LACTATE AND PYRUVATE METABOLISM IN ISOLATED HUMAN KIDNEY TUBULES

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1. Introduction

To our knowledge, uptake of lactate and pyruvate by the human kidney in vivo in normal subjects has not as yet been studied. These substrates are not taken up from the blood by the kidneys of patients afflicted with diabetes mellitus [1]. However, they are removed by the human kidney in vivo in certain other pathological states. This was first demonstrated in patients with various renal diseases [2] and, later observed [3] in obese patients following prolonged starvation. Since most of our knowledge concerning the metabolism of lactate and pyruvate has been derived from arterio–venous measurements, the metabolic fate of these substrates in the human kidney remains uncertain. Complete oxidation of lactate was calculated [2] to account for 35% of renal oxygen consumption. However, it was concluded [3] that all the lactate and pyruvate carbon skeletons removed by the kidney were converted to glucose.

In an attempt to clarify this subject, we studied the metabolism of lactate and pyruvate in isolated human kidney tubules. The data indicate that lactate and pyruvate may serve as the carbon sources of glucose and alanine in human kidneys, and that, at physiological concentration (1 mM), complete oxidation of lactate is very limited.

2. Methods and materials

2.1. *Isolation of human kidney tubules*

Fresh normal kidney cortex was obtained from the uninvolved pole of kidneys removed for neoplasm from 18 h fasted patients. Specimens of cortex were immediately dissected and placed in ice-cold Krebs–Henseleit buffer [4] gassed with a mixture of O_2–CO_2 (95–5%) until the beginning of the isolation technique period (usually within 10 min). Kidney cortex tubules were isolated by the procedure in [5] as modified [6], with the exception that the concentration of collagenase was increased 3-fold.

2.2. *Incubations*

Incubations were carried out at 37°C in a shaking water bath in 25 ml stoppered Erlenmeyer flasks in an atmosphere of 95% O_2–5% CO_2. In all experiments, tubules were incubated in Krebs–Henseleit buffer in paired flasks containing lactate or pyruvate at equimolar concentrations (1 mM or 5 mM). The incubation volume was 4 ml, when substrate was 1 mM. In order to increase the proportion of substrate removed by the tubules, the incubation volume was decreased to 2 ml when substrate was 5 mM. Reduction of the incubation volume from 4–2 ml did not affect the results obtained with 5 mM lactate or 5 mM pyruvate, except that pyruvate accumulation from 5 mM lactate was reduced in the same proportion. Incubations were terminated by adding perchloric acid (2% final conc.). In all experiments, zero-time flasks were prepared with and without substrates by adding perchloric acid before the tubules. After removing the denaturated protein by centrifugation, the supernatant was neutralized with 20% KOH for metabolite determinations.

2.3. *Analytical methods*

Lactate, pyruvate, glucose, alanine, aspartate, citrate, 2-oxoglutarate, fumarate, malate, glutamate, as well as the dry weight of the amount of tubules added to the flasks, were determined as in [6]. Ammonia was measured by the method in [7].

2.4. *Calculations*

Net substrate utilization and product formation were calculated as the difference between the total flask contents at the start of the experiment and after the period of incubation. The metabolic rates are expressed in micromoles of substance removed or produced per gram dry weight of tubules per unit time. The results were analysed by the Student's *t* test for paired data, comparing values obtained in the presence of lactate with those in the presence of an equimolecular concentration of pyruvate. The metabolic fates of lactate and pyruvate were established by calculating carbon balances.

2.5. *Chemicals*

L-lactate and pyruvate (sodium salts), enzymes, and coenzymes were from Boehringer Mannheim GmbH. Other chemicals were of analytical grade.

3. Results and discussion

3.1. *Time courses of the metabolism of lactate and pyruvate at low (1 mM) and high (5 mM) concentrations*

The rates of lactate and pyruvate utilization were virtually constant during 60 min incubation under all conditions (fig.1). In these in vitro experiments, as is the case under in vivo conditions [2], the utilization of lactate and pyruvate was found to increase, when the substrate concentration was raised. At equimolecular concentrations, the utilization of pyruvate was observed to be ~2-times that of lactate. This was probably due to greater stimulation of pyruvate dehydrogenase and (or) pyruvate carboxylase by higher pyruvate concentrations when pyruvate was employed as the substrate.

The rates of glucose synthesis from lactate and pyruvate were also constant with time (fig.1); this was also true without any substrate added. This linearity, as well as that of substrate utilization, clearly indicates that our human kidney tubules were metabolically active. On a wet weight basis (using a wet wt/dry wt ratio of 5.28 ± 0.10, $n = 16$), it can be calculated that our tubules synthesized 7–8-times more glucose from 5 mM pyruvate than reported for human kidney tubules prepared [8]. Our tubules synthesized 4–5-times more glucose from 5 mM lactate than has been cited [8] from 10 mM lactate.

At both substrate concentrations, the formation of glucose from lactate was significantly higher than that from pyruvate (fig.1); in this respect, human kidney resembles the dog kidney [6], but differs from the rat kidney in which pyruvate is a better gluconeogenic precursor than lactate [5,9–11]. Such observations imply differences between species in the mechanism of glucose synthesis. Human kidney cortex [12], as well as dog kidney cortex [12,13], but not rat kidney cortex [12], contain both a cytosolic and a mitochondrial phosphoenol pyruvate carboxykinase. As hypothesized for the dog kidney [6], we believe that, in the human kidney, intramitochondrially formed phosphoenol pyruvate could contribute to the supply of carbon skeletons for gluconeogenesis, when lactate, but not pyruvate, is the substrate. Such an additional source of carbon skeletons for glucose synthesis from lactate could then explain why, in human and dog kidney, lactate is more gluconeogenic than pyruvate.

Figure 1 also shows that, despite an increased removal of substrates, glucose synthesis was not greater with 5 mM than with 1 mM lactate and pyruvate; this indicates that the gluconeogenic pathway is already saturated with 1 mM substrate.

When lactate was the substrate, the accumulation of pyruvate was not found to be linear with time (fig.1). Pyruvate re-utilization was observed after 45 min with 1 mM lactate, and after 15 min with 5 mM lactate. Such kinetics clearly indicate that, in order for the lactate dehydrogenase reaction to reach near-equilibrium, the pyruvate concentration had to be adjusted to that of lactate according to the equation:

$$[PYR] = K\,[LAC]\,[NAD^+]/[NADH]\ [14]$$

Lower pyruvate accumulation from 5 mM than from 1 mM lactate was found to be due to the reduction of the medium volume from 4–2 ml with 5 mM lactate as substrate.

When pyruvate was the substrate, the rate of lactate synthesis, was found to be constant with time (fig.1). This implies that the rate of transport of reducing equivalents in the form of malate [15,16] from the mitochondria to the cytosol was constant with time. No increase in lactate synthesis was observed when pyruvate was used at 5 mM instead of 1 mM.

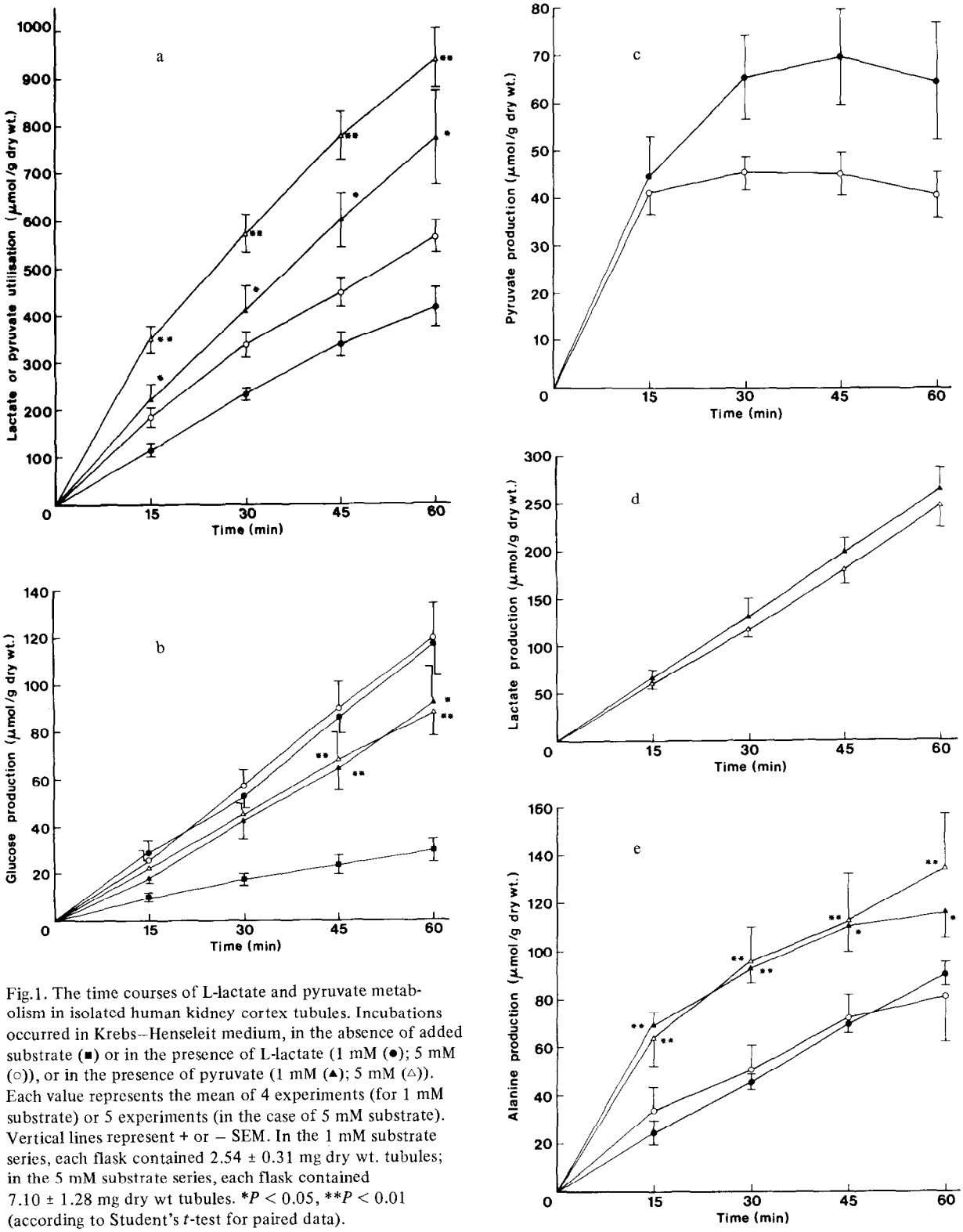

Fig.1. The time courses of L-lactate and pyruvate metabolism in isolated human kidney cortex tubules. Incubations occurred in Krebs–Henseleit medium, in the absence of added substrate (■) or in the presence of L-lactate (1 mM (●); 5 mM (○)), or in the presence of pyruvate (1 mM (▲); 5 mM (△)). Each value represents the mean of 4 experiments (for 1 mM substrate) or 5 experiments (in the case of 5 mM substrate). Vertical lines represent + or − SEM. In the 1 mM substrate series, each flask contained 2.54 ± 0.31 mg dry wt. tubules; in the 5 mM substrate series, each flask contained 7.10 ± 1.28 mg dry wt tubules. $*P < 0.05$, $**P < 0.01$ (according to Student's t-test for paired data).

This indicates that, despite an increased availability of carbon skeletons for lactate synthesis, the supply of reducing equivalents from 5 mM pyruvate was not higher than that from 1 mM pyruvate. Thus, there are at least one, or perhaps several rate-limiting steps in the sequence of reactions involved in the supply of reducing equivalents to the cytosol; these may include pyruvate carboxylase, mitochondrial malate dehydrogenase, transport of malate across the mitochondrial membrane, or cytosolic malate dehydrogenase.

The formation of alanine from lactate and pyruvate (fig.1) is consistent with the demonstration of glutamate–pyruvate transaminase activity in the human kidney [17]. As expected, the rate of alanine synthesis was higher from pyruvate than from lactate. Alanine synthesis raises the question of the origin of the alanine amino group. The production of ammonia observed in the absence of added substrate was reduced in the presence of lactate and, to a greater extent, in the presence of pyruvate. This indicates that amino acid nitrogens were incorporated into the alanine molecule (presumably by transamination of glutamate), instead of being released via the glutamate dehydrogenase reaction. In the presence of pyruvate and lactate, and after 60 min, the utilization of glutamate, which was present at the start of the incubation period, could account for ~33% and ~20%, respectively, of the alanine nitrogen found. Aspartate was neither found to be used nor produced in net amounts. Thus, other amino acids, which remain to be identified, must have contributed to the alanine amino group.

3.2. *Fate of lactate and pyruvate in human kidney tubules*

Table 1 shows that, after 60 min, glucose was the main product of lactate metabolism at both 1 mM and 5 mM. This suggests that lactate may significantly contribute to the renal production of glucose, which occurs in patients with chronic obstructive airway diseases [18] and in obese subjects undergoing prolonged starvation [3]. Glucose synthesis from lactate might appear to be of no importance in patients with renal diseases, because the kidneys of these patients do not release glucose [2]. However, this does not necessarily mean that glucose is not an end-product of lactate metabolism in these patients; it is conceivable that glucose synthesis from lactate, occurring in the cortex, is balanced by glucose utilization in the medulla, which is believed to have a predominantly glycolytic metabolism [19]. This view is supported by the recent demonstration [20] that, in vivo, the superficial cortex of cat kidney produces glucose in net amounts, whereas the deep cortex and the medulla take it up.

Table 1 also shows that 20% of the lactate (1 mM) removed was accounted for by alanine. Thus, lactate appears to be a potential carbon source of the alanine which is added to renal venous blood by the human kidney [21].

It should be pointed out that the lactate 'not accounted for' represented a significant fraction of lactate metabolism (which was presumably completely oxidized in the tricarboxylic acid cycle) only at 5 mM (table 1). Although one should be cautious in extra-

Table 1
The fates of lactate and pyruvate carbon skeletons in isolated human kidney tubules

Substrate added	Products formed				
	Glucose (%)	Pyruvate (%)	Lactate (%)	Alanine (%)	Not accounted for (%)
1 mM L-lactate	56.6	15.4	–	21.7	6.3
1 mM Pyruvate	23.7	–	34.3	15.0	27.0
5 mM L-lactate	42.0	7.1	–	14.3	36.6
5 mM Pyruvate	18.7	–	26.4	14.3	40.6

The values, which are derived from those of fig.1 are means. They are expressed in percentages of the substrate removal. The calculations were made on the assumption that the added substrate are the sole sources of the products measured, and that two lactate or pyruvate molecules are needed for the synthesis of each glucose molecule. No net formation of citrate, 2-oxoglutarate, glutamate, fumarate and malate was observed

polating in vitro results to the in vivo situation, this suggests that, at physiological concentration (1 mM), lactate does not supply energy for the renal reabsorptive work.

Since the normal blood concentration of pyruvate is ~0.1 mM, it is doubtful that pyruvate metabolism by the human kidney in vivo is of great physiological importance. The data of table 1 suggest, however, that, in situations where the blood pyruvate concentration is increased (e.g., in hyperlactatemia and lactic acidosis), pyruvate might become an important energy supplier of the human kidney.

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