Activation of oxoglutarate dehydrogenase in the kidney in response to acute acidosis

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1. Activation by H^+ and by Ca^{2+} of 2-oxoglutarate dehydrogenase extracted from mitochondria of normal or acidotic rat kidney is described. This effect, first shown for the enzyme from heart by McCormack & Denton [*Biochem. J.* (1979) **180**, 533–544], is of a regulatory importance in kidney, in which organ, in contrast with heart, increased flux occurs during acute acidosis. 2. In renal-cortical tubules, 2-oxoglutarate concentration fell within 1 min of decreasing the pH and rose again 1–3 min after increasing the pH of the medium. The extent of the decrease in 2-oxoglutarate was directly related to the decrease in pH. A similar fall in the oxoglutarate concentration in the whole perfused kidney was noted within 5 min of inducing acidosis. 3. In tubules, the rates of gluconeogenesis and ammoniogenesis from 1 mM-glutamine were increased by 64 and 33% respectively on decreasing pH to 7.0, the increase in rates being proportional to the fall in pH between 7.4 and 7.0. 4. The increased rates of renal ammoniogenesis and gluconeogenesis seen in acute acidosis *in vitro* can be accounted for by the increased activity of 2-oxoglutarate dehydrogenase and the tissue concentrations of 2-oxoglutarate when calculated from the K_m determined at normal and acidotic pH. 5. The decrease in 2-oxoglutarate concentration seen in acute acidosis implies a fall in intramitochondrial pH in kidney, and is the result of two phenomena, accelerated disposal via 2-oxoglutarate dehydrogenase and maintenance of near equilibrium of glutamate dehydrogenase.

The effect of acidosis on the concentration of certain key metabolites in the kidney has been recognized for several years (Alleyne, 1968; Simpson & Sherrard, 1969; Hems & Brosnan, 1971; Narins & Relman, 1974; Goldstein, 1976), but the reasons for these changes, particularly the decrease in 2-oxoglutarate, are poorly understood. It has been suggested (Goldstein, 1966, 1967) that the decrease in glutamate is due to the increased rate of gluconeogenesis that occurs in acidosis (Goodman *et al.*, 1966) facilitated by the increased activity of phosphoenolpyruvate carboxykinase (Alleyne & Scullard, 1969; Longshaw & Pogson, 1972). A similar explanation has been offered for the much greater fall in tissue 2-oxoglutarate content (Alleyne, 1968, 1970; Hems & Brosnan, 1971; Bennett & Alleyne, 1978). This is not the only mechanism, however, as Ross & Tannen (1979), using an isolated perfused kidney model which responds to acidification of the medium in a similar way to the kidney *in vivo*, showed that, when gluconeogenesis was inhibited with mercaptopicolinate, the concentration of 2-oxoglutarate still decreased when the

medium was acidified. This led to the conclusion that the decrease in 2-oxoglutarate was a reflection of the maintenance of near equilibrium by glutamate dehydrogenase, as originally proposed by Krebs & Vinay (1975) (see Ross & Tannen, 1979, for further discussion). Such a mechanism would indicate that intramitochondrial events regulate 2-oxoglutarate concentration throughout the cell. The importance of extramitochondrial 2-oxoglutarate concentration in regulating the rate of ammoniogenesis by control of glutamine entry into the mitochondria has been emphasized (Goldstein, 1976; Boyd & Goldstein, 1979).

The lowered concentration of 2-oxoglutarate in acidosis may be indicative of a lower intramitochondrial pH (Ross & Tannen, 1979). In view of the demonstration by McCormack & Denton (1979) that oxoglutarate dehydrogenase from heart muscle can be activated *in vitro*, with lowering of the K_m for 2-oxoglutarate at low pH, we have examined the properties of the enzyme isolated from rat kidney. In this tissue, activation of oxoglutarate dehydrogenase could have physiological importance in explain-

ing the acceleration of ammoniogenesis in acute acidosis.

In either case, it may be expected that the change in 2-oxoglutarate concentration in response to pH changes would be a rapid event; it may also be an initiating event in the acceleration of ammoniogenesis by acidosis. The present studies were designed to examine this relationship.

Methods

Animals

Male Wistar rats of weight 300–350 g were used throughout the study. They received a normal diet *ad libitum* and were allowed free access to water.

Perfusion

Surgical procedures were similar to those described previously (Ross *et al.*, 1973), with the exception that the ureter was not catheterized and mannitol was not given before surgery.

The kidneys were perfused with bicarbonate-buffered saline (Krebs & Henseleit, 1932) containing 7.5% dialysed bovine serum albumin (fraction V; Miles Laboratories, Slough, Bucks., U.K.) with 5 mM-glucose and 0.5 mM-glutamine as substrates. The pH of the medium was changed by the addition of an appropriate volume of 1 M-HCl, and was allowed to stabilize for 10 min before the beginning of each perfusion.

At various intervals after the beginning of perfusion, kidneys were freeze-clamped; the earliest time at which a kidney could be clamped under these conditions was approx. 90 s after the start of flow of medium into the renal artery.

Extraction of the freeze-clamped tissue was carried out as described previously (Nishiitsutsuji-Uwo *et al.*, 1967).

Preparation of isolated kidney tubules

Tubules were prepared from the cortex of fed rats by a modification (Tange *et al.*, 1977) of the method of Guder *et al.* (1971). They were incubated in 25 ml Erlenmeyer flasks at a final concentration of 3–5 mg dry wt./ml in 2 ml of Krebs–Henseleit buffer, containing 2.5% dialysed bovine serum albumin and the required substrates, added as neutral solutions to the final concentrations indicated. Incubations were continued for 30 min at 37°C. No attempt was made to separate tubules and incubation medium in these experiments.

Ca²⁺ concentration

In order that the experiments with tubules would be comparable with those with perfused kidneys, the Ca²⁺ concentration of the Krebs–Henseleit buffer was lowered from 2.54 mM to 1.27 mM. The use of this lower concentration in perfusion experiments is

required because of the binding of Ca²⁺ ions by bovine albumin during its preparation by dialysis. It has been reported (Alleyne *et al.*, 1973) that pH accelerates gluconeogenesis only at a low Ca²⁺ concentration, less than 1.5 mM. A control experiment at the normal Ca²⁺ concentration of 2.54 mM showed that the pH effect on the 2-oxoglutarate concentration of tubules was of the same magnitude as that at 1.27 mM-Ca²⁺.

Mitochondrial extracts

These were prepared from the kidney cortex only. Bisected kidneys were placed in ice-cold preparation buffer, consisting of 280 mM-sucrose, 2 mM-EGTA and 10 mM-Tris/HCl, pH 7.35. The cortex was chopped with scissors and resuspended in 5 vol. of buffer. The suspension was homogenized with ten strokes of a loosely fitting Teflon pestle and then centrifuged for 10 min at 500 g to remove debris, and the supernatant was centrifuged for a further 10 min at 10 000 g. Lysosomes, which might interfere with subsequent steps, were removed by resuspending the mitochondrial pellet in the same buffer with added digitonin (1 mg/ml; B.D.H.), leaving in ice for 2 min, and centrifuging once again for 10 min at 10 000 g. The mitochondrial pellet was washed twice in homogenization buffer and resuspended finally in 100 mM-potassium phosphate/1 mM-dithiothreitol, pH 7.0, and broken by freeze-thawing in liquid nitrogen. This extract was then centrifuged again at 15 000 g for 15 min, and the supernatant was used for subsequent assays of oxoglutarate dehydrogenase. Assays of the relevant fractions obtained after freeze-thawing showed 88.5 ± 8.3% recovery of activity; a further 10% could be recovered by washing the final pellet in the extraction buffer.

Assay of 2-oxoglutarate dehydrogenase

Conditions for the assay of 2-oxoglutarate dehydrogenase closely followed those described by McCormack & Denton (1979). NADH production was measured in a Gilford recording spectrophotometer at 30°C in 50 mM-Mops (4-morpholinepropanesulphonic acid) buffer. Assays were conducted at saturating concentrations of NAD⁺ (0.75 mM), thiamin pyrophosphate (1 mM) and CoA (0.25 mM), with other components as described by McCormack & Denton (1979). 2-Oxoglutarate concentration was varied between 6 μM and 21 mM, and enzyme rate was determined at between 9 and 13 different concentrations of the substrate. *K_m* was calculated by using a weighted linear least-squares regression program prepared by Dr. Paul England (Department of Biochemistry, University of Bristol). In order to examine the concurrent effects of Ca²⁺ concentration and pH on the *K_m* of 2-oxoglutarate dehydrogenase, EGTA buffers were used. A computer program based on the mathematics of Ca²⁺

dissociation prepared by Flodgaard & Fleron (1974) and Portzehl *et al.* (1964) was used to determine free and bound Ca^{2+} in each incubation, since dissociation constant is dependent on pH. Activities (means $\pm$ S.E.M.) are expressed per g dry wt. of kidney, to permit comparison with calculation of flux in whole kidney tissue. Statistical comparisons were by Student's *t* test, paired or unpaired as indicated.

Assays of metabolites

Metabolites were measured in both the kidney extracts and tubules plus incubation medium by standard enzymic procedures: glutamate (Bernt & Bergmeyer, 1974), 2-oxoglutarate (Bergmeyer & Bernt, 1974), ammonia (Kun & Kearney, 1974), glucose (Bergmeyer *et al.*, 1974*a*), pyruvate (Czok & Lamprecht, 1974), lactate (Gutmann & Wahlefeld, 1974*a*), aspartate (Bergmeyer *et al.*, 1974*b*) with 50 mM-triethanolamine as the buffering species, malate (Gutmann & Wahlefeld, 1974*b*), citrate (Dagley, 1974) and adenine nucleotides (Jaworek *et al.*, 1974; Lamprecht & Trautschold, 1974).

2-Oxoglutarate in tubules plus incubation medium was measured with the use of an Aminco-Chance DW2 spectrophotometer, operated in split-beam mode. Malate was measured in tubule extracts by linking the formation of NADH by malate dehydrogenase to the production of light by bacterial luciferase, as described by Ashcroft & Christie (1979).

Results

Time course of metabolite responses to changing pH in perfused kidney

Changes in the renal content of the reactants of glutamate dehydrogenase occur rapidly in response to falling pH of the medium, as shown in Table 1. The major and most rapid effect was the fall in the concentration of 2-oxoglutarate, which was 78% of the control value after only 1.5 min of perfusion and fell further to 34% of its original value at 6.5 min ($P \leq 0.001$). The concentration remained low after 16.5 min of perfusion ($P \leq 0.01$). Some of this effect is attributable to the progressive increase in tissue 2-oxoglutarate concentration in kidneys perfused at pH 7.4. After 16.5 min of perfusion, glutamate was significantly lower in kidneys perfused at the more acid pH. At pH 7.4, the glutamate concentration of the kidneys progressively increased with time. Ammonia concentration, on the other hand, showed no significant difference between kidneys perfused at pH 7.4 or 7.0, and increased progressively with the duration of perfusion, reflecting the progressive formation of ammonia from glutamine.

The changes in the concentrations of 2-oxo-glutarate and glutamate at 6.5 min result in a significant increase in the equilibrium ratio of

Table 1. Effect of pH of medium on renal metabolites during perfusion

Kidneys were perfused with the medium described in the Methods section at either pH 7.4 or 7.0. At various times they were freeze-clamped and the metabolites measured. Results are expressed as $\mu\text{mol/g}$ dry wt. and are shown as means $\pm$ s.e.m., with n being the number of kidneys perfused. Significant differences are shown by $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. For clarity, only comparisons between the two pH values are shown. Equilibrium constant of glutamate dehydrogenase (GldH) for $I = 0.25$, $t = 38^\circ\text{C}$ and $\text{pH} 7.0$ is $3.87 \times 10^{-6}\text{M}$ (Williamson *et al.*, 1967); the ratio $[\text{oxoglutarate}]/[\text{NH}_3]/[\text{glutamate}]$ for wet kidney is calculated.

Condition	...	Non-perfused	Time	...	pH 7.4			pH 7.0		
					1.5 min	6.5 min	16.5 min	1.5 min	6.5 min	16.5 min
<i>n</i>	...	6	...	...	8	5	4	8	5	4
2-Oxoglutarate		1.32 ± 0.15			0.71 ± 0.07	1.25 ± 0.13	1.40 ± 0.19	0.55 ± 0.06	0.43 ± 0.07**	0.71 ± 0.14*
Glutamate		5.84 ± 0.49			5.32 ± 0.37	6.57 ± 0.65	11.03 ± 0.88	5.30 ± 0.42	5.43 ± 0.71	6.74 ± 0.21*
Ammonia		4.31 ± 0.52			4.46 ± 0.54	5.48 ± 0.57	13.45 ± 0.28	5.23 ± 0.64	5.39 ± 0.98	12.96 ± 0.68
10 ³ × Equilibrium ratio (M ⁻¹) (GIDH)		0.27 ± 0.06			0.15 ± 0.02	0.26 ± 0.02	0.45 ± 0.09	0.14 ± 0.03	0.10 ± 0.01**	0.36 ± 0.09
10 ³ × $\frac{[\text{NADH}] \cdot [\text{H}^-]}{[\text{NAD}^+]}$ (M)		14.3			26.0	14.9	8.6	26.9	38.7	10.8

glutamate dehydrogenase, indicating an increase in the ratio $[\text{NADH}][\text{H}^+]/[\text{NAD}^+]$ (Table 1).

No significant differences in the renal content of other metabolites developed when the kidney was perfused at pH 7.0 instead of pH 7. Metabolites measured were: lactate, pyruvate, malate, citrate and adenine nucleotides. This result is in contrast with the findings after 45 min, when both malate and citrate were significantly decreased at the more acid pH (Ross & Tannen, 1979).

Effects of pH on metabolites of isolated kidney tubules

In isolated cortical tubules, the tissue concentration of 2-oxoglutarate closely reflected the pH of the incubation medium, being inversely related to $[\text{H}^+]$ (Table 2). A decrease in the incubation medium pH from 7.4 to 7.0 resulted in a decrease of 85% in the 2-oxoglutarate concentration. This is of the same magnitude as the decrease seen during perfusion. An increase in the final concentration of ammonia was observed at pH 7.0, but, in contrast with the perfusion experiments, there was no difference in the glutamate concentrations. The decrease in 2-oxoglutarate concentrations was observed in both the presence and absence of 1 mM-glutamine, but no increase in ammonia was seen in the absence of substrate. No differences in the concentration of malate were observed, whether glutamine was present or absent.

When tubules were incubated at pH 7.7, there was a 6-fold increase in the concentration of 2-oxoglutarate. The 50% increase in glutamate concentration did not reach statistical significance.

Titration the pH of the medium to values intermediate between 7.4 and 7.0 decreased the concentration of 2-oxoglutarate significantly from the control value, even in medium of pH 7.3. Significant decreases in 2-oxoglutarate concentration were observed for each further fall in pH of 0.1 unit. These changes occurred without any significant change in the concentration of glutamate (Fig. 1).

The rapidity of the tubular response to acid was demonstrated in an experiment to follow the time course of the decrease in 2-oxoglutarate concentration (Fig. 2). At zero time the concentration of 2-oxoglutarate in the tubules was very low, but

Fig. 1. Titration of 2-oxoglutarate with HCl in tubules. Tubules were incubated for 30 min with 1 mM-glutamine as substrate at the various pH values as described in the Methods section. Glutamate (●) and 2-oxoglutarate (○) were measured in the medium plus tubules and concentrations are expressed as a percentage of the respective control value at pH 7.4 in the same experiment. (For absolute values see Table 2.) The error bars indicate 1 S.E.M.; significant differences between adjacent points are shown by * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Table 2. Effect of pH on the metabolite content of isolated kidney tubules

Tubules were incubated in the presence or absence of 1 mM-glutamine as described in the Methods section. The results are expressed as $\mu\text{mol/g}$ dry wt. and are shown as means $\pm$ S.E.M. with the numbers of separate preparations shown in parentheses. Significant differences with respect to pH 7.4 are shown by * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Incubation conditions	Time (min)	pH	2-Oxoglutarate	Glutamate	Ammonia	Malate
No glutamine	0	7.4	0.73 ± 0.07 (23)	20.2 ± 1.8 (22)	84.8 ± 6.5 (22)	1.65 ± 0.14 (3)
	30	7.0	0.94 ± 0.06 (11)***	5.59 ± 0.56 (11)	168.7 ± 9.6 (11)	0.07 ± 0.03 (4)
	30	7.4	5.71 ± 0.27 (11)	7.16 ± 0.80 (11)	168.2 ± 8.8 (11)	0.13 ± 0.02 (4)
1 mM-Glutamine	30	7.0	0.95 ± 0.07 (18)***	27.1 ± 2.1 (17)	421.9 ± 14.1 (17)**	0.51 ± 0.10 (4)
	30	7.4	7.03 ± 0.29 (18)	30.1 ± 2.7 (17)	368.3 ± 11.4 (17)	0.61 ± 0.10 (4)
	30	7.7	41.6 ± 2.0 (3)**	46.1 ± 13.0 (3)	363.6 ± 19.9 (3)	

increased as the incubation continued. On addition of acid, to decrease the pH to approx. 7.0, there was a rapid decrease in 2-oxoglutarate concentration, reaching 32% of the control value within 1 min. The effect of acid was readily reversed; an equally rapid increase in 2-oxoglutarate concentration was observed once the acid had been neutralized by the addition of NaHCO₃ (Fig. 2).

Effect of pH on rates of metabolism in isolated kidney tubules

An increase in the rate of glucose production of about 50% was observed in both the presence and

absence of glutamine when the pH was lowered to 7.0. At pH 7.7, gluconeogenesis from glutamine was significantly inhibited (Table 3). This is in accordance with the inhibition of gluconeogenesis by elevated bicarbonate concentrations (Alleyne *et al.*, 1973; Baverel & Lund, 1979). In keeping with the increase in renal ammonia production in perfused kidney (Tannen & Ross, 1979), ammonia formation was increased by 33% (Table 3) when tubules were incubated with glutamine at pH 7.0, compared with pH 7.4. This effect was absent when no substrate was available. No significant depression of ammonia production occurred when the pH was raised to 7.7.

An increase in glucose production was apparent when the pH was lowered by 0.1 unit, but statistically significant increase in ammonia production was seen only when the pH fell below 7.1 or 7.2 (results not shown).

Effect of some inhibitors of oxoglutarate metabolism on the response to pH

The fall in concentration indicates possible inhibition of production or acceleration of the removal of 2-oxoglutarate. In experiments to test the involvement of some of these reactions in the response to acidosis, amino-oxyacetate, an inhibitor of transaminase, and mercaptopicolinate, an inhibitor of phosphoenolpyruvate carboxykinase and hence of gluconeogenesis, were added to tubules and the incubation pH was altered. The response of 2-oxoglutarate was a significant decrease in both instances when pH was lowered to 7.0 (Table 4). The effectiveness of the relevant inhibitors was confirmed by the decreased accumulation of aspartate in the presence of amino-oxyacetate, and total inhibition of gluconeogenesis by mercaptopicolinate.

Involvement of tricarboxylic acid-cycle activity in the response of tissue 2-oxoglutarate to pH was examined by adding a second substrate to tubules incubated in the presence of 1 mM-glutamine. Both succinate and malate produced a dramatic increase in 2-oxoglutarate concentration, which, however, was decreased to the expected extent when pH was lowered from 7.4 to 7.0. Other substrates (Table 5)

Fig. 2. Time course of the effect of acid on tubule 2-oxoglutarate concentration (representative experiment) Tubules were incubated in 250 ml conical flasks containing 25 ml of medium at pH 7.4, continuously gassed with O₂/CO₂ (19:1); 1 mM-glutamine was provided as substrate. After 15 min of incubation, HCl (0.37 ml of 1 M; ○) was added to the experimental flask, followed 15 min later by the addition of sufficient NaHCO₃ (0.25 ml of 1 M; ■) to restore the incubation to pH 7.4. The control flask remained at pH 7.4 throughout the incubation (●). Samples were withdrawn from the flasks at the times indicated and immediately added to HClO₄; 2-oxoglutarate was assayed as described in the Methods section. Similar results were found in four separate experiments.

Table 3. Effect of pH on gluconeogenesis and ammoniogenesis in isolated kidney tubules

Details of the incubation procedure are given in the Methods section. The rates are expressed as μmol/h per g dry wt. and are shown as means ± S.E.M. with the numbers of separate preparations shown in parentheses. Statistical significance with respect to pH 7.4 is shown by *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

pH ...	With no substrate		With 1 mM-glutamine			Net production from added substrate	
	7.0	7.4	7.0	7.4	7.7	7.0	7.4
Gluconeogenesis	41.0 ± 3.8 (11)*	29.3 ± 1.8 (11)	125.7 ± 4.7 (18)***	81.0 ± 2.7 (18)	35.5 ± 9.2 (3)***	84.4 ± 7.3 (11)**	50.5 ± 3.1 (11)
Ammoniogenesis	183.5 ± 19.1 (11)	183.3 ± 19.3 (11)	702.4 ± 31.4 (18)***	574.1 ± 21.8 (18)	575.4 ± 42.9 (3)	495.1 ± 31.7 (11)**	371.9 ± 22.2 (11)

Table 4. *Effect of inhibitors on the response of 2-oxoglutarate to pH*

Tubules were incubated as described in the Methods section with 1 mM-glutamine and the inhibitors at the concentrations indicated. Results are expressed as $\mu\text{mol/g}$ dry wt., showing the means $\pm$ S.E.M. for the numbers of separate flasks indicated in parentheses. Statistical significance is indicated by $***P \leq 0.001$. In the presence of 3-mercaptopycolinate, also at pH 7.4, glucose synthesis from glutamine was -7.3 ± 5.8 (4) $\mu\text{mol/h}$ per g dry wt. (cf. Table 3).

Inhibitor	Concn. (mM)	2-Oxoglutarate	
		pH 7.4	pH 7.0
None		6.60 ± 0.50 (6)	1.21 ± 0.23 (8)***
3-Mercaptopycolinate	0.6	6.75 ± 0.41 (4)	1.39 ± 0.31 (4)***
Amino-oxyacetate	1	9.38 ± 3.40 (4)	0.84 ± 0.17 (4)

Table 5. *Effect of additional substrates on the response of 2-oxoglutarate to pH*

Tubules were incubated as described in the Methods section, with 1 mM-glutamine and additional substrates at the concentration indicated. Results are expressed as $\mu\text{mol/g}$ dry wt., as the means $\pm$ S.E.M. for the numbers of separate flasks indicated in parentheses. Statistical significance is indicated by $**P \leq 0.01$, $***P \leq 0.001$.

Additional substrate	Concn. (mM)	2-Oxoglutarate	
		pH 7.4	pH 7.0
None		6.31 ± 0.34 (10)	0.82 ± 0.12 (14)***
Succinate	5	118.9 ± 3.17 (4)	15.19 ± 2.01 (6)***
Malate	5	125.7 ± 7.19 (3)	7.23 ± 0.58 (6)**
Citrate	2	35.9 ± 0.19 (2)	5.96 ± 2.01 (4)**
Lactate	2	27.6 ± 10.5 (4)	2.94 ± 0.97 (4)**
Oleate	1	23.5 ± 12.1 (4)	2.23 ± 0.93 (4)**

produced smaller but still significant elevations of oxoglutarate concentration at pH 7.4 and a corresponding fall in concentration at pH 7.0.

Effect of modifying pH at constant $[\text{HCO}_3^-]$

In view of the different effects of HCO_3^- on gluconeogenesis (Alleyne *et al.*, 1973; Baverel & Lund, 1979) and ammoniogenesis, the effect of pH changes brought about by changing $p\text{CO}_2$ at constant $[\text{HCO}_3^-]$ was examined in isolated kidney tubules. A consistent fall in [2-oxoglutarate] [6.64 ± 0.60 to 2.04 ± 0.07 ($n=4$) $\mu\text{mol/g}$ dry wt. ($P < 0.05$)] accompanied a fall in pH from 7.4 to 7.0, when 25% CO_2 was substituted for 5% CO_2 in the gassing mixture. Ammoniogenesis increased [515 ± 68 to 784 ± 145 $\mu\text{mol/h}$ per g dry wt. ($n=4$; $P < 0.05$)], but the rate of gluconeogenesis did not. In perfused kidney a similar decrease in 2-oxoglutarate concentration and increase in ammonia production have been obtained when pH was lowered by changing $p\text{CO}_2$ of the gassing mixture (results not shown).

Effect of pH on activity of 2-oxoglutarate dehydrogenase in kidney mitochondrial extracts

The effect of pH on the rate of disposal of 2-oxoglutarate was examined by determining the V_{max} and K_m of the enzyme in extracts of mito-

chondria prepared from kidneys from normal and acidotic rats.

The rates of oxoglutarate dehydrogenase activity were linear with time and with protein concentration, and they fell to less than 2% of the maximum rate when substrate was omitted. The total activity and the response to variations in H^+ concentration for oxoglutarate dehydrogenase from kidneys of normal fed or acidotic rats were identical, and values have therefore been pooled in Table 6.

A marked increase in enzyme activity was demonstrable at low pH. In seven consecutive titrations there was a significant fall in K_m of the enzyme for 2-oxoglutarate as pH was lowered in 0.3 unit steps, between 7.7 and 6.8; the K_m was 0.67 ± 0.05 mM at pH 7.7 and 0.096 ± 0.017 mM at pH 6.8. Changes were highly significant; $P \leq 0.01$ for the differences for each 0.3 pH-unit change ($n=7$). Thus, at the lowest pH tested, which may be close to that seen *in vivo* in acute acidosis, the K_m of oxoglutarate dehydrogenase was only 20% of that at pH 7.7, equivalent to the kidney pH in alkalosis. Maximum velocity (V_{max}) calculated from the same data was 10.61 ± 0.61 $\mu\text{mol/min}$ per g dry wt. (mean of 16 preparations) and was unchanged by variations in H^+ (pH 6.8 to 7.7), Ca^{2+} (0 to 30 μM), or by change in the acid-base status of the animals.

A further effect on K_m of renal oxoglutarate

Table 6. Effect of pH and Ca^{2+} on the K_m of 2-oxoglutarate dehydrogenase for 2-oxoglutarate in the rat kidney

Activity of the enzyme in mitochondrial extracts prepared from rat renal cortex was assayed at pH values indicated in the presence of 5 mM-EGTA (free $\text{Ca}^{2+} < 1 \text{ nM}$) or with added Ca^{2+} . The K_m , calculated as described in the Methods section, is given as means $\pm$ S.E.M. for the numbers of preparations in parentheses. Tissue concentrations of 2-oxoglutarate are taken from Ross & Tannen (1979) for the isolated perfused rat kidney, and flux (v) for the given substrate concentration (s) was calculated from the relationship $v = V_{\text{max}} \cdot s / (K_m + s)$. A wet-wt./dry-wt. ratio of 3.94 for kidney was applied. Statistical significance between adjacent columns is shown by $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, and of effect of Ca^{2+} compared with that of (free $\text{Ca} < 1 \text{ nM}$) is shown by $^aP \leq 0.05$, $^bP \leq 0.01$, $^cP \leq 0.001$.

	$[\text{Ca}^{2+}]$	pH 6.8	pH 7.1	pH 7.4	pH 7.7
Tissue 2-oxoglutarate (mM)		0.11 ± 0.01 (6)		0.49 ± 0.03 (8) ^{***}	
K_m of oxoglutarate dehydrogenase (mM)	$< 1 \text{ nM}$	0.36 ± 0.06 (4)	1.71 ± 0.24 (6) ^{**}	4.26 ± 0.35 (3) ^{***}	11.11 ± 2.64 (3)
	Endogenous	0.096 ± 0.017 (10) ^b	0.23 ± 0.04 (12) ^{**b}	0.39 ± 0.07 (10) ^{ab}	0.67 ± 0.11 (9) [*]
	$\sim 30 \mu\text{M}$	0.072 ± 0.015 (4) ^b	0.23 ± 0.04 (6) ^{ab}	0.50 ± 0.13 (3) ^{bc}	1.67 ± 1.15 (3) ^a
Flux through oxo-glutarate dehydrogenase ($\mu\text{mol/min}$ per dry wt.)	$< 1 \text{ nM}$	2.14 ± 0.32 (4)		0.94 ± 0.03 (3) [*]	
	Endogenous	4.65 ± 0.57 (8)		5.15 ± 0.54 (7)	
	$\sim 30 \mu\text{M}$	4.77 ± 1.38 (3)		4.56 ± 0.20 (3)

Fig. 3. Effect of Ca^{2+} on the activity of 2-oxoglutarate dehydrogenase from rat kidney cortex

Results from a single mitochondrial preparation are presented; the same result was obtained in several separate experiments. 2-Oxoglutarate dehydrogenase activity in mitochondrial extracts from kidney cortex of a fed rat was assayed and Ca^{2+} concentrations were varied by the addition of appropriate amounts of EGTA and EGTA- Ca^{2+} (see the Methods section). The initial concentration of 2-oxoglutarate in the incubation mixture was varied, to obtain each of the curves shown: ($\blacktriangle$) 0.12 mM-, ($\square$) 0.23 mM-, ($\blacksquare$) 0.46 mM-, ($\circ$) 0.9 mM- and ($\bullet$) 21 mM-2-oxoglutarate were used and the reaction was started by addition of the enzyme. Results are presented in terms of dry weight of kidney.

dehydrogenase was found when the Ca^{2+} concentration of the incubation mixture was varied. As with the enzyme from heart (McCormack & Denton, 1979), the effects of pH on K_m were more profound when Ca^{2+} was entirely removed by addition of EGTA (Table 6). Under these conditions, the change in K_m observed between pH 7.4 and 6.8 represents a 12-fold activation of the enzyme. At every pH the K_m for oxoglutarate was significantly higher in the absence of Ca^{2+} than in its presence (Table 6). The effect of various Ca^{2+} concentrations on the enzyme from kidney at a variety of 2-oxoglutarate concentrations and at constant pH (7.1) are shown in Fig. 3. This shows that the K_m of the enzyme is sensitive to variations in calcium content at the 2-oxoglutarate concentrations seen in both normal and acidotic kidneys, *in vivo* and *in vitro*.

Discussion

Mechanism of fall in tissue 2-oxoglutarate concentration

The pH-dependent fall in 2-oxoglutarate concentration in kidney tubules and in perfused kidney appears for a number of reasons to be a pH indicator, as follows.

(1) The rates of change are rapid, occurring within 1 min in cortical tubules, and 1.5–6.5 min in intact kidney.

(2) No difference in effect is seen when pH is lowered by decreasing $[\text{HCO}_3^-]$ or increasing CO_2 .

(3) The changes are completely reversible by simply adding HCO_3^- , even after prolonged incubation with glutamine.

(4) Addition of substrates expected to generate 2-oxoglutarate or inhibit oxoglutarate dehydrogenase results in a significant increase in 2-oxoglutarate concentration, which is then promptly reversed by lowering pH.

(5) Inhibition of two of the principal pathways whereby 2-oxoglutarate might be removed does not prevent the rapid fall in concentration of 2-oxoglutarate in response to a fall in pH.

One possible method whereby 2-oxoglutarate might reflect pH has been proposed (Krebs & Vinay, 1975; Ross & Tannen, 1979); maintenance of near equilibrium of the mitochondrial enzyme glutamate dehydrogenase might be the cause of a fall in 2-oxoglutarate concentration. For this to occur, the ratio $[NADH][H^+]/[NAD^+]$ would be expected to be increased under acidotic conditions. The pH of renal cells has been determined by using ^{31}P n.m.r. (Radda *et al.*, 1980). Intracellular pH fell by 0.3 unit on acidification of the medium perfusing the kidney from pH 7.4 to 7.0. Glutamate dehydrogenase is located in the mitochondria. No information is yet available about the changes in intramitochondrial pH, but it may be close to the fall of 0.3 pH unit indicated for the whole kidney.

Tissue distribution of 2-oxoglutarate

Guder & Purschell (1980) propose that the distribution of 2-oxoglutarate between cytosol and mitochondrion may be different at acid pH, and caution against conclusions drawn from whole-tissue measurements. In experiments with cortical tubules, as much as 80% of 2-oxoglutarate is extracellular (Guder & Purschell, 1980). Nevertheless, rapid changes occur on acidification, and must at least be initiated within the cell. Thus the larger pool of extracellular fluid is a reflection of events at the site of the enzyme, and we may assume that the direction, if not the magnitude, of the changes in 2-oxoglutarate content is the same in the mitochondrion, cytosol and extracellular fluid.

Effect of the pH-dependent activation of 2-oxoglutarate dehydrogenase

New light is thrown on the fall in tissue oxoglutarate concentration in response to acute pH changes by the present finding that in kidney the K_m of 2-oxoglutarate dehydrogenase is sensitive to pH.

Although the difference in true pH of kidney mitochondria under normal and acute-acidotic conditions is not accurately known, a fall of either 0.3 pH unit (obtained in n.m.r. measurements with the whole perfused rat kidney; Radda *et al.*, 1980) or of 0.6–0.7 pH unit (calculated from equilibrium of glutamate dehydrogenase; Ross & Tannen, 1979) has been estimated. In the first case, an activation of oxoglutarate dehydrogenase of 4-fold would be

expected, and in the second case the activation would be nearer to 12-fold (Table 6).

The physiological relevance to ammonia production of this marked activation of oxoglutarate dehydrogenase by H^+ depends, in addition, on the concentration of its substrate. As mentioned in the introduction, the whole-tissue concentration of 2-oxoglutarate falls in acute acidosis. In perfused kidney, 2-oxoglutarate concentration fell from 0.49 to 0.11 mM (Ross & Tannen, 1979), when the pH of the surrounding medium was lowered by 0.4–0.5 unit. Assuming a more-or-less even distribution of 2-oxoglutarate between all compartments of the cell, an assumption that is supported by the preliminary observations of Guder & Purschell (1980), flux through oxoglutarate dehydrogenase under normal and acidotic conditions in the kidney can be calculated from the expression

$$v = V_{\max} s / (K_m + s).$$

The flux through the enzyme calculated at pH 7.4 is $0.9 \mu\text{mol/min per g dry wt.}$ This increased to $2.1 \mu\text{mol/min per g}$ when the pH fell to 6.8.

These fluxes are both within the range expected from calculations based on measured rates of ammonia production in the intact kidney ($1.3 \mu\text{mol/min per g dry wt.}$ at pH 7.4, and $1.7 \mu\text{mol/min per g}$ at pH 6.9; Tannen & Ross, 1979). Since two ammonia molecules are released for each molecule of 2-oxoglutarate oxidized, flux through oxoglutarate dehydrogenase would be increased from 0.6 to $0.85 \mu\text{mol/min per g dry wt.}$ in these studies. The changes in flux are thus in the range expected from the pH-sensitive change in the activity of 2-oxoglutarate dehydrogenase, and the measured change in overall substrate concentration.

However, only when Ca^{2+} is omitted from the reaction (K_m is determined in the presence of EGTA) does the change in pH alone account for the observed increase in flux. With Ca^{2+} in the reaction mixture between $0.5 \mu\text{M}$ and approx. $30 \mu\text{M}$, the pH-induced activation of the enzyme appears to be rather precisely balanced by the accompanying fall in the tissue concentration of its substrate. At pH 7.4, flux through oxoglutarate dehydrogenase in the presence of Ca^{2+} is calculated to be $4.6\text{--}5.2 \mu\text{mol/min per g dry wt.}$ of kidney, whereas at pH 6.8, when the enzyme was activated 4–7-fold, the calculated flux is $4.7\text{--}4.8 \mu\text{mol/min per g dry wt.}$ Thus, in the presence of Ca^{2+} , calculated flux is in excess of the observed rate of ammonia production in perfused rat kidney (see also Ross & Bullock, 1978, for maximum rates), and the activation of the enzyme by the fall in pH, together with the accompanying fall in 2-oxoglutarate concentration, is sufficient to maintain flux in the pathways of gluconeogenesis and ammoniogenesis, but not to increase it further.

Significance for the regulation of ammoniogenesis

In vivo (Pitts, 1964; Emmett *et al.*, 1976), in the perfused kidney (Tannen & Ross, 1979), and now in isolated cortical tubules, acidosis results in increased ammoniogenesis from glutamine. This is evidence of increased flux through glutamate dehydrogenase, and therefore increased production of 2-oxoglutarate. This, together with increased gluconeogenesis, implies an increased flux through 2-oxoglutarate dehydrogenase. However, this is accomplished at a much lower concentration of substrate, 2-oxoglutarate. It is therefore difficult to escape the conclusion that 2-oxoglutarate dehydrogenase is activated *in vivo* and in intact kidney preparations.

Whether 2-oxoglutarate dehydrogenase activation is obligatory for enhanced ammoniogenesis remains to be established, but this event precedes, by several hours, the induction of phosphoenolpyruvate carboxykinase and glutaminase, which have hitherto been seen as regulatory in adaptation to acidosis. The present results exclude activation of phosphoenolpyruvate carboxykinase as the cause of decreased tissue oxoglutarate concentration, and support the idea of an earlier event in the initiation of accelerated renal ammoniogenesis. It is tempting to see this event as one closely related to a change in intramitochondrial $[H^+]$. Other regulators of intramitochondrial 2-oxoglutarate dehydrogenase activity appear to be over-ruled by the effects of pH. Thus $[ATP]/[ADP]$ ratios do not change; $[NAD^+]/[NADH]$ ratios may change, but as a function of pH, and even in the presence of high concentrations of succinate the effect of $[H^+]$ on oxoglutarate concentration persists. However, the intramitochondrial concentrations of these other effectors and of Ca^{2+} remain to be determined.

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