Effects of Malonate Administration on Renal Ammoniagenesis in Intact Dogs (42996)

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Abstract. **In the dog kidney *in vivo*, malonate augmented ammoniagenesis from both amide and nonamide nitrogen sources, similar to previous *in vitro* investigations using incubating canine renal tubules. This was highly significant in alkalotic dogs, where it was accompanied by decreased renal tissue concentrations of glutamate. Changes in renal ammonia metabolism were less evident in acidotic dogs where a markedly decreased glomerular filtration rate was noted following malonate administration. Under conditions of complete ureteric obstruction which effectively abolished glomerular filtration, malonate significantly augmented ammoniagenesis above baseline in acidotic dogs. These *in vivo* results with malonate have similarities to those seen in dogs subjected to an acid challenge alone and suggest that the adaptation in renal ammoniagenesis under both circumstances occurs via enhanced deamination of glutamate pools.**

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The influence of the metabolic inhibitor malonate on renal ammoniagenesis has been examined extensively *in vitro*. Addition of malonate to incubating renal tissue of rats (1) and dogs (2) increases ammoniagenesis, and evidence to date suggests that this occurs by enhancing intramitochondrial glutamate removal via deamination though the enzyme glutamate dehydrogenase (GDH) (Equation 2) (3). Deamination not only increases ammoniagenesis itself, but the resultant decreased glutamate concentrations lead to deinhibition of phosphate-dependent glutaminase (PDG) (4) and more ammonia formation via deamidation (Equation 1) (1, 5). Similar to GDH, PDG is also located within mitochondria.

$$\text{Glutamine} \xrightarrow{\text{PDG}} \text{Glutamate} + NH_3 + H+ \quad (1)$$

$$\text{Glutamate} \xrightarrow[\text{NAD+}]{\text{GDH}} \alpha\text{-ketoglutarate} + NH_3 + H+ \quad (2)$$

Studies concerned with the effects of malonate on renal ammoniagenesis in intact animals are limited. Malonate injected into rats increases urinary NH_4+ excretion (6), but the mechanisms responsible for this were not examined. *In vitro* work outlines the possible metabolic pathways and potential mechanisms involved in ammoniagenesis; however, the actual pathways and relative contributions to ammonia production can be defined only by studies on intact functioning kidneys. Accordingly, the objective of this study was to examine the influence of malonate on the renal ammoniagenesis of intact acidotic and alkalotic dogs.

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Materials and Methods

Experiments were performed on 31 dogs of either sex weighing between 15 and 24 kg. We induced chronic metabolic alkalosis or acidosis by daily administration for 5 days of 10–15 g of sodium bicarbonate or 10 g of ammonium chloride, respectively, mixed with a standard laboratory diet. After being fasted overnight, the dogs were anesthetized with intravenous pentobarbital (approximately 20 mg/kg) and additional doses were given intravenously as needed.

The operational procedures, laboratory analyses, and methods to assess renal function, renal ammoniagenesis and renal metabolism of glutamine, glutamate, and alanine have been described previously (5). Clearance studies were performed on the left kidney. Glomerular filtration rate (GFR) was measured by exogenous creatinine clearance. Renal blood flow (RBF), determined with *p*-aminohippurate, was measured by the Wolf modification (7) of the Fick principle; i.e., renal blood flow was calculated by V(U−Prv/Pa − Prv), where V is urine flow (ml/min), Pa is plasma arterial concentration, and Prv is the renal venous concentration (mol/ml). All concentrations in the renal vein were corrected for urine formation (8). Total renal ammonia

production was calculated from the urinary excretion plus the amount added to the renal venous blood (8). The pNH_3 calculation has been described (8). The addition or extraction of glutamine amide nitrogen and other amino acids by the kidneys was calculated and expressed as μmoles per minute, by multiplying the difference between the arterial and renal venous concentration by the renal plasma flow. Nonamide ammonia formation was calculated by subtracting the renal extraction of glutamine amide nitrogen from the total ammonia formation. Malonate infusions did not significantly affect urinary excretion of glutamine, glutamate, and alanine, and they were not measured routinely in the urine.

Two different protocols for administering malonate were carried out. The first group received less malonate than the second.

Group 1—Low Dose Malonate. Through an abdominal midline incision, renal cortex from the right kidney was removed (100–200 mg) and frozen in liquid nitrogen for the eventual determination of basal metabolite concentrations. Then, clearance studies were performed on the remaining kidney. A 60-min equilibration period followed closure of the abdomen. Two control 20-min clearance periods on the left kidney were observed in acidotic and alkalotic dogs. Then, 8 mmol of sodium malonate were administered as a bolus into an antecubital vein, followed by sustained infusion at 200 μmol/min. Two experimental clearance periods of 20 min each were then observed. At the end of the experiment, 100–200 mg of renal cortex from the left kidney was obtained and treated in the same manner as the control tissue.

Group 2—High Dose Malonate. In these groups after two control clearance periods, 160 mmol of sodium malonate were administered as two equal boluses 30 min apart. The first bolus preceded clearance period 3 by 10 min and the second preceded period 4 by 10 min. The four clearance periods observed in these experiments were each of 20-min duration. In comparing major differences between the design of the two types of malonate studies, cortical glutamate levels were assessed in the first and renal glutamine amide nitrogen extraction and alanine and glutamate release in the second.

A variation of the high dose studies was carried out in another six acidotic dogs. Malonate was given after the ureter of one kidney was completely occluded to halt GFR. In these studies, RBF was assessed with a Biotronics Laboratory B1 620 electromagnetic flow probe. The left renal artery was gently isolated within its pedicle to ensure intact innervation and fitted with the noncannulating electromagnetic flow probe. After an initial control clearance period of 20 min, the left ureter was completely occluded with two ligatures. After another 30-min interval, a second 20-min clearance period was observed. Ten minutes prior to a third 20-min clearance, 40 mmol of sodium malonate (half the initial dose used in the previous study) were administered as a bolus. Again, 10 min prior to a fourth clearance period, 80 mmol of sodium malonate were administered as a second bolus. Blood samples were collected as before.

Appropriate statistical analyses were performed with either paired or group analysis by using Student's *t* test. Regression lines were determined by the least squares method.

Results

Low Dose Malonate Studies. Infusion of malonate into acidotic and alkalotic dogs (Table I) did not significantly change urinary volume and pH, arterial pH and $[HCO_3^-]$, GFR, renal plasma flow (RPF), or RBF. Total renal ammoniagenesis was not significantly increased in acidotic dogs, nor was cortical glutamate concentration changed by malonate in this group. Dif-

Table I. Various Parameters before and after Low Dose Malonate Infusions into Alkalotic (six) and Acidotic (six) Dogs

Parameters	Acidosis		Alkalosis	
	Control	Malonate	Control	Malonate
Urine volume (ml/min)	0.71 ± 0.08	0.90 ± 0.06	0.54 ± .07	0.71 ± 0.10
Urine pH	5.9 ± 0.04	5.9 ± 0.04	7.9 ± .07	7.9 ± 0.07
Arterial pH	7.18 ± 0.01	7.18 ± 0.01	7.46 ± .02	7.46 ± 0.02
Arterial HCO_3^- (mEq/liter)	15.4 ± 0.5	15.8 ± 0.7	32.1 ± 1.0	32.6 ± 1.0
GFR (ml/min)	35.2 ± 1.4	30.7 ± 2.0	37.0 ± 2.5	34.2 ± 2.2
RPF (ml/min)	182.2 ± 11.4	164.4 ± 13.5	189.0 ± 15.4	175.9 ± 11.3
RBF (ml/min)	289.2 ± 18.9	265.2 ± 21.6	309.8 ± 25.0	288.4 ± 18.3
Ammonia production (μmol/min)	52.1 ± 3.0	50.6 ± 3.2	16.9 ± 2.1	35.2 ± 2.9[a]
Cortical glutamate (μmol/g wet wt)	4.2 ± 0.1	4.3 ± 0.1	8.2 ± 1.3	4.0 ± 0.7[a]

Note. Average ± SEM is shown. Statistics by paired Student's *t* test.
[a] $P < 0.001$.

Table II. Various Parameters before and after High Dose Malonate Infusions into Alkalotic (seven) and Acidotic Dogs (six)

	Alkalotic			Acidotic		
	Control	Malonate (80 mmol)	Malonate (80 mmol)	Control	Malonate (80 mmol)	Malonate (80 mmol)
Urine volume (ml/min)	2.2 ± 0.3	3.1 ± 0.3[a]	2.8 ± 0.4	2.8 ± 0.4	3.8 ± 0.6[b]	3.7 ± 0.8
Urine pH	7.80 ± 0.04	7.37 ± 0.11[c]	7.07 ± 0.13[a]	5.48 ± 0.28	6.12 ± 0.20[c]	6.30 ± 0.17
Arterial pH	7.45 ± 0.04	7.49 ± 0.06	7.46 ± 0.06	7.22 ± 0.03	7.20 ± 0.03	7.14 ± 0.03
Arterial HCO_3^- (mEq/liter)	25.0 ± 1.0	25.0 ± 0.9	24.6 ± 0.7	17.0 ± 2.4	16.8 ± 1.8	17.2 ± 1.6
GFR (ml/min)	37.2 ± 2.4	26.2 ± 4.4[c]	17.8 ± 3.2[c]	35.5 ± 3.5	18.1 ± 3.0[a]	13.5 ± 3.4[c]
RPF (ml/min)	140 ± 25	123 ± 24	112 ± 2	192 ± 30	161 ± 24[b]	143 ± 34
RBF (ml/min)	216 ± 34	188 ± 31	171 ± 34	306 ± 45	247 ± 41[b]	227 ± 61
Total ammonia production (μmol/min)	12.0 ± 2.8	29.2 ± 13[a]	31.3 ± 3.6	47.1 ± 5.4	41.8 ± 6.1	35.4 ± 8.4
Extracted glutamine (μmol/min)	5.4 ± 2.4	14.8 ± 13[c]	15.2 ± 2.4	21.4 ± 3.2	15.4 ± 3.4	16.4 ± 2.9
Nonamide ammonia[d] (μmol/min)	8.2 ± 0.9	16.3 ± 2.4[c]	15.5 ± 4.7	25.7 ± 4.0	26.5 ± 4.4	18.8 ± 7.2
Glutamate release (μmol/min)	1.32 ± 0.44	1.59 ± 0.18	1.40 ± 0.14	2.42 ± 0.46	2.39 ± 0.34	2.44 ± 0.37
Alanine release (μmol/min)	9.9 ± 2.8	9.6 ± 3.6	5.5 ± 2.0	10.1 ± 2.4	6.9 ± 2.4	7.0 ± 1.8

Note. Average ± SEM shown.
[a] $P < 0.01$.
[b] $P > 0.05 < 0.1$ compared with value to the left.
[c] $P < 0.02$.
[d] Total ammonia − glutamine extraction.

ferently, total ammoniagenesis in alkalotic dogs was increased significantly. Baseline renal cortical glutamate concentrations, which were higher in alkalotic compared with acidotic dogs, decreased significantly after malonate infusions in the alkalotic dogs.

High Dose Malonate Studies. To accentuate the metabolic changes, higher doses of malonate (approximately 10×) were given (Table II). Blood pH and plasma $[HCO_3^-]$ did not change significantly after malonate, while urinary pH rose in acidotic and fell in alkalotic dogs compared with baseline. GFR decreased consistently in both acidotic and alkalotic dogs, while renal plasma and blood flow were affected significantly only in acidotic dogs after malonate. In acidotic dogs, total ammonia production, glutamine amide nitrogen extraction, nonamide ammonia formation and alanine release did not change significantly. If anything, there was a tendency for those parameters to decrease after malonate. In contrast, total ammonia production, glutamine amide nitrogen extraction (amide ammonia), and nonamide ammonia production increased in alkalotic dogs after high dose malonate.

To determine whether the effects of malonate on renal ammoniagenesis in acidotic dogs were influenced by changes in GFR, these studies were repeated in chronically acidotic dogs following total ureteral obstruction with virtual cessation of glomerular filtration (Table III). Compared with baseline, blood pH and plasma $[HCO_3^-]$ did not change following ureteral obstruction. However, RBF rose significantly shortly after clamping.

After 30 min of ureteric clamping, renal ammoniagenesis, and nonamide ammonia formation decreased. After the infusion of malonate, total ammonia production and nonamide ammonia formation increased significantly, whereas there was a lesser effect on amide ammonia formation (glutamine amide nitrogen extraction). When renal venous pNH_3 was calculated, it was found to rise after ureteric clamping alone and increase even more after malonate infusion (Fig. 1).

Discussion

In vitro studies using malonate, an agent producing a block in the Krebs cycle principally at the level of succinic dehydrogenase (9), provide information concerning the regulation of renal ammoniagenesis (1–3). In 1968, we showed that malonate augments renal ammoniagenesis in incubating canine tubules from both amide and amino nitrogen sources of glutamine (3). The effect was attributed to the ability of malonate to enhance glutamate deamination (nonamide ammonia formation) via GDH by increasing the availability of intramitochondrial oxidized pyridine nucleotides required for this reaction (3). In turn, the lowered intramitochondrial glutamate concentrations led to greater ammonia formation through deamidation (amide ammonia formation) via PDG (Equations 1 and 2) (1).

We have postulated that this same sequence of

Table III. Effects of Malonate on Various Parameters of Acidotic Dogs Undergoing Ureteral Obstruction

	Control	Obstruction	Malonate (40 mmol)	Malonate (80 mmol)
Arterial pH	7.25 ± 0.06	7.26 ± 0.03	7.25 ± 0.04	7.16 ± 0.04
Arterial HCO_3^- (mEq/liter)	17.5 ± 2.0	16.3 ± 2.0	15.7 ± 1.9	15.3 ± 2.6
GFR (ml/min)	29.1 ± 8.8	0	0	0
RPF (ml/min)	116 ± 28	171 ± 44[a]	169 ± 56	160 ± 42
RBF (ml/min)	181 ± 44	252 ± 66	247 ± 85	235 ± 61
Renal ammonia production (μmol/min)	27.0 ± 3.6	18.8 ± 4.7[b]	35.0 ± 7.5[b]	35.2 ± 5.1
Nonamide ammonia production (μmol/min)	12.8 ± 2.5	5.0 ± 2.8[b]	20.2 ± 5.3[b]	20.5 ± 4.8
Glutamine extraction (μmol/min)	14.3 ± 1.6	13.8 ± 2.5	14.8 ± 3.0	14.7 ± 1.5
Glutamate release (μmol/min)	1.9 ± 0.4	2.4 ± 0.4	2.6 ± 0.4	3.1 ± 0.6

Note. Average ± SEM shown.
[a] $P < 0.02$ compared with value to left.
[b] $P < 0.05$ compared with value to left.

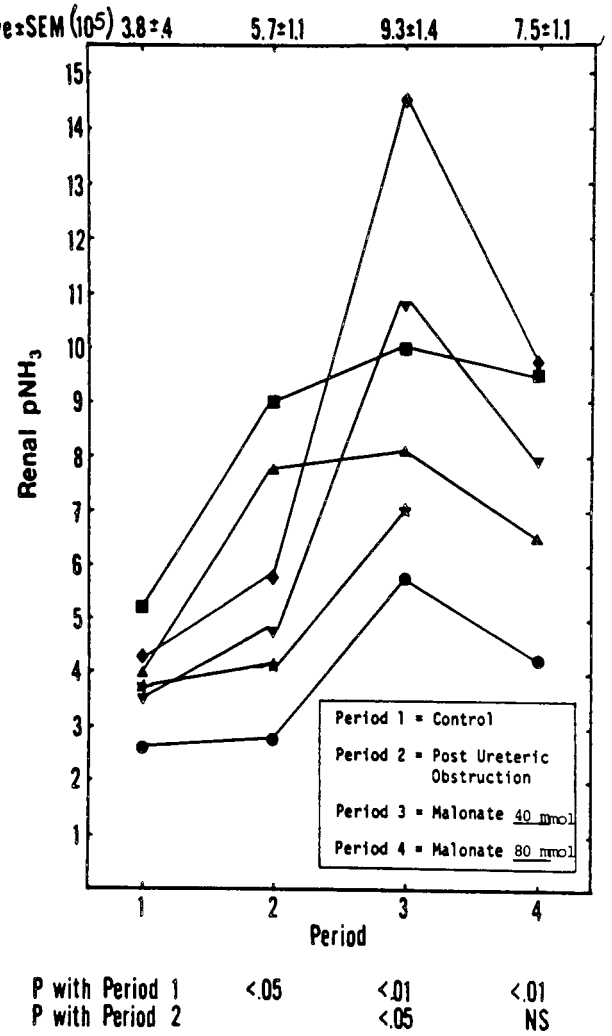

Figure 1. Renal pNH_3 in kidneys undergoing ureteral obstruction. First period is control. Second period is 0.5 hr after total obstruction. Periods 3 and 4 depict results in obstructed kidneys after two boluses of malonate (40 mmol and 80 mmol).

events may contribute to the physiologic adaptation whereby renal ammoniagenesis is enhanced during acid challenge (5). The present study was designed to determine whether administration of malonate might enhance renal ammoniagenesis in intact dogs in a manner similar to that of isolated canine tubules. If so, such stimulation secondary to malonate infusion might be compared with that produced by acidosis.

What is known about ammoniagenesis in the intact dog? Pitts (10) had previously established that the majority of renal ammonia derives from the amide nitrogen of glutamine and the amino nitrogen of glutamine and other amino acids. Since the work of Braunstein and Kritzman (11) demonstrating the ubiquity of tissue transaminases, the concept has developed that those amino acids that contribute their amino nitrogens as urinary ammonia do so indirectly by first transaminating α-ketoglutarate to form glutamate (10). Glutamate is subsequently deaminated oxidatively to yield ammonia and regenerate α-ketoglutarate. Because very little renal amide nitrogen extraction ends up in anything other than ammonia (12), as a first approximation, it is assumed that renal amide nitrogen extraction is synonymous with "amide ammonia formation." Therefore, total ammonia formation minus amide nitrogen extraction is an estimate of "nonamide" ammoniagenesis. The majority of nonamide ammonia comes from amino nitrogen sources. Thus, Pitts *et al.* (13) could account for 95% of renal ammonia formed during acidosis and 80% formed during acute alkalosis by the extraction of amide and amino nitrogens.

Similar to *in vitro* findings (1–3), the present study indicates that malonate administration to intact dogs can increase ammoniagenesis both from amino and amide nitrogen groups. In alkalotic dogs, this occurred along with decreased cortical glutamate concentrations. It was unclear whether ammoniagenesis was affected in acidotic dogs where glutamate concentrations were al-

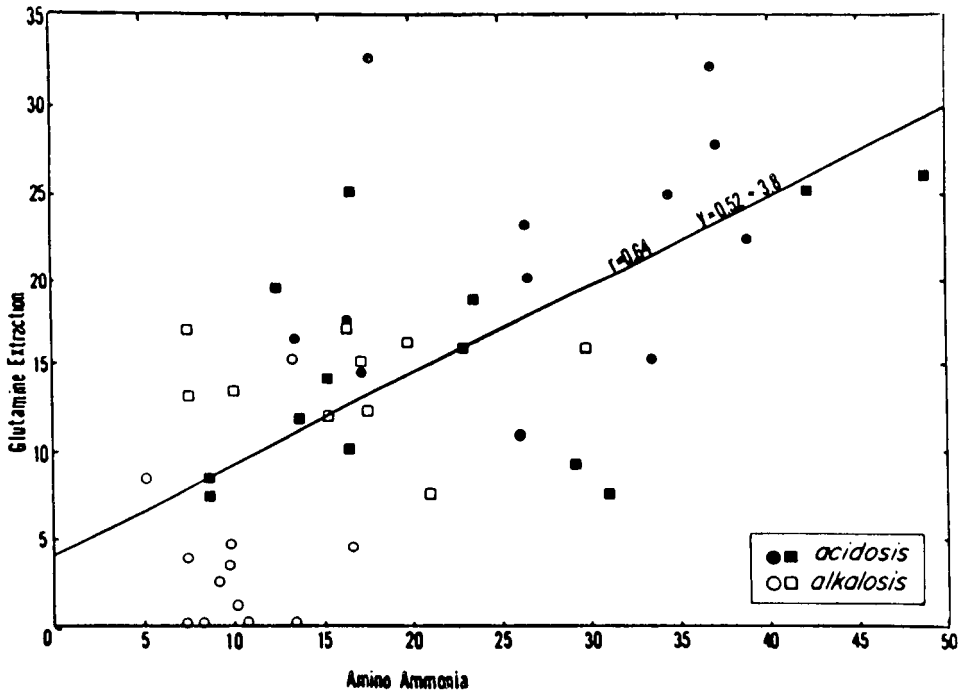

Figure 2. Correlation between amino (nonamide) ammonia formation (deamination) and glutamine extraction (deamidation) in intact acidotic and alkalotic dogs. Circle denotes control period, square denotes results after malonate infusions ($P < 0.01$).

ready low. It seemed possible that any changes could have been masked by the decrease in GFR seen with higher doses of malonate since GFR per se has been associated with decreased ammoniagenesis (14, 15). To investigate this possibility we repeated our studies on dogs with obstructed kidneys. Complete obstruction essentially eliminates glomerular filtration and luminal uptake of glutamine. Obviously, malonate infusions would not be influenced by a changing GFR. Despite virtual cessation of glomerular filtration, malonate increased ammonia production above baseline in the acidotic dog.

The elevation in ammonia production in obstructed kidneys was created largely by increased nonamide ammonia production. The effects on amide ammonia formation were less apparent in this model. Why this occurred is not entirely clear. Two possibilities exist. First, pNH_3 rises markedly in the obstructed kidney, more so after malonate infusion (Fig. 1). A rise in pNH_3 is associated with decreased deamidation (17). Second, the supply of glutamine for ammoniagenesis may become rate limiting without the luminal contribution to the cellular glutamine pool. With a rate-limiting supply of amide nitrogen, a decreased concentration of glutamate could not fully hasten deamidation significantly. Nonetheless, malonate was able to increase ammoniagenesis in acidotic kidneys.

These present findings in intact dogs agree with *in vitro* results which indicate that an increased deamination is driving the enhanced deamidation following malonate (1–3). In Figure 2, a plot of amino ammonia formed, i.e., nonamide ammonia, versus glutamine extraction, i.e., amide ammonia formation, shows a significant positive correlation. In light of the decrease or at least no increase in cortical glutamate concentrations and the lack of increase in glutamate or alanine release following malonate infusions, these correlations like the *in vitro* findings (3) are most consistent with increased amino ammonia formation leading to increased deamidation, rather than increased glutamine deamidation leading to more nonamide ammonia formation (5).

The results corroborate previous findings that metabolic inhibitors increase ammoniagenesis *in vivo* (17, 18). They confirm that metabolic inhibitors work primarily through enhancing glutamate removal via greater deamination through the glutamate dehydrogenase system (1–3). Since these changes in glutamine metabolism mirror the findings in acidotic kidneys (5), they support the hypothesis that acidotic adaptation takes place through a similar mechanism (1–3, 5, 6, 17).

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