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Relationship between lactate and glutamine metabolism in vitro by the kidney: Differences between dog and rat and importance of alanine synthesis in the dog

GUY LEMIEUX, PATRICK VINAY, GABRIEL BAVEREL, RAYMOND BRIÈRE, and ANDRÉ GOUGOUX
With the technical assistance of PIERRETTE FOURNEL

The Nephrology-Metabolism Division and the Renal Laboratory, Hôtel-Dieu Hospital, and the Department of Medicine, University of Montreal, Montreal, Quebec, Canada

Relationship between lactate and glutamine metabolism in vitro by the kidney: Differences between dog and rat and importance of alanine synthesis in the dog. Interaction between lactate (1 or 5 mM) and glutamine (1 or 5 mM) metabolism was studied with renal cortical slices incubated at a pH of 7.0 and obtained from acidotic (ammonium chloride) dogs and rats. The effect of amino-oxyacetate (0.2 mM), dichloroacetate (3 mM), and fluoroacetate (0.05 mM) was also studied. Significant differences were observed between dog and rat. In the dog, lactate had no effect on glutamine uptake and vice versa, but gluconeogenesis increased. Ammonia production, however, decreased by 13 to 21%, whereas a significant increase in alanine production was noted. In the rat, glutamine extraction and ammonia production dropped by 33% with 5 mM lactate. In contrast to the observation in the dog, no production of alanine was noted, but significant accumulation of glutamate took place. Amino-oxyacetate inhibited alanine production in the dog and reestablished ammoniagenesis, and it led to a marked decrement in the uptake of lactate and glucose production in both species. Dichloroacetate in the dog resulted in a reduction in pyruvate, alanine, glucose, and ammonia production while glutamate accumulation was observed. In both species, fluoroacetate stimulated glutamine uptake and ammonia production. With lactate alone, fluoroacetate decreased lactate uptake and glucose production. With both lactate and glutamine in the medium, fluoroacetate prevented any effect of lactate on ammoniagenesis. The present study demonstrates that lactate has a modest depressing effect on renal ammonia production by dog slices through increased synthesis of alanine and redistribution of nitrogen from glutamine. In the rat, the depressing effect of lactate on ammonia production in the alanine amino-transferase deficient kidney occurs through accumulation of glutamate. The data also reveal that oxidation of lactate to carbon dioxide is greater in the dog than it is in the rat, but that gluconeogenesis from lactate is more important in the rat.

Relations entre les métabolismes du lactate et de la glutamine in vitro par le rein: Différences entre le chien et le rat et importance de la synthèse d'alanine chez le chien. L'interaction entre le métabolisme du lactate (1 ou 5 mM) et celui de la glutamine (1 ou 5 mM) a été étudiée sur des tranches de cortex rénal incubées à pH 7,0 et obtenues à partir de chiens ou de rats en acidose. L'effet de l'amino-oxyacétate (0,2 mM), du dichloroacétate (3 mM) et du fluoroacétate (0,05 mM) a aussi été étudié. Des différences significatives entre le rat et le chien ont été observées. Chez le chien, le lactate n'a pas d'effet sur la captation de glutamine, et réciproquement, mais la gluconéogenèse augmente. Cependant la production d'ammoniaque est diminuée de 13 à 21% alors qu'une augmentation significative de la production d'alanine est observée. Chez le rat, l'extraction de la glutamine et la production d'ammoniaque diminuent de 33% avec le lactate 5 mM. A l'opposé de ce qui est constaté chez le chien, il n'est pas observé de production d'alanine mais une accumulation significative de glutamate. L'amino-oxyacétate inhibe la production d'alanine chez le chien et restaure l'ammoniogenèse, cependant qu'il détermine une diminution importante de la captation de lactate et de la production de glucose dans les deux espèces. Le dichloroacétate a pour conséquence, chez le chien, une diminution de la production de pyruvate, d'alanine, de glucose et d'ammoniaque, cependant qu'une accumulation de glutamate est observée. Dans les deux espèces le fluoroacétate stimule la captation de glutamine et la production d'ammoniaque. Avec le lactate seul, le fluoroacétate diminue la captation de lactate et la production de glucose. Quand à la fois du lactate et de la glutamine sont ajoutés au milieu, le fluoroacétate empêche les effets du lactate sur l'ammoniogenèse. Ce travail démontre que le lactate a un effet dépresseur modeste sur la production rénale d'ammoniaque par les tranches de rein chez le chien par l'intermédiaire d'une augmentation de la synthèse de l'alanine et de la redistribution de l'azote à partir de la glutamine. Chez le rat, l'effet dépresseur du lactate sur la production d'ammoniaque dans le rein privé d'alanine amino-transférase s'exerce au moyen d'une accumulation de glutamate. Ces résultats révèlent que l'oxydation du lactate en CO_2 est plus importante chez le chien que chez le rat alors que la gluconéogenèse à partir du lactate est plus importante chez le rat.

It is well established that ketone bodies [1, 2], fatty acids [3, 4], glutamine [5], and lactate [6, 7] are normally extracted by the dog kidney. Our labora-

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tory has demonstrated that the renal utilization of glutamine and production of ammonia are markedly depressed by ketone bodies and fatty acids both in vivo [1, 4] and in vitro [4, 8]. This depressing effect is not due to a modification of glutamine transport into the renal tubular cell [4, 8]. As ketone bodies and fatty acids enhance renal gluconeogenesis from lactate [9], the present study was undertaken to investigate if gluconeogenesis from lactate affects glutamine metabolism in vitro. Lactate is a highly oxidizable substrate and a reliable source of pyruvate, acetyl coenzyme A, and oxaloacetate in the kidney. It has already been shown in the rat in vitro that lactate while stimulating renal gluconeogenesis inhibits ammoniagenesis from glutamine [10]. Significant depression in ammoniagenesis with stimulated gluconeogenesis was also demonstrated with cortical slices from the kidney of chronically acidotic dogs incubated with glutamine and lactate [11]. In the same species, an infusion of lactate resulted in decreased ammonia excretion [12]. We have demonstrated that following an infusion of DL-sodium lactate sufficient to raise blood lactate by 5 mM/liter in acidotic dogs, renal ammoniagenesis does not fall by more than 15% [1]. In the present study we demonstrate that lactate inhibits renal ammoniagenesis only slightly in the renal cortex of acidotic dogs. This inhibition is related to redistribution of the nitrogen of glutamine without any change in glutamine uptake. This occurs through significant transamination resulting in striking alanine production. In the rat, lactate also has a modest antiammoniagenic effect but through a different mechanism. It induces an accumulation of glutamate with reduced uptake of glutamine. The effect of lactate on glutamine metabolism was further clarified in both species by the use of aminooxyacetate, dichloroacetate, and fluoroacetate, which modify the metabolic fate of lactate and glutamine. Our study also shows that the fraction of lactate that undergoes complete oxidation in the kidney is more important in the dog than it is in the rat, but that renal gluconeogenesis from lactate is higher in the rat.

Methods

Twenty experiments were performed on cortical slices from ten dogs (each weighing 15 to 20 kg) made acidotic following ammonium chloride administration as previously described [13] and from Sprague-Dawley rats (each weighing 350 to 400 g) (three rats per experiment) also made acidotic by drinking 1.5% ammonium chloride during 7 to 10 days. Mean plasma bicarbonate at the time of nephrectomy was 13.8 ± 0.53 mM/liter for the dogs and 19.0 ± 0.95 mM/liter for the rats. The slices were incubated for 60 min at a pH of 7.0 according to a technique previously reported [13]. In a first group of five experiments in dogs and rats, 1 mM L-glutamine (Sigma Chemicals, Saint Louis, Missouri) and 1 mM L-lactate (Boehringer Mannheim Corporation, New York, New York) were used as substrate alone or in combination and with or without 0.2 mM aminooxyacetate (Sigma Chemicals, Saint Louis, Missouri), 3 mM dichloroacetate (Fisher Scientific Company, Montreal, Quebec), or 0.05 mM fluoroacetate (Fluka, Buchs, Switzerland). In a second group of five experiments, in each species, L-glutamine and L-lactate were used at 5 mM, and the same maneuvers were repeated. These data are mentioned in the text but are not presented in detail in tables. Time-course experiments in dogs and rats have established the linearity of lactate and glutamine uptake as well as ammonia and glucose production over a 60-min period. Dose-effect studies were also performed to determine the optimal dose of aminooxyacetate, dichloroacetate, and fluoroacetate to be used. In all experiments, ammonia, glutamine, glucose, glutamate, citrate, lactate, pyruvate, alanine, and aspartate were measured enzymatically by previously reported methods [13, 14]. Net production was taken as the difference between the products measured at zero time and after 60 min of incubation. The metabolic fate of lactate when the latter was used as the sole substrate was estimated by the production of pyruvate, alanine, glucose, and citrate. The maximal conversion of lactate to carbon dioxide was calculated by subtracting the sum of intermediates released in the incubation medium from total lactate uptake. In these calculations, it is understood that the formation of glucose requires two molecules of lactate and that one alanine is derived from one molecule of pyruvate. Statistical analysis was performed using Student's *t* test for paired data. Results are presented as means ± SEM.

Results

Interaction between lactate and glutamine metabolism: (a) In the dog (Table 1). The addition of 1 mM lactate to 1 mM glutamine had no effect on glutamine uptake in slices from acidotic dogs. The same was true at 5 mM concentration. Conversely, glutamine had no effect on lactate uptake. Ammonia production, however, fell by 21% with 1 mM and by 13% with 5 mM concentration of lactate and glutamine. Alanine production increased significantly

Table 1. Metabolism of lactate and glutamine, 1 mM, by renal cortical slices from acidotic dogs ($N = 5$)[a]

	Glutamine uptake	Lactate uptake	NH_4^+ production	Glucose production	Glutamate production	Alanine production	Aspartate production	Citrate production	Pyruvate production
No substrate	—	−1.32 ±0.62	10.21 ±0.46	1.85 ±0.23	−0.97 ±0.30	0.99 ±0.17	−1.03 ±0.40	−0.36 ±0.09	0.09 ±0.03
Lactate, 1 mM	—	18.58 ±0.98	7.29 ±0.62^{++}	4.67 ±0.59	−0.98 ±0.41^{++}	3.29 ±0.23^{++}	−0.93 ±0.33	−0.14 ±0.20	1.03 ±0.54
Glutamine, 1 mM	12.95 ±0.90	−1.22 ±0.62^{+}	27.80 ±1.48^{+}	2.98 ±0.57^{+}	3.56 ±0.39	1.65 ±0.17^{+}	−0.10 ±0.27	−0.45 ±0.15	0.16 ±0.02^{+}
Lactate, 1 mM, + glutamine, 1 mM	12.55 ±0.45	18.34 ±0.69	21.89 ±1.09	5.78 ±0.47	3.41 ±0.54	7.14 ±0.41	−0.19 ±0.60	−0.40 ±0.09	1.21 ±0.10
Lactate, 1 mM, + aminooxyacetate, 0.2 mM	—	10.19 ±1.01*	8.83 ±0.55	3.42 ±0.49*	−1.16 ±0.29	1.10 ±0.22*	−1.25 ±0.33*	−0.58 ±0.19	0.12 ±0.04*
Lactate, 1 mM, + dichloroacetate, 3 mM	—	18.72 ±0.83	7.61 ±0.49	3.67 ±0.57*	−0.05 ±0.41	1.44 ±0.30*	−0.94 ±0.40	−0.34 ±0.09	0.37 ±0.70*
Lactate, 1 mM, + fluoroacetate, 0.05 mM	—	12.23 ±0.49*	10.06 ±0.93*	2.25 ±0.64*	−1.48 ±0.37	2.04 ±0.35*	−0.92 ±0.33	1.48 ±0.54	0.42 ±0.04*
Glutamine, 1 mM + aminooxyacetate, 0.2 mM	12.93 ±0.93	−0.52 ±0.61	29.62 ±1.42	2.87 ±0.40	3.91 ±0.51	1.02 ±0.26*	−1.21 ±0.34*	−0.45 ±0.15	0.24 ±0.47
Glutamine, 1 mM + dichloroacetate, 3 mM	13.52 ±0.72	−1.10 ±0.63	27.48 +1.06	2.10 +0.51*	4.72 ±0.44*	0.92 ±0.27*	−0.21 ±0.43	−0.46 ±0.18	0.08 ±0.04
Glutamine, 1 mM, + fluoroacetate, 0.05 mM	16.44 ±1.24*	−0.33 ±0.58*	36.70 ±1.38*	2.97 ±0.47	2.53 ±0.35*	2.33 ±0.28	−0.29 ±0.34	2.39 ±0.80*	0.24 ±0.04
Lactate, 1 mM, + glutamine, 1 mM, + aminooxyacetate, 0.2 mM	12.22 ±0.74	11.62 ±1.24*	26.95 ±1.67*	4.82 ±0.29	4.15 ±0.37	1.43 ±0.45*	−1.22 ±0.34	−0.44 ±0.17	0.41 ±0.80*
Lactate, 1 mM, + glutamine, 1 mM, + dichloroacetate, 3 mM	10.52 ±1.04	18.53 ±0.90	21.40 ±0.77	4.09 ±0.47*	5.29 ±0.60*	2.79 ±0.29*	−0.57 ±0.37	−0.26 ±0.10	0.37 ±0.06*
Lactate, 1 mM, + glutamine, 1 mM, + fluoroacetate, 0.05 mM	17.70 ±1.57*	13.07 ±0.70*	34.31 ±0.75*	5.43 ±0.76	2.07 ±0.41*	6.77 ±0.85	−0.56 ±0.35	2.01 ±0.65*	0.99 ±0.14

[a] All values are expressed as micromoles per gram wet wt per hour and corrected for zero time. Values are means ± SEM. Asterisks (*) denote values significantly different ($P < 0.05$) from corresponding conditions with lactate, glutamine or lactate + glutamine. Lactate + glutamine was also compared to glutamine alone ($^{+}$) or to lactate alone ($^{++}$). N denotes number of animals.

when glutamine was added to lactate at either 1 or 5 mM concentrations. The rise in alanine production was more than adequate to explain the fall in ammonia production. Glutamate production was either unchanged or decreased slightly at 5 mM lactate and glutamine. No stimulation of aspartate production was observed. Renal gluconeogenesis was significantly enhanced by the combination of lactate and glutamine. Citrate production was unchanged, and the expected production of pyruvate from lactate was noted.

(b) In the rat (Table 2). Glutamine and lactate uptake in the rat was significantly higher than it was in the dog. No effect of 1 mM lactate on glutamine uptake or ammoniagenesis was noted. In contrast, however, to the dog, the addition of 5 mM lactate depressed glutamine uptake by 23%. Accordingly, ammonia production decreased by 32%. This phenomenon was not accompanied by any release of alanine or aspartate. Significant accumulation of glutamate took place, however. As in the dog, glucose production was enhanced by the addition of lactate to glutamine. Interestingly, lactate appeared to be a better gluconeogenic substrate than glutamine in the dog, whereas the reverse was observed in the rat (Tables 1 and 2).

Effect of aminooxyacetate: (a) In the dog (Table 1). In the dog slices, aminooxyacetate, a well-known inhibitor of aminotransferases [15], had no effect on glutamine uptake and ammonia production. At 5 mM glutamine concentration, a slight decrease in glucose production was observed. The most striking effect of aminooxyacetate on glutamine alone was the significant reduction in alanine and aspartate production. In these experiments, we noted a tendency for glutamate to accumulate. Aminooxyacetate had a striking effect on the uptake of lactate with a decrement of 45% at 1 mM and a decrement of 43% at 5 mM lactate concentration. Simultaneously, glucose, alanine, and aspartate production decreased. A striking reduction in pyruvate production was also noted. The fall in ammonia production noted with the addition of lactate to glutamine at 1 mM concentration was entirely corrected by the addition of aminooxyacetate. At all concentrations of lactate plus glutamine, glucose, alanine, and aspartate production fell significantly.

Table 2. Metabolism of lactate and glutamine, 1 mM, by renal cortical slices from acidotic rats ($N = 5$)[a]

	Glutamine uptake	Lactate uptake	NH_4^+ production	Glucose production	Glutamate production	Alanine production	Aspartate production	Citrate production	Pyruvate production
No substrate	—	−0.82	15.20	3.54	−2.28	1.26	−0.90	−0.80	0.39
		±0.15	±1.19	±0.75	±0.44	±0.21	±0.18	±0.05	±0.10
Lactate, 1 mM	—	19.87	12.34	7.26	−2.28	1.53	−0.68	−0.54	1.73
		±0.94	±0.83^{++}	±0.54^{++}	±0.43^{++}	±0.19	±0.21	±0.09	±0.36
Glutamine, 1 mM	22.32	−1.17	52.06	8.26	4.06	1.27	−0.26	−0.46	0.61
	±1.12	±0.15^{+}	±2.22	±0.76^{+}	±0.85	±0.13^{+}	±0.19	±0.08	±0.12^{+}
Lactate, 1 mM, + glutamine, 1 mM	22.36	19.81	51.11	12.81	3.21	1.77	−0.39	−0.55	1.85
	±1.35	±0.75	±1.27	±1.39	±0.57	±0.18	±0.21	±0.17	±0.25
Lactate, 1 mM	—	12.72	13.28	2.32	−2.02	1.56	−0.96	−0.62	1.00
+ aminooxyacetate, 0.2 mM		±1.69*	±1.22*	±0.47*	±0.49	±0.13	±0.18*	±0.06	±0.19
Lactate, 1 mM	—	20.08	10.13	4.15	−2.24	0.94	−0.72	−0.63	1.14
+ dichloroacetate, 3 mM		±1.75	±0.87	±0.14*	±0.50	±0.04*	±0.15	±0.09	±0.21*
Lactate, 1 mM,	—	16.71	13.33	4.14	−2.42	1.26	−0.77	0.24	1.99
+ fluoroacetate, 0.05 mM		±0.98*	±0.75	±0.78*	±0.48	±0.15	±0.09	±0.44	±0.36
Glutamine, 1 mM,	21.86	−3.68	53.97	7.65	4.08	1.97	−0.89	−0.55	0.29
+ aminooxyacetate, 0.2 mM	±1.05	±0.21	±1.16	±0.40	±0.45	±0.13*	±0.14	±0.12	±0.08*
Glutamine, 1 mM	23.80	−0.67	51.69	5.42	5.23	0.92	−0.30	−0.51	0.37
+ dichloroacetate, 3 mM	±1.06	±0.20	±1.54	±0.62*	±0.53	±0.11*	±0.27	±0.10	±0.12*
Glutamine, 1 mM	23.92	−2.01	56.86	7.26	2.67	1.42	−0.90	0.91	0.90
+ fluoroacetate, 0.05 mM	±0.88	±0.12	±0.61	±0.69	±0.30	±0.07	±0.16	±0.45*	±0.20
Lactate, 1 mM, + glutamine, 1 mM,	21.58	10.32	52.45	7.35	4.32	1.69	−0.77	−0.59	1.08
+ aminooxyacetate, 0.2 mM	±0.94	±1.70	±1.98	±0.70*	±0.55	±0.18	±0.07	±0.10	±0.17*
Lactate, 1 mM, + glutamine, 1 mM,	21.01	19.70	47.08	8.61	4.51	1.01	−0.31	−0.64	1.10
+ dichloroacetate, 3 mM	±1.06	±1.16	±2.44	±0.73*	±0.72	±0.10*	±0.22	±0.10	±0.22*
Lactate, 1 mM, + glutamine, 1 mM,	23.92	17.08	54.36	12.33	1.92	1.73	−0.44	0.90	2.05
+ fluoroacetate, 0.05 mM	±1.18	±0.65	±1.60	±1.26	±0.15	±0.14	±0.14	±0.42*	±0.31

[a] All values are expressed as micromoles per gram wet wt per hour and corrected for zero time. Values are means ± SEM. Asterisks (*) denote values significantly different ($P < 0.05$) from corresponding conditions with lactate, glutamine or lactate + glutamine. Lactate + glutamine was also compared to glutamine alone ($^{+}$) or to lactate alone ($^{++}$). N denotes number of experiments (3 rats per experiment).

(b) In the rat (Table 2). As in the dog, aminooxyacetate had little effect on the metabolism of glutamine in the rat slices. As expected, no effect on alanine production was noted. The uptake of lactate was also markedly depressed by the addition of aminooxyacetate. It fell by 36% at 1 mM and 67% at 5 mM lactate. Glucose production fell in proportionate fashion. Again no effect on alanine, aspartate, or glutamate production was observed. When aminooxyacetate was added to lactate plus glutamine 5 mM, the inhibition of glutamine uptake previously noted was reversed, glutamine uptake and ammonia production returning to the values observed with glutamine alone. Glucose production fell by 50% in accordance with the fall in lactate uptake and utilization for gluconeogenesis. No effect on alanine, aspartate, or glutamate production was noted.

Effect of dichloroacetate: (a) In the dog (Table 1). Dichloroacetate, a stimulator of the pyruvate-dehydrogenase complex [16, 17], had little effect on glutamine metabolism. Only a slight reduction in glucose and alanine production was noted. With lactate alone, the addition of dichloroacetate led to significant reduction in pyruvate accumulation and in glucose and alanine production. The addition of dichloroacetate to the combination of lactate plus glutamine at 1 and 5 mM resulted in similar depression in pyruvate, glucose, and alanine production, whereas glutamate accumulated. In addition, at 5 mM lactate plus glutamine, dichloroacetate led to further decrease in glutamine uptake and ammonia production.

(b) In the rat (Table 2). The addition of dichloroacetate to glutamine, lactate, or lactate and glutamine had the same effect in the rat as it did in the dog with the exception that no influence on glutamine uptake and ammonia production in the presence of lactate was noted.

Effect of fluoroacetate: (a) In the dog (Table 1). Fluoroacetate, a well-known inhibitor of the Krebs cycle at the aconitase level [18], led to citrate accumulation in all situations studied. Glutamine uptake and ammonia production were stimulated. Glucose production did not change at 1 mM glutamine, but it increased at 5 mM. In the presence of lactate alone, fluoroacetate depressed the uptake of lactate at both 1 and 5 mM. Glucose production decreased in a proportionate fashion. In the presence of lactate

plus glutamine, fluoroacetate stimulated glutamine uptake and ammonia production, whereas glutamate release fell at 1 mM only.

(b) In the rat (Table 2). In the rat, in contrast to the dog, fluoroacetate had only a slight stimulating effect on glutamine uptake and ammonia production. A diminution in citrate utilization or net release of citrate was observed, however, in all instances. Fluoroacetate had the same inhibiting effect on lactate uptake as it did in the dog. In the presence of lactate plus glutamine, fluoroacetate had the same stimulating effect on glutamine uptake and ammonia production.

Fate of lactate: (a) In the dog (Table 3). Forty to fifty percent of lactate was transformed in glucose, and less than 33% underwent complete oxidation. The uptake of lactate was diminished by aminooxyacetate and fluoroacetate, but not by dichloroacetate. This was true at both 1 and 5 mM of lactate. All the metabolic effectors used led to a reduction in pyruvate, alanine, and glucose production from lactate. Citrate release was noted only with fluoroacetate. The oxidation of lactate to carbon dioxide was diminished at 1 mM by aminooxyacetate and fluoroacetate, whereas it was stimulated by dichloroacetate. The same was true at 5 mM with the exception of aminooxyacetate, which had no effect.

(b) In the rat (Table 4). The observations made in the rat were similar to those made in the dog, but gluconeogenesis appeared to be more important (68 to 73%) and oxidation less important (11 to 23%) than they are in the dog. The apparent stimulation of oxidation induced by fluoroacetate probably reflects accumulation of metabolic intermediates of a greater degree than that occurring in the dog. Indeed, oxygen consumption (not presented) was depressed in both species by fluoroacetate.

Discussion

The present study demonstrates that lactate has only a slight depressing effect on renal ammoniagenesis in vitro in the acidotic dog and rat. The mechanism of this depressing effect is different, however, in each species. In the dog, lactate has no effect on glutamine uptake. The depressing effect on ammoniagenesis observed in this species when lactate is added to glutamine is entirely explained by the

Table 3. Fate of lactate utilized by renal cortical slices from acidotic dogs ($N = 5$)[a]

	Lactate, 1 mM				Lactate, 5 mM			
	Lactate alone	Lactate + amino-oxyacetate	Lactate + dichloro-acetate	Lactate + fluoro-acetate	Lactate alone	Lactate + amino-oxyacetate	Lactate + dichloro-acetate	Lactate + fluoro-acetate
Lactate uptake	18.58	10.19	18.72	12.23	35.79	20.35	35.58	26.91
Lactate to:								
pyruvate	1.03	0.12	0.37	0.42	4.03	0.01	1.14	2.16
alanine	3.29	1.10	1.44	2.04	5.66	1.38	3.29	4.32
C_3-glucose	9.34	6.84	7.34	4.50	14.30	6.96	12.76	9.50
citrate	—	—	—	2.96	—	—	—	3.24
Maximum lactate to CO_2	4.92	2.13	9.57	2.31	11.79	11.76	18.02	7.69

[a] Values are expressed as micromoles per gram wet wt per hour and are derived from Table 1 and data at 5 mM. N denotes number of animals.

Table 4. Fate of lactate utilized by renal cortical slices from acidotic rats ($N = 5$)[a]

	Lactate, 1 mM				Lactate, 5 mM			
	Lactate alone	Lactate + amino-oxyacetate	Lactate + dichloro-acetate	Lactate + fluoro-acetate	Lactate alone	Lactate + amino-oxyacetate	Lactate + dichloro-acetate	Lactate + fluoro-acetate
Lactate uptake	19.87	12.72	20.08	16.71	56.18	19.13	52.67	46.47
Lactate to:								
pyruvate	1.78	1.00	1.14	1.99	4.06	1.07	2.45	4.15
alanine	1.53	1.56	0.94	1.26	1.44	1.22	0.94	1.28
C_3-glucose	14.52	4.64	8.30	8.28	37.94	24.12	15.40	20.96
citrate	—	—	—	0.24	—	—	—	—
Maximum lactate to CO_2	2.09	5.52	9.70	4.94	12.74	7.28	33.88	20.08

[a] Values are expressed as micromoles per gram wet wt per hour and are derived from Table 2 and data at 5 mM. N denotes number of experiments (3 rats per experiment).

striking increase in alanine production that is observed under these conditions. The availability of pyruvate from lactate, of glutamate from glutamine, and the presence of alanine aminotransferase in the dog kidney represent ideal conditions for the renal synthesis of alanine. Direct transamination of glutamine (glutaminase II pathway) could be operative also. Thus, the presence of lactate induces only a redistribution in the metabolic fate of glutamine. In the rat, no effect of lactate on ammoniagenesis from glutamine is observed unless 5mM concentration is used. Under these circumstances, a significant depression of glutamine extraction and ammoniagenesis is observed. This is related to the accumulation of glutamate observed in this condition. It is well-known that glutamate is a potent inhibitor of glutaminase I activity [19, 20]. The accumulation of glutamate is probably related to the generation of reducing equivalents which occurs during lactate metabolism. Although lactate is oxidized into pyruvate in the cytosol, the secondary reduction of the mitochondrial compartment will influence glutamate dehydrogenase and favor accumulation of glutamate. Such an effect probably also occurs in the dog, but no glutamate accumulation is observed because of rapid transamination to alanine. As the rat kidney is devoid of alanine aminotransferase [21], no alanine synthesis is observed, and glutamate accumulation occurs. The effect of lactate in the rat is similar, but to a lesser degree, to that observed with ketone bodies [8] and fatty acids [4]. Failure of lactate, 1 mM, to increase glutamate production in the rat and depress ammoniagenesis might be related to the fact that this physiologic concentration in an animal with high metabolic rate [22] and oxygen consumption [23] may not be sufficient to induce accumulation of reducing equivalents.

Lactate stimulates gluconeogenesis both in the dog and the rat. When lactate is added to glutamine, an additive effect on gluconeogenesis is observed. This indicates that the activity of phosphoenolpyruvate carboxykinase is not rate-limiting for gluconeogenesis from either substrate. Also, it can be inferred that the generation of oxaloacetate through pyruvate carboxylase does not interfere with gluconeogenesis from glutamine in both species. Therefore, the inhibitory effect of ketone bodies and fatty acids on glutamine metabolism [1, 4, 8] cannot be attributed to the stimulation of oxaloacetate generation through the pyruvate carboxylase system and inhibition of pyruvate dehydrogenase complex. No accumulation of oxaloacetate was observed in both rat and dog because no accumulation of aspartate could be demonstrated.

Our studies are different but not in conflict with those of Pilkington and O'Donovan who reported a 38% fall in ammoniagenesis in acidotic dog kidney slices incubated at a pH of 7.0 but with 10 mM glutamine and 5mM lactate [11]. The difference may be related to the length of acid loading which was 3 days in their case [11] and 5 in ours. Roxe, Schreiner, and Preuss reported a 30% fall in ammoniagenesis in renal cortical slices from normal rats incubated at a pH of 7.40 with 0.6 mM glutamine and 1.2 mM lactate [10]. The experimental conditions of these studies are different from ours where equimolar amounts of both glutamine and lactate were used at the same time with cortical tissue obtained from acidotic animals to insure maximal utilization of glutamine and ammonia production. Interestingly, Preuss has recently reported that lactate shows a more depressing action on ammoniagenesis by slices from normal rats than it does by slices from acidotic animals [24]. During infusion of lactate in normal dogs, Churchill and Malvin reported a striking decrease in ammonia excretion [12]. Their results are not surprising because these authors probably created extracellular alkalosis, which would readily explain the marked decrement in ammonia excretion. Our present in vitro data are in accord with our own observations in vivo where an infusion of lactate to acidotic dogs just sufficient to correct metabolic acidosis and raise plasma lactate concentration to 6.6 mM/liter resulted only in a 15% decrease in total ammonia production by the kidney [1].

Aminooxyacetate is a well-known inhibitor of aminotransferases including alanine (GPT), aspartate (GOT), and glutamine (glutaminase II) aminotransferases [15]. In the dog, aminooxyacetate induced a striking inhibition of alanine production. At the same time the depressing effect of lactate on ammoniagenesis from glutamine was abolished completely. This observation reinforces the point that the slight inhibition on ammoniagenesis by lactate was due to increased alanine synthesis in this species. Under these circumstances, one could have expected an accumulation of glutamate in the dog as significant as that which occurred in the rat following inhibition of alanine synthesis. This did not occur and is readily explained by the effect of aminooxyacetate on the transfer of reducing equivalents into the mitochondria through the aspartate shuttle [15, 25, 26]. This also explains the reduction in lactate utilization observed with aminooxyacetate in

both dog and rat. These results are in full accord with those of Lardy, Paetkan, and Walter [27] and with those of Rognstad and Katz [15].

Dichloroacetate stimulates the pyruvate dehydrogenase complex, which transforms pyruvate into acetyl coenzyme A in the mitochondria [16, 17]. This effector was used to stimulate the oxidation of pyruvate and the generation of intramitochondrial reducing equivalents. Such an effect should simulate that of ketone bodies and fatty acids [1, 4, 8]. In the present study, it is clear that dichloroacetate stimulated the metabolism of pyruvate from lactate as evidenced by a fall in pyruvate production and a secondary reduction in glucose and alanine production. Ammoniagenesis from glutamine, already depressed by lactate, decreased further in the dog, whereas glutamate accumulated, an effect similar to that observed with ketones [8] and fatty acids [4, 8].

Fluoroacetate, a Krebs cycle inhibitor at the aconitase level [18], was used to induce the reverse effect of dichloroacetate by inhibiting the oxidation of pyruvate. Indeed, fluoroacetate depresses the uptake of lactate when the latter is used alone. Because glucose production from lactate decreased significantly, an effect of fluoroacetate on pyruvate carboxylase is also observed, as already reported by Mehlman with fluorocitrate [28]. With glutamine alone, fluoroacetate stimulated glutamine uptake and ammonia production, whereas glucose production either increased or remained unchanged. This effect is attributable to an intramitochondrial redox effect opposite to that observed with ketone bodies [1, 8] and fatty acids [4, 8]. In the presence of glutamine and lactate combined, fluoroacetate completely reversed the depressing effect of lactate on ammoniagenesis in both dog and rat, indicating that the effect of lactate occurs through its oxidation. The observed increment in gluconeogenesis following fluoroacetate can only be generated from glutamine, because fluoroacetate has an inhibiting effect on pyruvate carboxylase. The reactions influenced by the various effectors are illustrated in Fig. 1.

The present studies have made it possible to evaluate the metabolic fate of lactate in vitro, with and without addition of various effectors. The analysis emphasizes that, with equivalent uptake, more lactate is transformed in glucose in the rat than it is in the dog, whereas the fraction that undergoes complete oxidation is greater in the dog than it is in the rat. Nevertheless, lactate appears to be a better gluconeogenic substrate than is glutamine in the dog, but the reverse is true in the rat.

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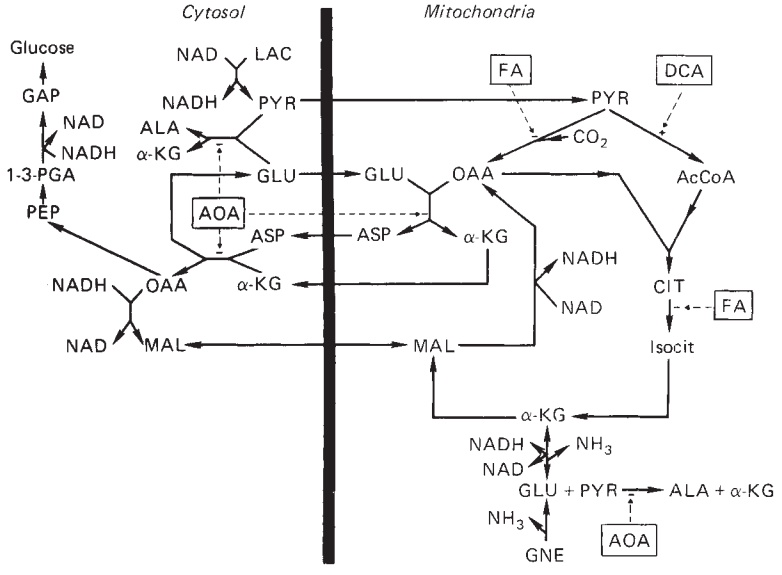

Fig. 1. *Mitochondrial and cytosolic reactions influenced by aminooxyacetate (AOA), dichloroacetate (DCA), and fluoroacetate (FA).* Other abbreviations are: PYR, pyruvate; OAA, oxaloacetate; ALA, alanine; GNE, glutamine; GLU, glutamate; MAL, malate; ASP, asparate; LAC, lactate; PEP, phospoenolpyruvate; 1-3-PGA, 1-3-phosphoglyceraldehyde; GAP, glyceraldehyde phosphate; CoA, coenzyme A.

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Reprint requests to Dr. G. Lemieux, Nephrology-Metabolism Division, Hôtel-Dieu Hospital, 3840 St. Urbain St., Montreal, Quebec, Canada, H2W 1T8

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