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THE EFFECT OF THE ADMINISTRATION OF SODIUM BICARBONATE AND AMMONIUM CHLORIDE ON THE EXCRETION AND PRODUCTION OF AMMONIA. THE ABSENCE OF ALTERATIONS IN THE ACTIVITY OF RENAL AMMONIA-PRODUCING ENZYMES IN THE DOG

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Research Article

THE EFFECT OF THE ADMINISTRATION OF SODIUM BICARBONATE AND AMMONIUM CHLORIDE ON THE EXCRETION AND PRODUCTION OF AMMONIA. THE ABSENCE OF ALTERATIONS IN THE ACTIVITY OF RENAL AMMONIA-PRODUCING ENZYMES IN THE DOG

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The regulation of the excretion of ammonia by the kidney has been studied extensively. It is clear that urine pH is an important determinant of ammonia excretion, with greater amounts of ammonia diffusing into acid urines than into alkaline urines (1–8). However, it is also evident that the magnitude of the rise in ammonia excretion during the chronic administration of strong acids or acidifying salts cannot be accounted for solely on the basis of "trapping" of greater amounts of ammonia in increasingly acid urines. Thus Pitts (4) found that dogs given NH_4Cl for two days excreted more ammonia at any given urine pH than did control animals, presumably due to increased production of ammonia by the renal tubule cells. Earlier, Van Slyke and co-workers (9) had found that the extraction of glutamine from renal arterial blood was greater in dogs receiving NH_4Cl than in those receiving $NaHCO_3$, and further that the extracted glutamine amide-N accounted for approximately 60 per cent of the ammonia produced by the kidney. Lotspeich and Pitts (10) postulated that the capacity of some renal mechanism, capable of extracting and hydrolyzing glutamine, increases during chronic metabolic acidosis to produce more ammonia. This was confirmed in rats when Davies and Yudkin (11) and others (7, 12) found that the administration of HCl increased and the administration of $NaHCO_3$ decreased the activity of rat kidney glutaminase. The observation of similar changes in acidotic guinea pigs and rabbits (13) has suggested that ammonia excretion in all mammals, including man, is regulated in part by adaptive changes in the activity of renal glutaminase.

There is evidence, however, that the regulation of ammonia excretion differs in dogs and rats.

Orloff and Berliner (6) observed that the relationship between urine pH and ammonia excretion in dogs was reproducible following the infusion of sodium bicarbonate and independent of acute alterations in acid-base balance. This is in contrast to the findings of Leonard and Orloff (7) in the rat. In this species a correlation between ammonia excretion and urine pH was not observed under all circumstances. Alterations in ammonia excretion occurred independently of urine pH changes in some circumstances. The converse was also observed: changes in urine pH without associated changes in ammonia excretion. In the rat variations in ammonia excretion in acute studies are conditioned not only by urine pH, the only determinant in the dog, but also by the state of acid-base balance. Dogs and rats also exhibit dissimilar urinary responses to the chronic inhibition of carbonic anhydrase. In rats treated chronically with acetazoleamide, ammonia excretion falls briefly, then rises to greater than normal levels. This elevated excretion of ammonia is associated with an increased activity of renal glutaminase (7, 14). In dogs, however, the chronic administration of acetazoleamide results in a persistent reduction in ammonia excretion, with a return towards normal values only as the urine becomes less alkaline (15). On the basis of the response of man (16) and dog (15) to acetazoleamide, it had been suggested (17) that an adaptive increase in the activity of renal glutaminase might not occur in these species.

The purpose of the present studies was to determine whether or not alterations in the activity of renal glutaminase participate in the regulation of ammonia excretion in dogs. The effects of NH_4Cl and $NaHCO_3$ on the relationship between

ammonia excretion and the activities of the various renal enzymes involved in the conversion of glutamine to ammonia were examined in dogs and the results compared with those from essentially analogous studies in rats.

PROCEDURES

The relationship between ammonia excretion and urine pH in four trained female dogs was determined both after the administration of 8 Gm. of NH_4Cl daily for 7 to 14 days and after the administration of an equivalent amount of $NaHCO_3$ for a similar period of time. At the end of each experimental period studies were performed in which the urine was acutely acidified by the infusion of 200 μM per minute of Na_2SO_4, then alkalinized by the infusion of 600 μM per minute of $NaHCO_3$, using a Bowman constant infusion pump. Urine was collected anaerobically in oiled syringes for pH and ammonia measurements.

To study the effect of NH_4Cl and $NaHCO_3$ on the activities of the renal enzymes involved in the production of ammonia, biopsies of kidney cortex were obtained surgically from 12 dogs and the activities of the enzymes compared after the administration of NH_4Cl and after the administration of $NaHCO_3$. Thus each dog served as its own control. Enzymatic activity was also determined in homogenates of kidneys from both acidotic and normal rats. Six of the 12 dogs were given 8 Gm. of $NaHCO_3$ daily for 5 to 21 days, after which time the kidney biopsies and enzyme analyses were performed. The dogs were then given 8 Gm. NH_4Cl daily for 5 to 21 days and the kidney enzyme determinations repeated. In the remaining 6 dogs the procedure was reversed so that NH_4Cl was given first and $NaHCO_3$ last. Four of the dogs received 100 mEq. of HCl intravenously daily instead of the oral NH_4Cl. This was done in order to circumvent any possible difficulties due to inadequate absorption. At the time of biopsies blood samples were drawn for plasma bicarbonate measurements. Immediately upon removal, the samples of kidney cortex were either weighed and homogenized in 19 volumes of ice cold distilled water to give 5 per cent homogenates, or sliced in the cold with a Stadie-Riggs slicer. The homogenates were assayed for phosphate-activated glutaminase I, pyruvate-activated glutaminase II, glutamic dehydrogenase and glutamic-oxaloacetic transaminase.

METHODS

The pH of the urine was measured in an internal glass electrode at 37° C. using a Cambridge research model pH meter. The concentration of ammonia in the urine was measured by the method of Conway (18). The total CO_2 content of plasma was determined by the method of Van Slyke and Neill (19). Dry weights of the kidney homogenates were determined by drying 5 ml. aliquots of the homogenates overnight at 90° C.

The activity of phosphate-activated glutaminase I was assayed using the method previously described (14). The pH optimum (8.0) of glutaminase I was the same in the kidneys of rats and dogs, and the rate of ammonia formation was linear over the same range of tissue concentrations (5 to 20 mg. wet kidney) for both dog and rat kidneys.

The activity of pyruvate-activated glutaminase II was measured by a modification of the method described by Goldstein, Richterich-van Baerle and Dearborn (20). Maximal activity for dog and rat kidney glutaminase II was observed at pH 8.8 to 9.0. The method was modified by substituting 0.5 M Tris buffer (pH 9.0) for the veronal buffer. At the end of the 30 minute incubation period the reaction was stopped by the addition of 0.2 ml. 37.5 per cent $HClO_4$. The samples were centrifuged and ammonia measured on 0.2 ml. aliquots of the supernatant by a modification of the microdiffusion method of Seligson and Seligson (21). Rather than using the Nesslers reagent as described by Seligson the ammonia was determined colorimetrically using hypo-chlorous acid and sodium phenate (22).

Glutamine synthetase activity was measured in several different ways: the uptake of ammonia by kidney homogenate in the presence of glutamate and ATP as described by Richterich-van Baerle and associates (23), the formation of γ-glutamyl hydroxamate as described by Levintow, Meister, Hogeboom and Kuff (24), and the method of Reiner and Hudson (25). Potent activity was demonstrable in rat kidney using all three methods, but no measurable activity was found in dog kidney with any of the methods. Since Levintow and co-workers (24) have adduced evidence that the same enzyme catalyzes both the synthesis of glutamine and the transfer exchange of the γ-glutamyl amide group, the formation of γ-glutamyl hydroxamate from glutamine, ADP, and hydroxylamine as described by Levintow and associates (24) was the method finally adopted for the assay of glutamine synthetase activity in rat kidney homogenates. Five-tenths ml. of a 1 per cent homogenate was used in each assay. γ-Glutamyl hydroxamate was measured by the method of Lipmann and Tuttle (26).

Glutamic dehydrogenase activity was assayed in 0.2 ml. of a 1:6 dilution of the 5 per cent homogenate using the method of Olson and Anfinsen (27). Glutamic-oxaloacetic transaminase was assayed in 0.2 ml. of a 1:25 dilution of the 5 per cent kidney homogenate according to the method of Steinberg and Ostrow (28).

The hydrolysis of glutamine by intact kidney slices was determined by incubating slices of kidney cortex, weighing 50 to 100 mg., in 3 ml. of oxygenated Ringer's phosphate solution (pH 7.4), containing 49 μM glutamine per ml. The reaction was stopped at the end of 30 minutes by the addition of 0.2 ml. 37.5 per cent $HClO_4$. The medium was centrifuged and ammonia was measured in 0.5 ml. aliquots of the supernatant.

RESULTS

The effect of the chronic administration of NH_4Cl and $NaHCO_3$ on the relationship between
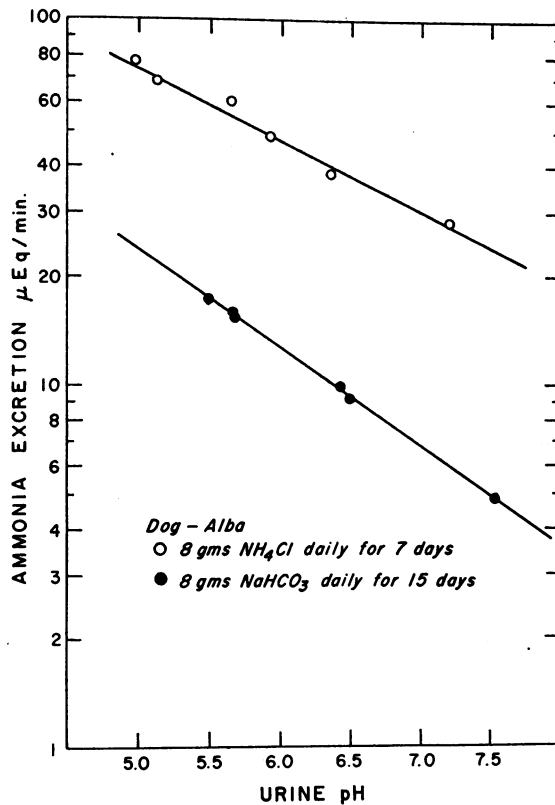
FIG. 1. THE EFFECT OF CHRONIC NH$_4$CL AND NaHCO$_3$ LOADS ON THE RELATION BETWEEN AMMONIA EXCRETION AND URINE pH IN DOGS

ammonia excretion and urine pH is shown in Figure 1. In both studies a linear relationship between the logarithm of ammonia excretion and urine pH was observed. Although the slopes of the two lines are approximately the same, the rate of ammonia excretion is three to four times greater at any given urine pH after NH$_4$Cl than after NaHCO$_3$. The results from three other dogs were similar. Adaptive changes in ammonia excretion have also been reported in rats (7) and guinea pigs (29). The results of these studies confirm the observations of Pitts (4) that increased excretion of ammonia after the chronic administration of NH$_4$Cl cannot be accounted for on the basis of increased acidity of the urine, but must in some way reflect accelerated production of ammonia by renal tubule cells.

As noted earlier, the studies of Van Slyke and associates (9) indicate that the increased production of ammonia during NH$_4$Cl acidosis is a consequence of enhanced hydrolysis of glutamine.

In rats (7, 11, 12) and guinea pigs (13) this accelerated conversion of glutamine to ammonia is apparently accomplished by an adaptive increase in the activity of renal glutaminase. The results of the present studies, however, indicate that such a mechanism does not account for the increased ammonia excretion in acidotic dogs.

The effect of the chronic administration of NH$_4$Cl and NaHCO$_3$ on the renal glutaminases is shown in Table I. In rats NH$_4$Cl increased the activity of phosphate-activated glutaminase I approximately fourfold, whereas in dogs NH$_4$Cl resulted in a 10 per cent decrease in activity. A similar decrease was noted in four dogs given HCl intravenously rather than NH$_4$Cl orally. Although this decrease in the activity of dog kidney glutaminase I was statistically significant, its physiological significance is obscure. Pyruvate-activated glutaminase II was also increased approximately fourfold in rats given NH$_4$Cl, but was unchanged in dogs. Glutamine synthetase activity, estimated indirectly from γ-glutamyl transferase activity, was decreased in rats receiving NH$_4$Cl, while no activity was measurable in dog kidneys. The depression of glutamine synthetase activity in rat kidneys is in contrast to the results of Richterich-van Baerle and co-workers in which glutamine synthetase activity was increased in guinea pig kidneys by the administration of NH$_4$Cl. It is unlikely that the failure of glutaminase I and II to increase in dogs during the administration of NH$_4$Cl was due to inadequate stimulation, since at the time of biopsy the plasma HCO$_3^-$ concentrations were 26 ± 2 mEq. per L. after NaHCO$_3$ and 21 ± 2 mEq. per L. after NH$_4$Cl, and the ammonia excretions were 8 mEq. per 24 hours after NaHCO$_3$ and 45 mEq. per 24 hours after NH$_4$Cl. Thus it is seen that the dogs were in fact slightly acidotic and were excreting increased amounts of ammonia after the administration of NH$_4$Cl.

Since the administration of NH$_4$Cl did not increase the activity of glutaminase I and II in the cortex of dog kidneys, the possibility of isolated changes occurring in other sections of the kidney was investigated. The activity of glutaminase I was measured in the cortex, medulla and papilla of kidney from dogs given either NH$_4$Cl or NaHCO$_3$ and in rats given either H$_2$O or 1.5 per cent NH$_4$Cl solution ad lib. In both dogs and

TABLE I

The effect of NH₄Cl and NaHCO₃ on the activity of glutaminase I, glutaminase II and glutamine synthetase in kidney cortex of dogs and rats

Group		Phosphate-activated glutaminase $\mu M\ NH_3/hr./100\ mg.$ dry kidney		Pyruvate-activated glutaminase $\mu M\ NH_3/hr./100\ mg.$ dry kidney		Glutamine synthetase units/hr./100 mg. wet kidney	
		Dogs	Rats	Dogs	Rats	Dogs	Rats
No. of animals		12	8	4	4	4	4
Controls	Mean activity		545		101		45.1
	S. D.		±77		±31		±3.9
NaHCO₃*	Mean activity	735		105			
	S. D.	±151		±15			
NH₄Cl†	Mean activity	665	2,120	108	471	0	38.0
	S. D.	±175	±412	±53	±146		±2.9
	Mean difference	−70	+1,595	+ 3	+370		−7.1
	S. E.	±30	±148	±22	± 57		±2.5
	p	<0.05	<0.01	>0.5	<0.01		<0.05

* The dogs received 8 Gm. NaHCO₃ daily for 5 to 21 days.
† The dogs received 8 Gm. NH₄Cl daily for 5 to 21 days; the rats received 1.5 per cent NH₄Cl in tap water *ad lib.* for 14 to 21 days.

rats the activity of glutaminase I in the papilla was approximately one-tenth the activity in the cortex. In rats the administration of NH₄Cl had no effect on the activity of glutaminase I in the papilla, while the activity increased approximately fourfold in the cortex. In dogs the administration of NH₄Cl did not increase the activity of glutaminase I in either cortex or medulla. A slight increase in activity was observed in the papilla of dogs. However the enzyme and tissue blank constituted such a large fraction of the total ammonia produced that the results were not interpretable. Furthermore the absolute amounts of ammonia produced were so small as to be physiologically insignificant.

Although the activity of the renal glutaminases, as assayed in homogenates, did not increase in dogs given NH₄Cl, the alternative possibility that chronic NH₄Cl acidosis induces changes in the intracellular environment which increases the effective activity of the enzyme resident in the cell, without changing its actual concentration, was considered. Changes of such a nature would not be detected by assays of tissue homogenates, but might be with intact kidney slices. To investigate this point the rate of glutamine hydrolysis by intact kidney slices was measured. The effect of NH₄Cl administration is shown in Table II. NH₄Cl increased the rate of hydrolysis in rats,

but decreased the rate by approximately 10 per cent in dogs. Although no evidence for an increase in the effective intracellular activity of dog kidney glutaminase was obtained, the true *in vivo* activity may not be reflected by the *in vitro* hydrolysis of glutamine by kidney slices.

Another process whereby the conversion of glutamine to ammonia could be accelerated involves the action of glutamic dehydrogenase. Glutamic

TABLE II

The effect of NH₄Cl and NaHCO₃ on the hydrolysis of glutamine by intact kidney slices

Group		Glutamine hydrolysis $\mu M\ NH_3/100\ mg.$ kidney/hr.	
		Dogs	Rats*
No. of animals		4	12
NaHCO₃†	Mean activity	7.7	5.5
	S. D.	±0.7	±0.8
NH₄Cl‡	Mean activity	7.2	14.0
	S. D.	±1.0	±2.3
	Mean difference	−0.45	+8.4
	S. E.	±0.36	±0.7
	p	>0.30	<0.01

* Unpublished data of J. Orloff.
† The dogs received 8 Gm. NaHCO₃ daily for 10 to 14 days; the rats received 1.5 per cent NaHCO₃ solution *ad lib.* for 14 days.
‡ The dogs received 8 Gm. NH₄Cl daily for 10 to 14 days; the rats received 1.5 per cent NH₄Cl solution *ad lib.* for 14 days.

TABLE III

The effect of NH_4Cl and $NaHCO_3$ on glutamic dehydrogenase and glutamic-oxaloacetic transaminase in kidney cortex of dogs and rats

Group		Glutamic acid dehydrogenase *units/mg. dry kidney*		Glutamic-oxaloacetic transaminase *units/mg. dry kidney*	
		Dogs	Rats	Dogs	Rats
No. of animals.................................		12	4	5	4
Controls	Mean activity		380		489
	S. D.		± 59		± 34
NaHCO₃*	Mean activity	326		285	
	S. D.	±80		±57	
NH₄Cl†	Mean activity	278	955	281	485
	S. D.	±92	±117	±32	±126
	Mean difference	−48	+575	− 4	− 4
	S. E.	±32	± 66	±18	± 65
	p	<0.2	<0.01	>0.5	>0.5

* Dogs received 8 Gm. $NaHCO_3$ daily for 5 to 21 days.
† Dogs received 8 Gm. NH_4Cl daily for 5 to 21 days; rats received 1.5 per cent NH_4Cl *ad lib.* for 14 to 21 days.

acid, an end product of glutamine hydrolysis, is known to inhibit glutaminase I (30). An increase in the activity of glutamic dehydrogenase, by increasing the rate of glutamic acid removal, would increase the effective activity of glutaminase I. Also the action of glutamic dehydrogenase would permit glutamine to contribute its amino-N as well as its amide-N to urinary ammonia. As seen in Table II the administration of NH_4Cl increased the activity of glutamic dehydrogenase by approximately threefold in rats, but decreased the activity slightly in dogs.

The participation of ammonia precursors other than glutamine in the increased formation of ammonia during NH_4Cl acidosis was also investigated. Davies and Yudkin (11) found that in addition to renal glutaminase, L-amino acid oxidase and glycine oxidase were increased in the kidneys of rats given HCl. Although, no L-amino acid oxidase has been found in dog kidney (31), infusions of amino acids are known to stimulate ammonia excretion in dogs (6, 10, 32). The probable pathway involved is transamination of the amino acid with γ-keto glutarate to form glutamate, which is then deaminated. The possibility that this pathway was responsible for the increased production of ammonia in acidotic dogs was studied. However, the administration of NH_4Cl produced no changes in the activity of glutamic-oxaloacetic transaminase in either dogs or rats (Table III).

DISCUSSION

Recent investigations of the physicochemical factors influencing ammonia excretion (6, 8, 33) support the hypothesis that ammonia transverses the tubule cell membrane as the unionized lipoid soluble NH_3, and that, except for very acid urines, equilibrium between cell and luminal NH_3 is attained. Since the NH_4^+ ion appears to be relatively nondiffusible across the cell membrane and is in rapid equilibrium with NH_3, the accumulation of NH_4^+ in the urine is dependent on urine pH, more accumulating in acid than in alkaline urines. In addition, any factor which changes the concentration of ammonia within the cell alters ammonia excretion more or less independently of urine pH. Thus, infusions of glutamine and other amino acids elevate ammonia excretion despite alkalinization of the urine (6, 32).

During the chronic administration of NH_4Cl cellular factors appear to be more important in regulating ammonia excretion than is urine pH. Animals receiving NH_4Cl excrete more ammonia at any given urine pH than do control animals, indicating that the concentration of ammonia in the tubule cell has risen, presumably due to increased production of ammonia.

The increased production of ammonia during NH_4Cl acidosis appears to be related primarily to the hydrolysis of glutamine (9), and to a lesser extent to the deamination of other amino acids

(9). In rats elevation of the activity of the renal ammonia-producing enzymes appears to be responsible for the accelerated production of ammonia (7, 11, 12). The administration of NH_4Cl produces striking increases in the activity of glutaminase I, glutaminase II, glutamic dehydrogenase, L-amino acid oxidase and glycine oxidase. In dogs, however, adaptation of the renal ammonia-producing enzymes does not participate in the regulation of ammonia excretion. In the present experiments, the administration of NH_4Cl failed to increase the activity of glutaminases I and II, glutamic dehydrogenase and glutamic oxaloacetic transaminase in dog kidney homogenates. Also the rate of glutamine hydrolysis by intact kidney slices was unchanged by the administration of NH_4Cl. These findings leave unexplained the mechanism for both the increased production of ammonia during acidosis and the increased extraction of glutamine from the renal arterial blood of acidotic dogs as reported by Van Slyke and associates (9).

Although the present studies would indicate that renal glutaminase is not an adaptive-type enzyme system in dogs, Iacobellis, Muntwyler and Griffin (34) have reported that glutaminase activity was increased in the kidneys of two potassium deficient dogs. This is similar to the adaptive increase in enzyme activity noted in hypokalemic rats by Seldin, Rector, Carter and Copenhaver (35) and Iacobellis and co-workers (36). However, the finding of elevated glutaminase activity in potassium deficient dogs could not be confirmed when the activity of glutaminase I in kidney biopsy samples from four hypokalemic dogs was compared with activity in the kidney biopsies from the same dogs during control periods (37).

In summary, the present studies reveal that the adaptation of renal enzymes, which constitutes an important regulatory mechanism of ammonia in rats and guinea pigs, is not a process common to all species. This finding may be of considerable significance with respect to the regulation of ammonia excretion in man. Ammonia excretion in man more closely resembles that of the dog in that the logarithm of ammonia excretion is linearly related to urine pH (8), and urinary ammonia is persistently reduced during chronic carbonic anhydrase inhibition (16). However, more direct studies are needed to clarify the role of the ammonia-producing enzymes in the regulation of ammonia excretion in man.

SUMMARY

An adaptive increase in ammonia excretion following the chronic administration of NH_4Cl is known to occur in the dog. This, as well as the decrease in ammonia excretion following chronic bicarbonate administration, has been confirmed.

The adaptive changes in ammonia excretion are not accompanied by alterations in the activities of any of the renal enzymes known to be involved directly or indirectly in the conversion of glutamine to ammonia. In contrast to findings in the rat, NH_4Cl did not increase the activity of glutaminase I, glutaminase II, glutamic dehydrogenase and glutamic oxaloacetic transaminase in kidney homogenates. Furthermore enzymatic hydrolysis of glutamine by intact kidney slices was unaltered.

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