suggested that this could be indicative of futile cycling of pyruvate. A cycle between phosphoenolpyruvate and pyruvate was excluded, since inhibition of phosphoenolpyruvate carboxykinase did not alter the rate of lactate production. It was concluded instead that the extra lactate may have been formed from oxaloacetate by an alternative cycle involving oxaloacetate decarboxylase (Rognstad, 1979). A final suggestion, not completely excluded by the studies of Janssens *et al.* (1980), was that lactate arose from endogenous sources such as glycerol or amino acids, present in isolated renal tubules.

This study has now been extended to the intact perfused rat kidney. The data in Table 1 show that in kidneys from fed rats the rate of lactate production in the absence of glucose was only 10% of that in isolated renal tubules and about 6% of the rate of lactate consumption. Furthermore, endogenous lactate production in kidneys from starved rats was undetectably low. There is therefore a discrepancy between the findings in isolated tubules and the intact perfused kidney. The explanation for this is not clear and requires further investigation. However, it seems that a futile cycle involving pyruvate in the intact kidney cortex of either fed or starved rats is unlikely.

In the intact kidney a lactate cycle between cortex and medulla is observed very clearly, as is demonstrated by a synchronous high rate of lactate consumption and production.

It is important to note that throughout this work it is assumed that only one lactate pool exists in the perfused kidney. We believe that by the recirculation of the perfusion medium compartmentalization of cortical and medullary lactate pools is avoided, since at each turn of the perfusion cycle lactate from the cortex will be delivered to the medulla, and vice versa. Thus glucose and lactate carbon will flow through one single pool of lactate.

Renal function and lactate metabolism

The relationship between renal metabolic processes and most tubular transport phenomena remains unclear. Here we are able to demonstrate a direct and significant correlation between lactate production from glucose and the rate of urine flow and GFR in the functioning kidney. On the other hand, there is no correlation between lactate consumption and these two indices of renal function. These results suggest that glycolysis rather than lactate oxidation (Cohen *et al.*, 1980) is required for some aspect of renal tubular function, probably for Na^+ reabsorption.

Striking confirmation of a significant role for lactate production from glucose in these transport phenomena was provided by two further experiments. In the presence of frusemide, which is known to inhibit tubular transport totally in the thick ascending limb of Henle's loop, lactate production fell to the value predicted from the regression shown in Fig. 2(*b*). Similarly, when filtration was abolished, lactate production was inhibited to an extent identical with that obtained when GFR is zero in the regression shown in Fig. 1(*a*) (intercept = 90 μmol/h per dry wt.).

The functioning kidney produces lactate from glucose at the same time as consuming lactate, and it also produces glucose at the same time as consuming it. The present findings indicate that lactate production is directly related to renal function. Thus glucose utilization would be a requirement for maintenance of renal function.

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