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Research Article

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Stimulation of Ammonia Production and Excretion in the Rabbit by Inorganic Phosphate

STUDY OF CONTROL MECHANISMS

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ABSTRACT The purpose of this study was to clarify the mechanism(s) responsible for regulation of ammonia production and excretion in the rabbit. The normally low ammonia excretion rate during acute metabolic acidosis was stimulated acutely and increased approximately ninefold after infusion of sodium phosphate, but remained low if sodium sulphate or Tris was substituted for phosphate. Ammonia production was increased significantly by phosphate in rabbit renal cortex slices and in isolated renal cortex mitochondria.

In isolated mitochondria, mersalyl, an inhibitor of both the phosphate/hydroxyl and phosphate/dicarboxylate mitochondrial carriers, inhibited the phosphate-induced stimulation, indicating that phosphate must enter the mitochondrion for stimulation. A malate/phosphate exchange seemed to be involved since *N*-ethylmaleimide, an inhibitor of the phosphate/hydroxyl exchange, did not inhibit phosphate-stimulated ammonia production, whereas there was inhibition by 2-*n*-butylmalonate, a competitive inhibitor of the dicarboxylate carrier. Phosphate itself was not essential since malonate stimulated ammoniagenesis in the absence of added phosphate. Similarly, citrate stimulated ammoniagenesis in isolated mitochondria in the absence of inorganic phosphate provided that it induced L-malate exit on the citrate transporter associated with inhibition of citrate oxidation by fluoroacetate. Similar results were also seen in mitochondria from rat renal cortex.

A fall in mitochondrial α-ketoglutarate level resulted in an increase in ammonia production. This could be achieved directly with malonate or indirectly via L-malate exit. Simultaneous measurements of glutamate showed that the rate of ammonia production was reciprocally related to the glutamate content. We conclude that phosphate-induced stimulation of ammoniagenesis in the rabbit kidney is mediated by removal of glutamate, the feedback inhibitor of phosphate-dependent glutaminase. Glutamate removal is linked to phosphate-induced dicarboxylate exit across the mitochondrial membrane.

INTRODUCTION

The regulatory mechanisms involved in increased renal glutamine extraction, deamidation, and deamination during metabolic acidosis play a major role in acid-base homeostasis by permitting increased urine excretion of hydrogen ions in the form of ammonium. Urine ammonia excretion increases during metabolic acidosis in man, rat, and dog. However, despite many attempts to gain an understanding of this phenomenon, confusion still exists as to the role various mechanisms play (1–3).

Previous studies have shown that ammonia excretion varies markedly from species to species, carnivores having a high rate of excretion (1–3) and herbivores a low rate of excretion (4). Several factors may be involved. Rats and dogs tend to have a neutral or acidic urine while rabbits, which are low ammonia excretors, have an alkaline urine. Renal phosphate-dependent glutaminase activity is high in the rat (5) and dog (6), whereas this activity is low in the rabbit (7). Metabolic acidosis

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results in only a small quantitative increase in urine ammonia excretion and phosphate-dependent glutaminase activity in rabbit kidney as compared to the rat (7).

We observed that phosphate infusion increased urine ammonia excretion 8- to 10-fold in the rabbit with acute metabolic acidosis. It was of interest, therefore, to probe the biochemical mechanism(s) which might be responsible for the acute control of ammonia excretion in the rabbit under these conditions. The mechanism appears to involve removal of feedback inhibition of glutamine deamidation as a result of phosphate-induced dicarboxylate countertransport across the mitochondrial membrane.

METHODS

Citrate, *N*-ethylmaleimide, oligomycin, and mersalyl were obtained from the Sigma Chemical Co. (St. Louis, Mo.); malonic acid from Matheson Coleman & Bell (East Rutherford, N. J.); fluoroacetic acid from Calbiochem (San Diego, Calif.); glutamic dehydrogenase, succinic, and α-ketoglutaric acids from Boehringer Mannheim Corp. (New York); 2-*n*-butylmalonic acid from Aldrich Chemical Co., Inc. (Milwaukee, Wis.), and 1,2,3-benzenetricarboxylate and *p*-iodobenzylmalonate from K and K Laboratories, Inc. (Plainview, N. Y.). Carbonyl-cyanide-*p*-trifluoromethoxyphenyl hydrazine (FCCP)[1] was the kind gift of Professor G. R. Williams, Department of Biochemistry, University of Toronto. Versilube F-50 silicone fluid was from the General Electric Co., Silicone Products Department, Waterford, N. Y.

Acute metabolic acidosis studies. Male rabbits (2.5–3 kg; Reiman's Fur Ranch, Agatha, Ontario) were premedicated for 30–90 min with intramuscular chloropromazine (2.5%, 1 ml/kg) before anesthesia with intravenous sodium pentobarbital (6%, 0.25 ml/kg). Xylocaine (1%; Astra Pharmaceutical Products, Inc., Worcester, Mass.) was injected subcutaneously at incision sites. After induction of anesthesia, isotonic saline was infused into the jugular vein at a rate of 160 μmol/min for 2 h. Hydrochloric acid (0.1 N) was then infused at a rate of 160 μmol/min until the blood bicarbonate approached 10 meq/liter. Urines were collected anaerobically from intra-ureteral catheters for measurement of ammonia after steady-state metabolic acidosis had been achieved and the urine pH was less than 6.0. Isotonic sodium phosphate (pH 7.4) was then infused at a rate of 130 μmol/min, and urine was collected at intervals for ammonia determinations. In separate experiments, after induction of acute metabolic acidosis, isosmotic sodium sulphate or Tris-HCl (neutralized to pH 7.4 with sodium hydroxide) was infused instead of sodium phosphate, and urine was again collected at intervals for ammonia determinations.

Kidney slice technique. Rabbits were killed by cervical dislocation, and the kidneys rapidly removed, decapsulated, and placed in ice-cold isotonic saline. Each kidney was quartered, and the cortex separated from medulla by scissor dissection through the corticomedullary junction. Cortical slices (approximately 0.5 mm thickness) were prepared by a microtome, weighed, and 100-mg portions transferred to Erlenmeyer flasks containing 2 ml bicarbonate-buffered incubation medium which had been flushed for 3 min with $O_2 + CO_2$ (95 : 5) to a final pH of 7.4 (8). The concentrations of all additions are given in the legends or text. Slices were incubated at 37°C for the times indicated. After incucation, the medium was deproteinized with an equal volume of 10% perchloric acid, and the precipitate removed by centrifugation.

Preparation of rabbit cortex mitochondria. Renal cortex mitochondria were isolated by differential centrifugation in a medium containing 250 mM sucrose, 50 mM Tris chloride, and 1.0 mM ethylene glycol-bis(β-aminoethyl ether)*N,N,N',N'*-tetraacetic acid (EGTA), pH 7.4 as previously described (9). The mitochondria were then suspended to give a final protein concentration of approximately 30 mg/ml.

Analytical methods. The blood and urine samples for pH and P_{CO_2} were collected anaerobically into heparinized capillary tubes and analyzed immediately on a Radiometer model PHM72 digital acid-base analyzer (Radiometer Co., Copenhagen). Urine ammonia was measured by formal titration (10) or the ammonia electrode (11) (Orion ammonia electrode, model 95-20, Orion Research Inc., Cambridge, Mass.). Ammonia was measured in neutralized perchloric acid extracts using the ammonia electrode. Urine phosphorus was measured by a modified method for the photoelectric colorimeter (12). Protein was determined by the method of Lowry et al. (13). Glutamate and citrate were assayed enzymatically (14). Oxygen consumption was measured polarographically with a Clark oxygen electrode (Yellow Springs, Ohio) as previously described (15). Mitochondria were separated from the incubation medium by rapid centrifugation through 3–5 mm thickness of silicone fluid (versilube F-50) (16).

RESULTS

Effect of phosphate, sulphate, and tris on ammonia excretion in rabbits. Rabbits were infused with hydrochloric acid to produce acute metabolic acidosis. Hydrochloric acid infusion was stopped and replaced by isotonic saline for 30±5 min, after which the blood and urine were analyzed as indicated in Table I. Isotonic sodium phosphate (pH 7.4) was then infused in experiment A, and the measurements repeated. The same procedures were repeated using isotonic sodium sulphate or Tris-chloride instead of the isotonic sodium phosphate. Urine ammonia excretion was low and increased approximately ninefold 80 min after infusion of phosphate to 3.52±0.94 μeq/min (experiment A), whereas ammonia excretion remained low when sodium sulphate (experiment B) or Tris (experiment C) was substituted for phosphate. There were five experiments in each group.

Effect of phosphate on ammonia production by the rabbit renal cortex slices. The production of ammonia increased in an almost linear fashion for at least 60 min (Fig. 1). After 30 min incubation, the rate of ammonia production was 87.7±5.0 nmol/min per g wet wt when glutamine (2 mM) was the substrate and increased to 156±2.7 nmol/min per g wet wt ($n = 6$, $P < 0.01$) when phosphate (10 mM) was also present. In the absence of glutamine and phosphate, the ammonia production rate was 17.0±0.7 nmol/min per g wet wt and did not increase in the presence of phosphate.

[1] *Abbreviation used in this paper:* FCCP; carbonyl-cyanide-*p*-trifluoromethoxyphenyl hydrazine.

TABLE I

Effect of Phosphate, Sulphate, and Tris on Ammonia Excretion in Rabbits with Acute Metabolic Acidosis

Infusion	Blood		Urine		
	pH	HCO_3^-	pH	Ammonia excretion	Flow rate
		meq/liter		*μeq/min*	*ml/min*
Exp. A					
NaCl	7.48±0.03	17.8±1.6	7.90±0.13	—	—
+HCl	7.23±0.04	9.6±0.4	5.33±0.13	0.40±0.06	0.47±0.10
+P_i (pH 7.4)	7.25±0.02	11.7±1.8	5.98±0.04	3.52±0.94*	1.38±0.33
Exp. B					
NaCl	7.49±0.02	19.2±2.0	7.94±0.18	—	—
+HCl	7.20±0.03	11.4±1.3	5.12±0.21	0.26±0.06	0.50±0.11
+Na_2SO_4	7.23±0.04	11.8±1.2	5.92±0.14	0.26±0.02	1.57±0.40
Exp. C					
NaCl	7.49±0.03	20.2±1.2	7.92±0.16	—	—
+HCl	7.27±0.03	11.8±1.5	5.28±0.12	0.06±0.010	0.43±0.13
+Tris HCl (pH 7.4)	7.29±0.02	12.0±1.6	5.86±0.12	0.06±0.003	1.82±0.60

Rabbits were infused with hydrochloric acid (0.1 N) to produce acute metabolic acidosis (see Methods). Five animals were used in each group (A, B, and C). In experimental group A, the quantity of isotonic neutral (pH 7.4) P_i administered was 10 mmol over the 80-min time period. Isosmolar quantities of sodium sulphate and neutral Tris-HCl (pH 7.4) were given in groups B and C, respectively, instead of phosphate for the same duration. The results are presented as the mean±SEM for each period.
$P < 0.01$ for ammonia excretion after administration of agent.

Mitochondrial oxygen-uptake studies. Coupling of oxidation to phosphorylation is demonstrated in Fig. 2. When mitochondria were incubated in the presence of inorganic phosphate, magnesium, and ADP, the rate of oxygen consumption was low. With the addition of an oxidizable substrate, succinate, the rate of oxygen consumption increased markedly. The rate of oxygen consumption remained rapid until an inhibitor of oxidative

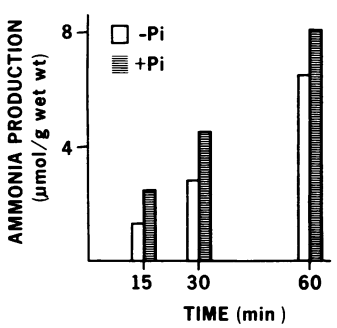


FIGURE 1 Effect of phosphate on ammonia production in rabbit renal cortex slices. For details, see kidney slice technique in Methods. The concentration of glutamine was 2.0 mM and phosphate 10 mM. Results are from a representative experiment. Data in the absence of phosphate is presented in the clear bars and data in the presence of phosphate in the shaded bars. The results of six separate experiments at the 30-min time intervals are summarized in the text.

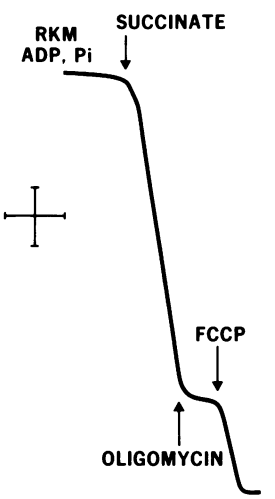


FIGURE 2 Polarographic measurements of oxygen consumption of rabbit kidney cortex mitochondria. Rabbit kidney cortex mitochondria (RKM) (1 mg protein) were suspended in 1.2 ml medium at pH 7.4 containing potassium chloride (125 mM), Tris chloride (20 mM), inorganic phosphate (2.5 mM), magnesium chloride (2 mM), and ADP (1.0 mM) at 25°C. The final concentration of additions were as follows: succinate 2.5 mM, oligomycin 10 μg/ml, and FCCP 1.0 μM. Time (1 min) is indicated on the horizontal axis and oxygen consumption (0.09 μg atoms of oxygen) on the vertical axis. The results are of a representative experiment.

TABLE II

Effect of Phosphate on Ammonia Production in Rabbit Renal Cortex Mitochondria

Additions	No. of experiments	Ammonia production
		nmol/min/mg protein
0	11	11.4±0.2
P_i	11	22.6±0.4*
P_i + mersalyl	4	14.4±1.0‡
P_i + *N*-ethylmaleimide	4	23.2±0.3
P_i + 2-*n*-butylmalonate	6	18.1±0.4‡

Rabbit renal cortex mitochondria (3 mg) were added to 4 ml incubation medium containing glutamine (2 mM), Tris-chloride (20 mM), potassium chloride (125 mM) at pH 7.4 and 37°C for 30 min. Incubations were terminated by the addition of 2 ml 10% perchloric acid. The concentrations of additions were as follows: P_i 10 mM, mersalyl 50 μM, *N*-ethylmaleimide 500 μM, and 2-*n*-butylmalonate 10 mM. Results are reported as mean±SEM.

* $P < 0.01$ for the effect of P_i.

‡ $P < 0.01$ as compared to P_i.

phosphorylation, oligomycin, was also added. This rate was again increased when the uncoupler, FCCP, was added.

Effect of metabolites and inhibitors on ammonia production in rabbit renal cortex mitochondria. The rate of ammonia production was linear over a 30-min period. This rate increased by 11.4±0.2 nmol/min per mg protein when 2 mM glutamine was substrate. Ammoniagenesis was further increased by approximately twofold in the presence of phosphate. This latter rate was inhibited by mersalyl, an inhibitor of phosphate transport (17) and to a lesser degree by an equimolar concentration of the competitive inhibitor of the dicarboxylate carrier (18), 2-butylmalonate, but not by the inhibitor of the phosphate/hydroxyl transporter, *N*-ethylmaleimide (16; Table II).

TABLE III

Effect of Malonate on Ammonia Production in Rabbit Renal Cortex Mitochondria

Additions	No. of experiments	Ammonia production
		nmol/min/mg protein
0	11	11.4±0.2
Malonate	7	23.1±0.4*
Malonate + P_i	6	22.6±0.8*
P_i	11	22.6±0.4*
Malonate +*p*-iodobenzylmalonate	4	16.9±0.3‡
p-Iodobenzylmalonate	4	11.2±0.5

For details of experiment, see Table II. The concentrations of P_i and malonate were 10 mM and *p*-iodobenzylmalonate was 5 mM.

* $P < 0.01$ for the effect of malonate or P_i.

‡ $P < 0.01$ as compared to malonate.

TABLE IV

Effect of Citrate on Ammonia Production in Rabbit Renal Cortex Mitochondria

Additions	No. of experiments	Ammonia production
		nmol/min/mg protein
0	11	11.4±0.2
Citrate	4	10.2±0.4
FAc	4	17.8±0.2*
Citrate + FAc	4	19.4±0.5* ‡
Citrate + FAc + B,1,2,3	4	15.3±0.3§
B,1,2,3	4	12.4±0.3

For details of experiment, see Table II. The concentration of citrate was 2 mM, fluoroacetate (FAc) was 5 mM, and 1,2,3-benzenetricarboxylate (B,1,2,3) was 10 mM.

* $P < 0.01$ as compared to control.

‡ $P < 0.01$ as compared to FAc.

§ $P < 0.01$ as compared to citrate + FAc.

Malonate also produced a twofold stimulation of ammoniagenesis from glutamine, however, the effects of both malonate and phosphate were not additive. If *p*-iodobenzylmalonate, an inhibitor of the α-ketoglutarate carrier (19), was added with malonate, the production of ammonia was decreased (Table III).

Citrate did not augment ammoniagenesis from glutamine. Fluoroacetate increased this rate, and a further stimulation was observed when both citrate and fluoroacetate were present. 1,2,3-Benzenetricarboxylate, an inhibitor of the mitochondrial citrate transporter (19), prevented this citrate-induced stimulation. 1,2,3-Benzenetricarboxylate did not alter the rate of ammoniagenesis in the absence of citrate (Table IV).

If glutamate (2 mM) replaced glutamine as substrate, the rate of ammonia production was 1.27±0.4 nmol/min per mg protein. This rate increased approximately twofold in the presence of 10 mM inorganic phosphate to 2.2±0.5 nmol/min per mg protein ($n = 4$, $P < 0.01$ on paired observations, each done in triplicate).

Correlation between ammonia production and glutamate levels. After a 30-min incubation the glutamate content was 138±6.8 nmol/mg mitochondrial protein. When phosphate, malonate, or citrate plus fluoroacetate was added to the incubation medium, the glutamate content decreased to 108±7.5, 103±6.8, and 110±9.0 nmol/mg mitochondrial protein, respectively (Table V). The intramitochondrial glutamate content was also decreased from 12.6±0.7 to 9.8±0.8, 8.8±0.7, and 10.0±0.7 nmol/mg, respectively. The ammonia production rate increased

TABLE V

Effect of Phosphate, Malonate, and Citrate Plus Fluoroacetate on the Glutamate Content of Rabbit Kidney Mitochondria

Additions	Glutamate		
	Total	Intramitochondrial	Δ‡
	nmol/mg protein		
0	138±6.8	12.6±0.7	—
P_i	108±7.5*	9.8±0.8	−2.8±0.2*
Malonate	103±6.8*	8.8±0.7	−3.8±0.3*
Citrate + FAc	110±9.0*	10.0±0.7	−2.6±0.6*

Rabbit renal cortex mitochondria were incubated as described in Table II. The concentration of additions were glutamine 2 mM and, where indicated, P_i or malonate 10 mM, citrate 2 mM, and fluoroacetate (FAc) 5 mM. Results are reported as mean±SEM for four separate experiments each assayed in triplicate.

* $P < 0.01$ for fall in glutamate as compared to the control.

‡ A minus sign indicates a fall in intramitochondrial glutamate.

from 11.4±0.2 to 22.6±0.4, 23.1±0.4, and 19.4±0.5 nmol/min per mg protein when phosphate (Table II), malonate (Table III), or citrate plus fluoroacetate (Table IV), respectively, were also present.

Effect of metabolites and inhibitors on ammonia production in rat renal cortex mitochondria. To demonstate that the acute control mechanisms described in Tables II–IV were not unique to the rabbit, similiar studies were performed in mitochondria derived from rat renal cortex. Again the rate of ammoniagenesis from glutamine was significantly increased by phosphate, malonate, or citrate plus fluoroacetate. These rates were specifically inhibited by the presence of inhibitors of the dicarboxylate, α-ketoglutarate, or citrate transporters, respectively (Table VI).

TABLE VI

Effect of Phosphate, Malonate, and Citrate on Ammonia Production in Rat Renal Cortex Mitochondria

Additions	Ammonia production
	nmol/min/mg protein
0	32.0±1.6
Phosphate	59.7±4.1*
Malonate	61.0±2.8*
Fluoroacetate	42.4±1.2*
Citrate + FAc	48.5±1.3* ‡
Citrate	30.2±1.8
Phosphate + *N*-ethylmaleimide	62.7±3.7
Phosphate + 2*n*-bulylmalonate	39.6±3.8§
Phosphate + mersalyl	32.8±2.2§
Malonate + *p*-iodobenzylmalonate	35.4±1.9§
Citrate + FAc + $B_{1,2,3}$	37.9±1.9§
N-ethylmaleimide	35.8±2.5
2*n*-butylmalonate	34.1±2.5
Mersalyl	32.2±2.7
p-iodobenzylmalonate	30.5±2.3
B,1,2,3	34.4±1.4

For details, see Tables II–IV.

* $P < 0.01$ as compared to control.

‡ $P < 0.01$ as compared to fluoroacetate.

§ $P < 0.01$ as compared to no inhibitor.

DISCUSSION

This study was designed to clarify acute mechanisms of control involved in phosphate-stimulated ammoniagenesis in the rabbit. A regulatory site can be identified by demonstrating a fall in substrate concentration(s) when the rate of flow through the pathway is increased. The duration of these experiments must be short enough to ensure that there is no new enzyme synthesis or degradation (20). We applied these criteria to the study of ammoniagenesis in the rabbit.

The biochemical basis for increased renal glutamine utilization and ammonia production in metabolic acidosis is not clear. Several hypotheses regarding this regulatory phenomenon have been proposed. Control of ammoniagenesis by glutaminase levels now seems unlikely (5, 21). Regulation by enhanced renal gluconeogenesis (22–25) with an adaptive increase in phosphoenolpyruvate carboxykinase (26) cannot solely account for this control. Another hypothesis which seems to have been excluded is control of ammoniagenesis by the mitochondrial NADH/NAD ratio (27, 28). Presently two theories of regulation have considerable merit. First, ammoniagenesis is controlled by an adaptive specific increase in the transport of glutamine into renal mitochondria in chronic acidosis (29, 30). This implies substrate control of the intramitochondrial phosphate-dependent glutaminase. The second theory is feedback inhibition of phosphate-dependent glutaminase by glutamate (31–34). Control by this mechanism implies regulation of the mitochondrial glutamate concentration. These hypotheses cannot be adequately tested in vivo as the concentrations of glutamine and glutamate in the renal cortex mitochondria have not been measured or even approximated with accuracy despite elegant whole cell measurements (35, 36). While the rabbit does not respond to metabolic acidosis with an adaptive increase in ammonia excretion (7), we observed large increases in ammoniagenesis after phosphate administration. Therefore, this model was used to identify the regulatory sites and to study the control mechanisms involved in this pathway.

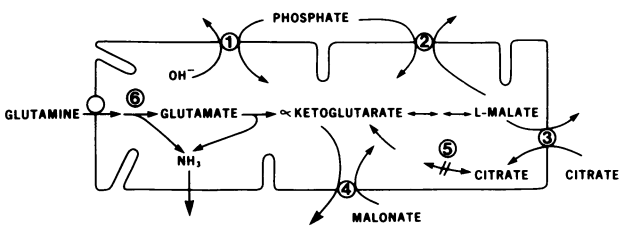
Specific transport systems are involved in translocation of metabolites across the inner membrane of mitochondria (37). Phosphate-dependent glutaminase, the primary enzyme involved in renal ammoniagenesis, is located within the inner mitochondrial membrane (38,

39). Therefore, control of ammoniagenesis is possible by regulation of mitochondrial transporters. Our experimental data favor the hypothesis that the stimulation of ammonia production in the rabbit resulted from removal of glutamate from the mitochondria, thereby augmenting glutamine deamidation (31–34). This decrease in feedback inhibition was stimulated by phosphate and other metabolites which resulted in exit of citric acid cycle intermediates from the mitochondria, thereby decreasing glutamate.

Stimulation of ammonia production by phosphate in the rabbit. Infusion of phosphate increased ammonia excretion but neither sulphate, another nonreabsorbable anion, nor Tris, a buffer with a pK in the range of phosphate, had a similar effect (Table I). Therefore, the effects of phosphate appeared to be specific. Since phosphate increased the rate of ammoniagenesis in renal cortex slices as well (Fig. 1), this tended to localize the effect to the kidney. Phosphate also increased the rate of ammonia production from glutamine in isolated mitochondria (Table II), indicating a mitochondrial locus of action. These mitochondria had coupled respiration but, in addition, contamination with an ATPase. Coupling was indicated by the fact that oligomycin, an inhibitor of mitochondrial ATP synthesis, inhibited respiration, and this was reversed by the subsequent addition of the uncoupler FCCP.

To determine the mitochondrial site of regulation of ammoniagenesis, inhibitors and alternate substrates for the mitochondrial anion transporters were employed. The rationale for these studies is seen in Scheme 1. Inorganic phosphate must enter the mitochondrion to stimulate ammoniagenesis, as shown by the finding that mersalyl, which blocks phosphate entry on both phosphate transporters (17), inhibited the phosphate-induced stimulation. It appears that phosphate must enter on the dicarboxylate carrier (in exchange for L-malate) because of the following: (*a*) the competitive inhibitor for this step, 2-*n*-butylmalonate (18) diminished the rate; (*b*) *N*-ethylmaleimide, an inhibitor of the phosphate-hydroxyl exchange (17), was without effect (Table II). Therefore, there are two possible mechanisms involved: phosphate entry and activation of phosphate-dependent glutaminase (4, 7, 32, 33, 39, 40) or, alternatively, the exit of L-malate. Two experimental protocols were employed to allow differentiation of these possibilities. The exit of α-ketoglutarate was promoted by adding malonate, a countertransporter for the α-ketoglutarate carrier (37). Ammoniagenesis was stimulated by malonate to the same maximum rate despite the absence of added phosphate. Augmented ammoniagenesis has been previously observed by others using malonate or phosphate but the results were interpreted differently (41, 42). Moreover, *p*-iodobenzylmalonate, an inhibitor of the α-ketoglutarate carrier (19), diminished the malonate-induced increase in ammoniagenesis. These data support our interpretation of the mode of action of malonate. Another experimental model was also tested because the malonate effects might not be specific. L-Malate exit was stimulated on the tricarboxylate carrier by adding citrate. However, the added citrate could be converted to α-ketoglutarate and L-malate via metabolism in the Krebs cycle. When this interconversion was inhibited by fluoroacetate (via conversion to fluorocitrate and inhibition of aconitase (43), citrate augmented ammoniagenesis. This was prevented by the simultaneous addition of 1, 2, 3-benzenetricarboxylate, a specific inhibitor of citrate-induced malate exit (19). It should also be noted that fluoroacetate itself stimulated ammoniagenesis somewhat, presumably as a result of a decrease in mitochondrial α-ketoglutarate and L-malate levels, together with a concomitant increase in citrate concentrations. To demonstrate that these acute control mechanisms are not unique to the rabbit, identical studies were performed using mitochondria derived from the renal cortex of the rat. The results were qualitatively similar (Table VI).

All the above results can be interpreted as follows: removal of α-ketoglutarate directly with malonate or indirectly via L-malate exit resulted in augmented ammoniagenesis. When glutamate contents were measured in simultaneous experiments, there was a reciprocal relationship between the rate of ammonia production and the glutamate content (Table V). This hypothesis is further strengthened by the fact that phosphate also stimulated ammonia production when glutamate rather than glutamine was the initial substrate. Therefore, control of ammoniagenesis in the rabbit kidney appears to be mediated by a feedback inhibition of glutaminase by glutamate. Renal cortex phosphate levels and blood phosphate levels were normal in rabbits (unpublished ob-



SCHEME 1 Sites of interaction in the regulation of ammoniagenesis. For description, see text. (1) Phosphate-hydroxyl countertransporter—inhibited by mersalyl and *N*-ethylmaleimide. (2) Dicarboxylate carrier—inhibited by mersalyl, 2-*n*-butylmalonate and *p*-iodobenzylmalonate. (3) Citrate transporter—inhibited by 1,2,3-benzenetricarboxylate and *p*-iodobenzylmalonate (4) α-Ketoglutarate transporter—inhibited by *p*-iodobenzylmalonate. (5) Aconitase—inhibited by fluorocitrate. (6) Phosphate-dependent glutaminase—inhibited by glutamate.

servations). The properties of the rabbit renal mitochondrial dicarboxylate carrier are under investigation.

In summary, ammonia excretion was stimulated ninefold by phosphate infusion during acute metabolic acidosis in the rabbit. This effect of phosphate could not be reproduced by a nonresorbable anion (sulphate) nor another buffer with a similar pK (Tris). This stimulation was also seen in cortex slices or intact mitochondria. The mechanism involves removal of α-ketoglutarate from the mitochondrion with a resultant decrease in glutamate levels. We offer the hypothesis that the decrease in glutamate concentration would result in relief of inhibition of phosphate-dependent glutaminase and account for the stimulation of ammonia production in rabbit kidney.

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