Renal Ammonia Production in the Presence of Citric Acid Cycle Blockade[1] (35354)

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Following acute or chronic acid challenge, kidneys produce more ammonia from glutamine (1). Increased production of this urinary buffer is the kidneys' chief means for excretion of the excess acid (2). Since the mechanisms involved in increasing ammoniagenesis following acidosis are presently unknown, it is important to examine biochemical means for stimulating renal ammoniagenesis.

Previous investigators have related ammoniagenesis to citric acid cycle activity in many ways (3–5). Recently, we reported that inhibitors of citric acid cycle activity could increase ammonia formation from glutamate by isolated dog tubules (6). To gain further knowledge concerning this phenomenon, a series of *in vitro* studies with rat kidney slices and *in vivo* studies with rats were performed. These studies show that interruption of the citric acid cycle with either fluorocitrate or malonate stimulates ammoniagenesis *in vitro* and that injection of these same inhibitors into rats results in increased ammonia excretion *in vivo*. The possible relevance of these findings to augmented ammoniagenesis following acid challenge is discussed.

Materials and Methods. Albino rats (200–250 g; Zivic-Miller, Allison Park, Pa.) were fed water and rat chow *ad libitum*. 24 hr prior to study, water was replaced with 1.5% $NaHCO_3$ to insure that no rat was acidotic at the time of study.

For *in vitro* studies, rats were sacrificed and the kidneys were rapidly placed in iced saline. Within 15 min, slices were cut on a Stadie-Riggs microtome. Only the first slice from the outer surface of the kidney was used. Each slice was halved, half was used as control, the other half was incubated in malonate or fluorocitrate (K and K Chemical). Eight slice pairs were used in the studies with malonate and 10 slice pairs with fluorocitrate. In the studies concerned with citrate gluconeogenesis (Fig. 2), six slices were halved, half was placed in citrate (10 m *M*) and the other half in citrate (10 m*M*) and barium fluorocitrate (1.0 m*M*). When glutamate or citrate was used as substrate the concentration was 10.0 m*M*. Inhibitors were added in the concentrations indicated in the text. Medium used for all experiments was a modified Krebs–Ringers solution buffered with 10 m*M* phosphate to a pH 7.4.

For *in vivo* studies, rats were injected with 1.2 ml of 1.0 *M* malonate/100 g of rat (7) or 1 ml of 4 *M* barium fluorocitrate/100 g of body wt. Control rats received an equivalent volume of saline intraperitoneally. To assure diuresis all rats received 5.0 ml of H_2O through a gastric tube. At the end of 4 hr, the bladder urine was evacuated by ether stimulation. Urines were collected under toluene; and pH, volume, and ammonia concentration were measured.

Ammonia and glucose were estimated by methods previously described (8). Citrate was measured by a citrate lyase technique (9). Statistics are by pair or group analysis using the Student's *t* test.

Results. In Vitro. In the presence of 10 m*M* malonate, ammoniagenesis from glutamate increased in 7 of 8 slice pairs with an average increase of 24% (Fig. 1A) ($p<.05$). The actual increase was from 63.3 ± 5.8 (SEM) μmoles/g/90 min to 81.3 ± 4.6

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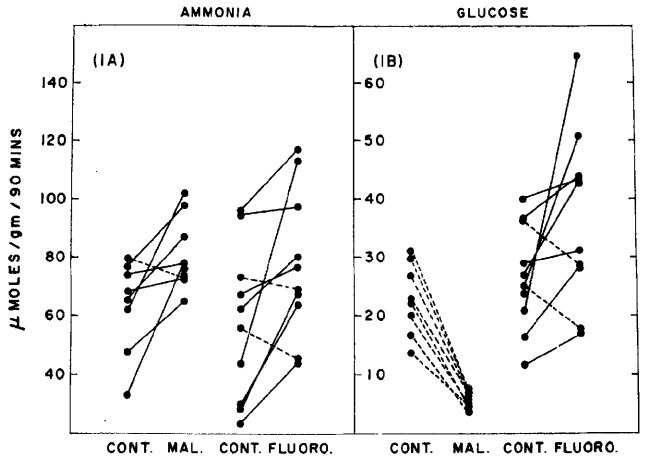

FIG. 1A. Ammonia production is followed in paired kidney slices in control medium or medium containing 10 m*M* malonate (MAL) or 1.0 m*M* fluorocitrate (Fluoro). (B) gluconeogenesis from paired rat kidney slices is followed in the medium without and with inhibitors. Slice pairs are connected by lines.

(SEM) μmoles/g/90 min. Figure 1B shows the effectiveness of the malonate block since gluconeogenesis decreased markedly in each case ($p<0.01$). The addition of 1.0 m*M* barium fluorocitrate also increased ammoniagenesis from glutamate (Fig. 1A) in 8 of 10 slice pairs. Ammoniagenesis increased from control values of 57 ± 8.4 (SEM) μmoles/g/90 min to 77.3 ± 4.8 (SEM) μmoles/g/90 min ($p<.02$). In contrast to the results with malonate, however, glucose production from glutamate increased 32% in the presence of fluorocitrate. Glucose production increased from a control value of 26.8 ± 2.9 (SEM) μmoles/g/90 min to 35.7 ± 2.9 (SEM) μmoles/g/90 min. That this concentration of fluorocitrate can block citrate metabolism, however, was demonstrated by showing that gluconeogenesis from citrate was blocked in the presence of this inhibitor. Glucose production in the presence of citrate in the control was 17.0 ± 1.3 (SEM) μmoles/g/90 min while it was 9.6 ± 1.0 (SEM) μmoles/g/90 min in the presence of fluorocitrate ($p<.001$).

In Vivo. The intraperitoneal injection of malonate resulted in increased ammonia excretion over the ensuing 4 hr. The individual results are depicted in Fig. 2 and the overall results in Table I. Figure 2 shows that, at any given urine pH, malonate causes greater ammonia excretion. Since urine flows were comparable in the two groups (Table I) this indicates that renal ammonia production increased in the presence of malonate. Similar results were seen following barium fluorocitrate injections. As shown in Fig. 2, ammonia excretion is increased at any given urinary pH by fluorocitrate despite no increase in urine flow (Table I). The efficiency of the metabolic block of fluorocitrate *in vivo* was evident since citrate excretion increased for the 4 hr following fluorocitrate

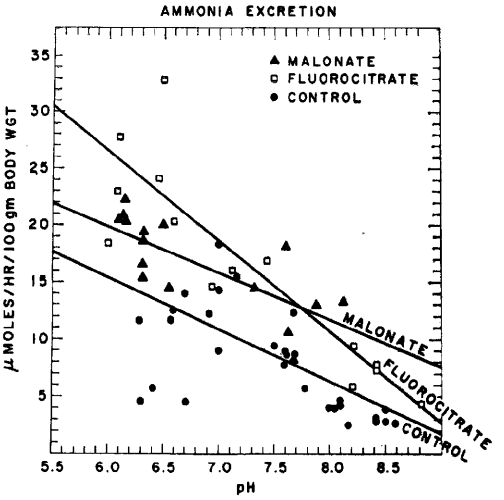

FIG. 2. Ammonia excretion from rats injected with saline, fluorocitrate or malonate plotted against urine pH. Lines are drawn by least squares method.

TABLE I. Ammonia Excretion Following ip Injections of Saline, Disodium Malonate, or Barium Fluorocitrate.

	No.[a]	Wt	V[b]	U pH	Art. pH	NH_4^+[c]	p[d]
Control	29	220	8.7	7.5	7.38	8.3 ± 0.9	
MAL	16	186	10.8	6.8	7.26	16.7 ± 1.0	<.001
Fluoro	14	257	8.2	7.0	7.43	18.9 ± 1.0	<.001

[a] Number of rats used.
[b] Urine volume for 4 hr.
[c] Ammonia excretion (μmoles/hr/100 g of rat ± SEM).
[d] Compared to control.

injections. Citrate excretion was 11.4 ± 0.3 (SEM) μmoles/hr/100 g of body wt in 5 fluorocitrate injected rats compared to 6.6 ± 1.4 (SEM) μmoles/hr/100 g of body wt ($p<.01$) for 5 control rats.

Discussion. That metabolic inhibitors increase ammoniagenesis in rat kidney slices confirms and extends previous studies performed with isolated dog tubules (6) since both 10 m*M* malonate and 1.0 m*M* fluorocitrate increase ammoniagenesis from glutamate in slices. In addition, injection of these same inhibitors into rats increases ammonia excretion. Since the urine flow and pH were of similar magnitude in the latter studies, it seems safe to presume that this increased excretion represents increased renal ammonia production.

This study yields some clues as to how citric acid cycle activity and ammoniagenesis may be related. The concentration of many organic anions in kidneys increases when metabolism is blocked by malonate and fluorocitrate (7, 10). O'Donovan and Lotspeich (11) have shown that some of these organic anions increase glutaminase I activity. However, the renal concentration of glutamate, the end product of glutamine deamidation, decreases after malonate injections (8) and each inhibitor increases ammonia production from glutamate *in vitro*. Both these findings indicate that there is a major effect of these inhibitors beyond the glutaminase I step at a point where glutamate is deaminated. It has been shown in isolated dog renal tubules that simultaneous oxidation of organic anions of the citric acid cycle (succinate, α-ketoglutarate, citrate, pyruvate) and glutamate results in decreased ammoniagenesis (6). Slowing the oxidation of the citric acid cycle anions with arsenite, partially overcomes this inhibition of ammoniagenesis from glutamate. Since, in this investigation, malonate and fluorocitrate, both *in vitro* and *in vivo*, were associated with increased ammonia production, it appears that a decrease in citric acid cycle activity stimulates renal ammoniagenesis.

Ammoniagenesis is probably regulated by a number of interrelated factors. Indeed, augmentation of ammoniagenesis produced by acute and/or chronic acid challenge may even be brought about by separate mechanisms (12). The activity of the citric acid cycle during acid challenge is, however, unknown. Citrate and α-ketoglutarate appear to be decarboxylated faster in acidosis (5), but whether formation of citrate to maintain cycle activity is increased, normal, or decreased has not been measured. One theory holds that the cycle may actually slow down in acidosis because of pH sensitivity of the condensing enzyme (3, 13). Thus, if a slow down in citric acid cycle activity does indeed occur then the increased ammoniagenesis shown in this study using Krebs cycle blockers may provide an explanation for at least a portion of acid-augmented renal ammoniagenesis.

Summary. The addition of 1.0 m*M* fluorocitrate to kidney slices markedly inhibited gluconeogenesis from citrate, while 10.0 m*M* malonate did the same to gluconeogenesis from glutamate. Both inhibitors increased ammonia production from glutamate by kidney slices. Injections of malonate and fluorocitrate into rats increased ammonia excretion *in vivo* despite no significant change in urine volume or pH. Therefore, both *in vivo* and *in vitro*, a slow down in citric acid cycle activ-

ity by two agents blocking at different points in the cycle resulted in augmented renal ammoniagenesis.

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