STIMULATION OF RAT RENAL PHOSPHOENOLPYRUVATE CARBOXYKINASE ACTIVITY AFTER AN INTRAVENOUS LACTIC ACID LOAD

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Received 12 March 1981

1. Introduction

It is well known that metabolic acidosis attained by ammonium chloride administration leads to an increase in renal gluconeogenesis [1,2] through the activation of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (PEPCK) [3–5]. We have reported that muscular exercise (swimming in water at 22°C for 2 h) results in an increase of renal gluconeogenic ability and PEPCK activity, most probably as a result of the metabolic acidosis caused by overproduction of lactate [6–9]. To obtain further information about the relations between lactic acidosis and renal metabolism we produced experimental lactic acidosis which could mimic in some way the physiological acidosis accompanying exercise. Then we have studied the activity of PEPCK, the gluconeogenic ability in kidney cortex and the renal content of some intermediates of gluconeogenesis from rats loaded intravenously with lactic acid or sodium lactate. We have also studied the renal response to the infusion of adrenaline, because of the involvement of this hormone in the metabolic events during exercise [10]. Our results indicate that induction of renal PEPCK is closely related to acidosis.

2. Materials and methods

Female rats of the Wistar strain (200–250 g body wt) were used. A load of L(+)-lactic acid or sodium lactate was infused into conscious rats throug a cannula pre-inserted into the jugular vein under ether anaesthesia. The infusion was given at 0.05 ml/min with a constant infusion pump over 7–9 min according the weight of the animals. Adrenaline was infused in the same way at 10 μg/kg body wt. The controls were loaded with saline solution (0.9%).

Blood samples were collected in heparinized syringes from the abdominal aorta after anaesthetizing the rats with sodium pentobarbital (60 mg/kg body wt). Kidneys were rapidly excised after death and rinsed in cold saline solution.

Glucose production by renal cortical slices was estimated according to [11] by incubating washed kidney cortical slices in saline medium to which 10 mM L-lactate had been added, at 40°C for 1 h, with O_2–CO_2 (95:5) as the gas phase. The tissue (2–4 mg dry wt) was suspended in 4 ml of the incubation medium and shaken in 25 ml conical flasks. After the incubation, the slices were removed and weighed after drying at 110°C. Glucose was determined in the medium.

Glucose was assayed by the glucose oxidase method [11,12]. Lactate was determined as in [13]. Phosphoenolpyruvate carboxykinase activity was measured in the direction of oxaloacetate synthesis using the modification [14] of the method in [15]. Protein was determined as in [16], with bovine serum albumin as standard.

For the determination of the renal content of gluconeogenic intermediates, the rats were sacrificed by cervical dislocation 2 h after the end of the infusion. One of the kidneys was rapidly excised and clamped between metal tongs pre-cooled in liquid nitrogen [17]. The time elapsing between dislocation of the neck and freezing the organ was 8–10 s. The frozen tissue was pulverized in a mortar, extracted with perchloric acid solution and half neutralized with KOH, as in [18].

Lactate was determined as in [13], aspartate as in [19], malate as in [20], pyruvate and phosphoenolpyruvate as in [21], glucose 6-phosphate and fructose 6-phosphate as in [22].

Table 1
Renal phosphoenolpyruvate carboxykinase activity, glucose production and blood metabolites 2 h after infusion of lactic acid or sodium lactate

Experimental condition	Phosphoenolpyruvate carboxykinase activity (nmol . min^{-1} . mg protein^{-1})	Glucose synthesis from 10 mM lactate (μmol . h^{-1} . g dry wt^{-1})	Blood	
			Lactate (mM)	Glucose (mM)
(a) 4 mmol/kg				
Control	43.2 ± 2.1 (10)	121 ± 6 (9)	2.06 ± 0.13 (9)	7.28 ± 0.20 (8)
Lactic acid	71.5 ± 5.1 (16)[b]	161 ± 9 (12)[a]	2.61 ± 0.21 (16)	7.53 ± 0.20 (15)
Sodium lactate	40.2 ± 2.3 (6)	134 ± 4 (4)	2.40 ± 0.48 (5)	6.91 ± 0.26 (6)
(b) 6 mmol/kg				
Control	50.7 ± 2.9 (3)	123 ± 7 (3)	2.11 ± 0.14 (3)	7.21 ± 0.14 (3)
Lactic acid	77.3 ± 3.2 (4)[a]	173 ± 10 (4)[a]	2.63 ± 0.18 (4)	7.72 ± 0.52 (4)
Sodium lactate	53.0 ± 14.1 (3)	107 ± 8 (3)	2.05 ± 0.64 (3)	6.93 ± 0.20 (3)

The results are given as means ± SEM with the number of observations in parentheses. *P*-Values were calculated by Student's *t*-test: [a] $P < 0.01$; [b] $P < 0.001$

3. Results and discussion

Renal PEPCK activity and gluconeogenic capacity were significantly increased above the controls 2 h after the lactic acid load (table 1). Blood glucose and lactate content were unchanged at this time in all conditions. However, at the end of the infusion lactate concentration had risen in the blood and pH and bicarbonate concentration were significantly reduced (table 2). These data correspond with a moderate acidosis [23]. Infusion of sodium lactate slightly increased both pH and bicarbonate content. These increases could be explained by assuming that at least part of lactate entry into the cells was in acid form [24,25].

Although the acidosis produced by this system is not as pronounced as that found after swimming (where blood lactate reaches 10 mM in 15 min [8]) the magnitude of renal PEPCK activity and gluconeogenic capacity increases was similar to that found after 2 h swimming [6,7]. These findings suggest that kidney cortex PEPCK is very sensitive to acidosis. However, the lack of effect of the sodium lactate load on the enzyme activity and gluconeogenic capacity (table 1) underlines the dependence of the renal metabolic response to acidosis and precludes an hypothetical role of lactate as substrate inducer of renal gluconeogenesis.

The renal content of gluconeogenic intermediates after the infusion of lactic acid (table 3) confirms 'in vivo' the increases in the enzymic activity and gluconeogenic capacity observed 'in vitro'. The concentration of malate was strongly decreased and that of phosphoenolpyruvate was increased 2 h after infusion, suggesting the acceleration of the reaction catalyzed by PEPCK. Likewise the renal concentration of hexose phosphates was increased, while none of these

Table 2
Lactate concentration, pH, pCO_2 and bicarbonate content in blood at the end of infusion of lactic acid or sodium lactate (4 mmol/kg)

Experimental condition	Lactate (mM)	pH	pCO_2	Bicarbonate (mM)
Control	1.88 ± 0.50 (3)	7.449 ± 0.030 (5)	22.1 ± 1.7 (5)	15.4 ± 0.8 (5)
Lactic acid	5.35 ± 0.21 (5)[b]	7.330 ± 0.035 (5)[a]	22.7 ± 0.9 (5)	11.5 ± 0.6 (5)[b]
Sodium lactate	5.73 ± 0.62 (3)[b]	7.541 ± 0.026 (5)[a]	25.2 ± 2.3 (5)	21.6 ± 0.8 (5)[b]

Samples were taken from the abdominal aorta after anaesthesia. The results are given as means ± SEM with the number of observations in parentheses. *P*-Values were calculated by Student's *t*-test: [a] $P < 0.05$; [b] $P < 0.001$

Table 3
Renal content of gluconeogenic intermediates 2 h after infusion of lactic acid or sodium lactate (6 mmol/kg)

Experimental condition	Concentrations of metabolites (nmol/g fresh kidney)						
	Lactate	Pyruvate	Malate	Aspartate	PEP	Fru-6-P	Glc-6-P
Control	634 ± 95 (5)	26 ± 3 (5)	181 ± 13 (4)	450 ± 35 (4)	24 ± 1 (5)	6 ± 1 (4)	36 ± 7 (4)
Lactic acid	461 ± 36 (4)	28 ± 2 (5)	93 ± 6 (5)[b]	521 ± 128 (4)	43 ± 2 (5)[b]	11 ± 1 (4)[a]	50 ± 7 (4)
Sodium lactate	638 ± 52 (6)	28 ± 2 (5)	184 ± 13 (5)	320 ± 76 (6)	24 ± 3 (5)	6 ± 2 (3)	39 ± 6 (3)

The results are given as means ± SEM with the number of observations in parentheses. *P*-Values were calculated by Student's *t*-test: [a] $P < 0.05$; [b] $P < 0.001$

Abbreviations: PEP, phosphoenolpyruvate; Fru-6-P, fructose 6-phosphate; Glc-6-P, glucose 6-phosphate

metabolites were significantly affected by the infusion of sodium lactate.

The symphato–adrenal system is involved in the metabolic events during exercise [10]; adrenergic amines rise in blood during severe exercise [26–28] including swimming [29]. To elucidate the importance of adrenaline in the enhanced renal gluconeogenic capacity observed during exercise, we administered 10 μg adrenaline/kg body wt to the animals by the same procedure. The renal PEPCK activity, gluconeogenic ability and blood lactate and glucose concentrations are shown in table 4. It is noticeable that gluconeogenic ability was enhanced after the infusion (1 and 2 h) to a similar extent to that produced by a lactic acid load whereas the enzymic activity was significantly increased only at 1 h.

The effect of adrenaline on renal gluconeogenesis seems to be independent of PEPCK activation. Adrenaline may facilitate the transport of substrates across the mitochondrial membrane [30]. Although catecholamines produce an increase of cyclic AMP in isolated kidney tubules, stimulation of adenylate cyclase is not involved in the acceleration of renal gluconeogenesis, characterized as a typical α-adrenergic event of the hormone [31,32]. It is probable that this effect of catecholamines may contribute to the stimulation of renal gluconeogenesis during swimming in addition to metabolic acidosis. On the other hand it can also explain the higher increase of gluconeogenesis in relation to PEPCK activity in our experimental conditions. The slight activation of this enzyme must be tentatively attributed to the mild acidosis produced by adrenaline infusion. In fact, lactate concentration in blood rises until 5.00 ± 0.56 mM (av. 3 obs. ± SEM) 2 min after the end of the infusion. The pH values were very similar to the normal at the different times tested but the bicarbonate content was decreased at 5 min from 16.3 ± 1.2 mM in controls to 10.8 ± 1.9 mM in treated animals (av. 3 obs. ± SEM in each case).

Acknowledgement

The authors are grateful to Dr G. A. O. Alleyne for critically reading the manuscript.

Table 4
Renal phosphoenolpyruvate carboxykinase activity, glucose production and blood metabolites after infusion of 10 μg adrenaline/kg body wt

Experimental condition	Phosphoenolpyruvate carboxykinase activity (nmol . min^{-1} . mg $protein^{-1}$)	Glucose synthesis from 10 mM lactate (μmol . h^{-1} . g dry wt^{-1})	Blood	
			Lactate (mM)	Glucose (mM)
Control	42.7 ± 4.1 (6)	114 ± 7 (6)	1.99 ± 0.13 (7)	7.33 ± 0.22 (6)
Adrenaline (1 h)	57.4 ± 5.2 (4)[a]	173 ± 10 (4)[b]	2.79 ± 0.42 (3)	7.22 ± 0.29 (3)
Adrenaline (2 h)	53.3 ± 4.8 (4)	167 ± 6 (8)[b]	2.89 ± 0.36 (7)	6.96 ± 0.17 (8)

The animals were sacrificed 1 or 2 h after the infusion. The results are given as means ± SEM with the number of observations in parentheses. *P*-Values were calculated by Student's *t*-test: [a] $P < 0.02$; [b] $P < 0.001$

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