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Adaptation of renal ammonia production in the diabetic ketoacidotic rat

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Adaptation of renal ammonia production in the diabetic ketoacidotic rat. Renal ammonia production was measured in diabetic, ketoacidotic rats. Rats were made diabetic by the i.v. injection of streptozotocin (150 mg/kg of body wt). They were maintained on insulin for 1 week. Ketoacidosis was induced by withdrawing insulin for 3 days. At that time, blood and kidney ketone body (D-β-hydroxybutyrate and acetoacetate) levels were markedly elevated (approx. 6 mM), and plasma total carbon dioxide concentration was strongly depressed (approx. 11 mM). Renal ammonia production (ammonia released into renal vein plus that excreted in the urine) was stimulated sevenfold by the diabetic ketoacidosis. In a separate study, we examined the effects of ketone bodies on renal ammonia production in ammonium-chloride-induced acidotic, nondiabetic rats. Infusion of β-hydroxybutyrate had no significant effect on either urinary ammonia excretion (at relatively constant urinary pH), total renal ammonia production, or renal glutamine extraction. In vitro studies showed that β-hydroxybutyrate (4.0 mM) markedly inhibited (61%) conversion of L-glutamine (0.6 mM) to ammonia by renal cortical slices prepared from normal rats. Inhibition was greatly reduced with slices prepared from kidneys of acidotic (ammonium-chloride-induced or diabetic ketoacidosis) rats. These results indicate that *(1)* renal ammonia production is markedly stimulated in diabetic ketoacidosis, and *(2)* in contrast to findings previously obtained by others in the acidotic dog, ketone bodies do not appear to inhibit renal ammonia production in vivo and only weakly in vitro in the acidotic rat.

Adaptation de la production rénale d'ammoniac chez le rat diabétique en acidocétose. La production rénale d'ammoniaque a été mesurée chez le rat diabétique en acidocétose. Les rats ont été rendus diabétiques par une injection i.v. de streptozotocine (150 mg/kg body wt) et traités par l'insuline pendant une semaine. L'acidocétose a été déclenchée par la suppresion de l'insuline pendant trois jours. A ce moment, les concentrations sanguines et rénales de corps cétoniques (D-β-hydroxybutyrate et acéto-acétate) étaient très augmentées (approximativement 6 mM) et le CO_2 total du plasma très abaissé (approximativement 11 mM). La production rénale d'ammoniac (ammoniac de la veine rénale plus ammoniaque de l'urine) était multipliée par 7. Dans un protocole différent, nous avons étudié les effets des corps cétoniques sur la production rénale d'ammoniac chez des rats non diabétiques, en acidose par le chlorure d'ammonium. La perfusion de β-hydroxybutyrate n'a eu d'effet significatif ni sur l'excétion urinaire d'ammoniaque (à pH relativement constant), ni sur la production rénale d'ammoniac ou l'extraction rénale de la glutamine. Des études in vitro ont montré que le β-hydroxybutyrate (4,0 mM) inhibe de façon importante (61%) la conversion de la L-glutamine (0,6 mM) en ammoniac par les tranches de cortex de rein préparées à partir de rats normaux. L'inhibition est moindre avec des tranches préparées à partir de rats en acidose (chlorure d'ammonium ou acidocétose diabétique). Ces résultats indiquent que *(1)* la production rénale d'ammoniac est fortement stimulée au cours de l'acidocétose diabétique et, *(2)* contrairement aux résultats obtenus par d'autres chez le chien en acidose, les corps cétoniques ne paraissent pas inhiber la production rénale d'ammoniac in vivo, et seulement faiblement in vitro, chez le rat en acidose.

The role of the kidney in combating metabolic acidosis has been a subject of considerable interest for many years. The classical experimental model for studying this problem has been metabolic acidosis induced by administration of acidifying salts such as ammonium chloride. In real life, this form of metabolic acidosis is rare. An important form of metabolic acidosis, however, is diabetic ketoacidosis. Although this disorder is a potential problem in many diabetic patients, little research has been done on the response of the kidney to this kind of acid-base imbalance.

In 1933, Atchley et al [1] measured the renal response of a patient to diabetic acidosis. They found that the kidneys responded to the acidosis with a marked increase in excretion of titratable acid and ammonia. Because ammonia excretion continued to increase as the ketoacidosis progressed but urine pH remained relatively constant, at a depressed level, it may be inferred that there was an adaptation of renal ammonia production. Because the level of blood ketone bodies was markedly elevated, it is reasonable to conclude that elevated levels of β-hydroxybutyric and acetoacetic acids contributed, at least in part, to the adaptation of ammonia excretion in diabetic acidosis. Lemieux et al [2] reported,

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however, that ketone bodies inhibit renal ammonia production in acidotic dogs, and Roxe, Schreiner, and Preuss [3] reported significant inhibition of ammonia production by a mixture of β-hydroxybutyrate and lactate in rat kidney slices incubated with glutamine. Lemieux et al [4] recently reported that both β-hydroxybutyrate and acetoacetate inhibited ammoniagenesis in dog cortical slices incubated with glutamine. Thus, it is of interest to determine whether ammonia production adapts in diabetic ketoacidosis. Therefore, we measured renal ammonia production in diabetic ketoacidotic rats. In addition, we assessed the effects of ketone bodies on renal ammonia production and renal glutamine extraction in nondiabetic, ammonium-chloride-induced acidotic rats.

Methods

Animals and treatments. Male, Sprague-Dawley rats (Caesarian derived), each ranging in weight from 170 to 360 g, were obtained from Charles River Labs and maintained on Purina Rat Chow and water supplied *ad lib.* Metabolic acidosis was induced by intragastric administration of ammonium chloride (10 mEq/kg of body wt) in a volume of water equal to 2.5% of the body weight. The rats were given morning and evening doses for 2 days and a final dose on the morning of day 3, just prior to anesthesia.

Rats were made diabetic by injection of streptozotocin (150 mg/kg of body wt; Sigma) dissolved in 0.01 M citrate buffer (pH, 4.4), and they were given 5% glucose as their sole drinking fluid for 48 hours [5]. They were weighed each day and fed *ad lib.* Twenty-four hours after the streptozotocin injection they were injected s.c. with insulin (3 U; Isophane®; Lilly). The rats were kept on insulin for the next 5 days with blood glucose levels monitored during this period. The dose of insulin was adjusted to maintain the gain in body weight as described by Blackshear and Alberti [5]. Diabetic ketoacidosis was induced by withdrawing insulin injections for 72 hours.

Renal ammonia production measured in vivo. Rats were anesthetized with Inactin (150 mg/kg of body wt; Promonta-Hamburg, Henley and Co., New York). An endotracheal tube was implanted, and the bladder, carotid artery, and jugular vein were cannulated with polyethylene tubing. Rectal temperature was maintained at 38° to 39° C by keeping the rats on a heated platform. One hour before the urine collection was started, ^{3}H-paraminohippurate (0.4 μCi/100 g of body wt; New England Nuclear) was injected i.v. as a priming dose, followed by a constant infusion (3 μCi/hr) in 0.9% sodium chloride (1.05 ml/hr).

Following the 1-hour equilibration period, urine was collected for two 30-min periods. The urine was weighed, assayed for pH with a pH meter, and analyzed for ^{3}H-paraminohippurate and ammonia. Blood samples (0.5 ml each) were taken from the carotid artery during the first and second urine collection periods. An aliquot was taken for hematocrit determination, and plasma was immediately separated from the remainder by centrifugation. An aliquot of plasma was analyzed for total carbon dioxide content. The remaining plasma was deproteinized with 10% trichloroacetic acid and the supernatant analyzed for ^{3}H-paraminohippurate. At the end of the second urine collection period, 1.5 ml of blood was taken from the carotid artery. An aliquot was mixed immediately with an equal volume of 20% cold trichloroacetic acid and centrifuged, and the supernatant was analyzed for ammonia. The other aliquot was centrifuged, and the plasma was deproteinized with trichloroacetic acid and analyzed for ^{3}H-paraminohippurate. The ^{3}H-paraminohippurate clearance was taken as a measure of effective renal plasma flow. Total renal blood flow was calculated with the corresponding hematocrit value [6]. The left kidney was then exposed with a midline incision, and 1.5 ml of blood was removed slowly (approximately 1 to 2 min) from the left renal vein by a 2.5-ml syringe fitted with a 25-gauge 0.5-inch needle. The blood was mixed immediately with an equal volume of cold 20% trichloroacetic acid and analyzed for ammonia. Immediately after blood was drawn from the left kidney, the right kidney was extirpated and freeze-clamped with aluminum tongs precooled in liquid nitrogen.

In experiments on the effects of β-hydroxybutyrate on renal ammonia production, immediately following the first 30-min urine collection period, we changed the infusate from 0.9% sodium chloride to 0.9% sodium chloride containing sodium DL-β-hydroxybutyrate (Sigma, approximately 98% pure), as shown in Fig. 1. Urine was collected for three 30-min periods, during which time β-hydroxybutyrate was infused at a rate of 350 μmoles/kg·min for the entire 90 min, or the rate was changed to 175 μmoles/kg·min after the first 30 min. Blood samples were taken and analyzed for plasma total carbon dioxide and ^{3}H-PAH and for blood ammonia and ketone bodies at the times indicated in Fig. 1. Urine samples were analyzed for ammonia and ^{3}H-PAH.

Renal glutamine extraction. Arteriovenous

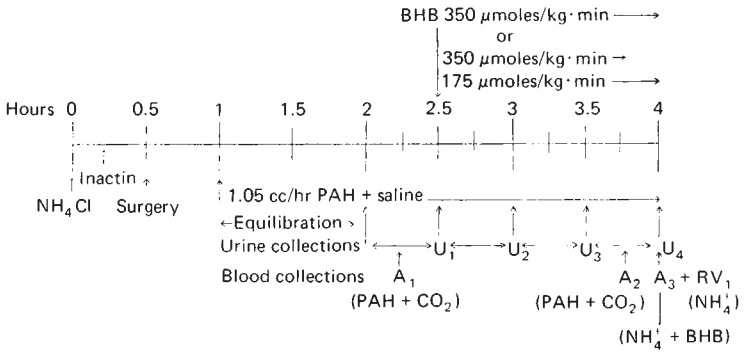

Fig. 1. *Experimental protocol for studies on effects of β-hydroxybutyrate (BHB) on renal ammonia excretion and production in NH_4Cl-acidotic rats.* Blood samples were analyzed for compounds shown in parentheses. Urine samples (U_1 to U_4) were analyzed for ammonia and PAH. Abbreviations are A, arterial blood; RV, renal venous blood; U, urine.

plasma glutamine concentration differences were measured in vivo. Rats were prepared and treated as shown in Fig. 1. Arterial samples were taken both at the midpoint (before switching to β-hydroxybutyrate) and at the end of the experiment. Renal venous samples were drawn only at the end of the experiment. The blood samples were centrifuged immediately (within 1 min), and the proteins were precipitated from the resulting plasma with perchloric acid. Perchloric acid supernatants were neutralized with potassium hydroxide, and the potassium perchlorate removed by centrifugation. The neutral supernatant solutions were assayed for glutamine with a bacterial glutaminase preparation (Sigma, Grade V) and glutamate dehydrogenase (Sigma, Type II) as described by Lund [7].

Ammonia production from glutamine by renal cortical slices. Rats were killed by a blow on the head and partially exsanguinated. The kidneys were removed quickly, decapsulated, and placed in ice-cold Krebs-Ringer bicarbonate (KRB) solution gassed with 95% oxygen and 5% carbon dioxide. Thin slices weighing 40 to 50 mg were cut free-hand with a safety razor blade, rinsed in cold KRB before being weighed, and added to 25-ml flasks containing 3 ml cold KRB and 0.6 mM L-glutamine (Sigma). The flasks were gassed for 1 min with 95% oxygen and 5% carbon dioxide and incubated for 60 min at 37° C in a shaking (120 oscillations per minute) water bath. In experiments with β-hydroxybutyrate, we added 4.0 mM sodium DL-β-hydroxybutyrate to the KRB along with L-glutamine.

At the end of the incubation, the reaction was stopped by addition of perchloric acid. The mixture was homogenized, centrifuged, and neutralized with potassium perchlorate. The potassium-perchlorate-free supernatant was analyzed for ammonia. Values for ammonia production from glutamine were corrected for ammonia production from endogenous substrates and ammonia generated by the spontaneous decomposition of glutamine.

Analyses. Ammonia was determined by a microdiffusion-colorimetric technique [8]. D-β-Hydroxybutyrate and acetoacetate were analyzed in perchlorate extracts of whole blood, and freeze-clamped kidneys were analyzed by enzymatic techniques [9, 10]. Enzymes and substrates were purchased from Sigma Chemical Co. (St. Louis). ^{3}H-paraminohippurate was assayed with a liquid scintillation counter (Packard Tri Carb) and corrected for differences in light quenching between samples. Total carbon dioxide in plasma was determined manometrically with a Natelson Microgasometer (A. H. Thomas). Statistical significance of differences between group means was determined by student's *t* test.

Results

General effects of diabetic ketoacidosis on blood and renal parameters. Table 1 shows data on some related blood parameters in control and diabetic rats. Data on ammonium-chloride-induced acidotic rats are included for qualitative, but not quantitative comparison with diabetic, acidotic rats. As shown in Table 1, hematocrit was somewhat elevated in diabetic rats taken off insulin for 3 days. The slightly elevated hematocrit was probably due to the loss of body fluids associated with glucosuria and ketonuria. Plasma total carbon dioxide was markedly depressed in this group. The carbon dioxide depression exceeded the elevation in blood ketone bodies (β-hydroxybutyrate and acetoacetate) by about 5 mM (Table 1). The relatively high concentration of plasma glucose in untreated rats was

Table 1. Effects of diabetic ketoacidosis and ammonium

Condition of rats	Weight *g*	Hct *%*	Plasma CO_2 *mM*	Plasma glucose *mg/dl*	Blood ketones D-β-Hydroxybutyrate *μmoles/ml*
Untreated	197 ± 6	43 ± 1	26.4 ± 0.5	177 ± 16	0.036 ± 0.002
Diabetic, off insulin 3 days	202 ± 8	45 ± 1	15.5 ± 1.4[b]	468 ± 37[b]	4.3 ± 0.6[b]
Diabetic, on insulin	267 ± 17[b]	42 ± 1	28.0 ± 1.0	109 ± 43	—
Acidotic (NH_4Cl) 2 days	250 ± 28	42 ± 1	19.1 ± 0.7[b]	158 ± 24	—

[a] Values are means ± SEM of 6 to 8 rats per group.
[b] $P < 0.01$ compared to values in untreated rats.
[c] $P < 0.05$ compared to values in untreated rats.

probably due to the postabsorptive state of the animals.

Table 1 also shows data on some related renal parameters in rats that were anesthetized with Inactin and infused with 0.9% sodium chloride (approximately 1.0 ml/hour). Urine pH was significantly lowered in diabetic rats off insulin for 3 days and in rats receiving ammonium chloride. Renal blood flow was not significantly altered in any of the treated groups. Urine flow was significantly elevated in diabetic rats off insulin; this diuresis was likely due to the elevated concentrations of glucose and ketone bodies in the glomerular filtrate. A less marked but significant elevation in urine flow was observed in rats made acidotic by ammonium chloride and in diabetic rats on insulin. As shown in Table 1, D-β-hydroxybutyrate concentration was elevated in the kidneys of diabetic, ketotic rats to about the same degree as it was in the blood. Renal acetoacetate concentrations, however, although markedly elevated in ketotic rats, were significantly lower than were the corresponding blood concentrations.

Renal ammonia production. Renal ammonia production was considered to be equal to the sum of ammonia released into the renal vein plus that excreted in the urine. Table 2 shows the effects of diabetic ketoacidosis on renal ammonia production. Ammonia production was elevated approximately sevenfold in diabetic rats off insulin for 72 hours compared to untreated rats or diabetic rats kept on insulin throughout the experimental period. Renal ammonia production in a group of acidotic (ammonium chloride) nondiabetic rats was measured for qualitative comparison with renal ammonia production in diabetic, ketoacidotic rats. Figure 2 shows that in both groups of acidotic animals, significant portions of the extra ammonia produced are released into both the urine and the renal vein. Thus, there appears to be no qualitative difference in the pattern of distribution of ammonia produced in the two acidotic groups.

Effect of β-hydroxybutyrate on renal ammonia excretion and production. The large increase in renal ammonia production in diabetic, ketotic rats seemed to contradict the idea that ketone bodies inhibit renal ammonia production [2–4]. Thus, we tested the effects of these compounds on renal ammonia production in nondiabetic rats made acidotic by ammonium chloride administration for 2 days and assayed on day 3 of acidosis. We found it technically impossible to sample blood from the renal vein of rats more than once without seriously impairing renal function. Therefore, we measured ammonia excretion before and after infusion of β-hydroxybutyrate. The solution of β-hydroxybutyrate infused was adjusted to a pH of 4.4 or 5.0 to prevent the development of alkalosis as a result of oxidation of β-hydroxybutyrate. Because urine pH remained

Table 2. Effects of diabetic ketoacidosis and ammonium chloride acidosis on renal ammonia production[a]

Condition of rats	Renal vein NH_4^+ release: V-A *μmoles/ml*	Renal vein NH_4^+ release: V-A × RBF *μmoles/100 g body wt · hr*	Urinary NH_4^+ release *μmoles/100 g body wt · hr*	Total NH_4^+ production *μmoles/100 g body wt · hr*
Untreated	0.037 ± .004	7.8 ± .8	11.2 ± 1.2	19.0 ± 1.7
Diabetic off insulin 3 days	0.354 ± .025[b]	76.2 ± 9.9[b]	65.4 ± 10.1[b]	141.6 ± 14.1[b]
Diabetic on insulin	0.027 ± .006	4.0 ± .8[b]	12.9 ± 3.9	16.9 ± 4.4
Acidotic (NH_4Cl) 2 days	0.179 ± .035[b]	34.8 ± 5.1[b]	51.4 ± .7[b]	86.2 ± 10.1[b]

[a] Values are means ± SEM of 6 to 8 rats per group.
[b] $P < 0.01$ compared to values in untreated group.

chloride acidosis on related blood and renal parameters[a]

Acetoacetate	Urine pH	RBF *ml/100 g body wt · min*	Urine flow rate *ml/100 g body wt · hr*	Renal ketones: D-β-Hydroxybutyrate *μmoles/g*	Renal ketones: Acetoacetate *μmoles/g*
0.020 ± 0.003	6.2 ± 0.1	3.72 ± 0.35	0.158 ± 0.013	0.052 ± .058	0.017 ± .010
2.0 ± 0.5[b]	5.7 ± 0.1[b]	3.53 ± 0.28	0.428 ± 0.057[b]	5.78 ± 1.23[b]	0.210 ± .060[b]
—	6.0 ± 0.1	2.63 ± 0.44	0.259 ± 0.037[c]	—	—
—	5.8 ± 0.1[c]	3.59 ± 0.68	0.266 ± 0.033[c]	—	—

relatively constant throughout the experiment, we assumed that under these conditions ammonia excretion reflected renal ammonia production. In addition, we measured renal ammonia production at the end of the infusion and compared it to production measured in a group of ammonium-chloride-induced acidotic rats treated in a similar manner but infused with saline. The experimental protocol is shown in Fig. 1. As shown in Table 3, infusion of DL-β-hydroxybutyrate elevated plasma D-β-hydroxybutyrate (to 2.5 mM) and acetoacetate (to 0.2 mM) with no significant effect on either plasma carbon dioxide or urine pH. Urine flow increased progressively during the infusion of ketone body. It can be seen in Table 3 and Fig. 3 that elevation of blood β-hydroxybutyrate concentration in acidotic rats had no significant effect either on ammonia excretion assessed by using each rat as its own control or on renal ammonia production measured in different rats (Fig. 3). Some hemolysis was observed in some rats infused with DL-β-hydroxybutyrate; there was, however, no apparent difference in renal function or ammonia production in rats with hemolysis and those without. Thus, ketone bodies do not appear to depress renal ammonia production in nondiabetic acidotic rats.

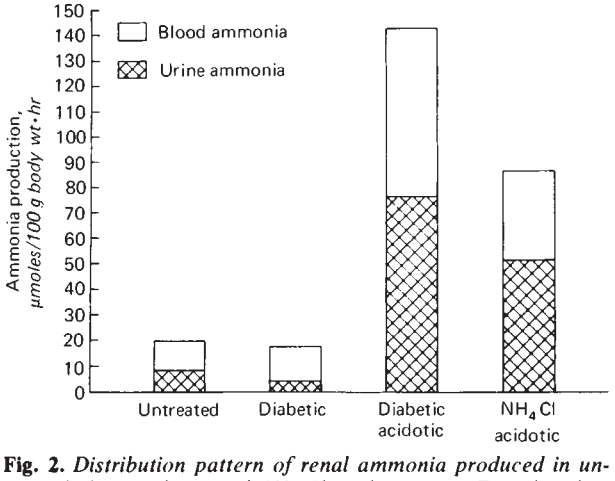

Fig. 2. *Distribution pattern of renal ammonia produced in untreated, ketoacidotic and NH_4Cl-acidotic rats.* Data is taken from Table 2.

Effect of β-hydroxybutyrate on renal glutamine extraction. Renal glutamine extraction was measured in ammonium-chloride-induced acidotic (2 days) rats prepared and infused with saline as shown in Fig. 1. As shown in Table 4, there was significant extraction of glutamine from renal arterial plasma; arteriorvenous glutamine concentration difference was 0.16 μmoles/ml. This value is nearly identical to the plasma glutamine arteriovenous concentration difference (0.17) obtained by Squires, Hall, and Brosnan [11] in chronically acidotic rats. A separate group of rats was prepared in a similar fashion, but the infusion was switched from saline to saline plus sodium β-hydroxybutyrate for the last 90 min of the experiment. Blood D-β-hydroxybutyrate concentration was markedly elevated in this group, but there was no change in renal glutamine extraction. Consistent with the lack of effect of the ketone body on renal glutamine extraction was the observation that plasma glutamine concentration did not change following infusion of β-hydroxybutyrate (period 1 vs. period 4, Table 4). There was significant extraction of D-β-hydroxybutyrate under these conditions (Table 4). The blood arteriovenous D-β-hydroxybutyrate concentration difference was 1.38 μmoles/ml, a value which is statistically significant when tested by paired-data analysis. The renal concentration of D-β-hydroxybutyrate at the end of the infusion period was 4.8 ± 0.8 μmoles/g of kidney (mean ± SEM).

In a separate set of experiments (not shown), we found that there was significant glutamine extraction by the kidneys of diabetic ketoacidotic rats despite the presence of a markedly elevated concentration of β-hydroxybutyrate. The arteriovenous plasma concentration difference of glutamine in the kidneys of these 7 rats was 0.13 ± 0.05 μmoles/ml,

Table 3. Lack of effect of β-hydroxybutyrate on renal ammonia excretion and production in acidotic rats[a]

	Period 1 Control	Period 2 BHB[b]	Period 3 BHB[b]	Period 4 BHB[b]
Plasma CO_2, mM	22 ± 1	—	—	19 ± 3
Blood D-β-hydroxybutyrate, mM	—	—	—	2.5 ± 0.5
Blood acetoacetate, mM	—	—	—	0.2 ± 0.1
Urine pH	5.6 ± 0.03	5.4 ± 0.1	5.3 ± 0.1	5.5 ± 0.1
Urine flow, *ml/100 g body wt · hr*	0.45 ± 0.09	0.45 ± 0.06	0.80 ± 0.16	1.36 ± 0.31
Urine NH_4^+ excretion[c], *μmoles/100 g body wt · hr*	61 ± 6	62 ± 5	66 ± 4	63 ± 2
RBF, *ml/100 g body wt · hr*	4.36 ± 0.24	—	—	4.18 ± 0.55
Renal V-A NH_4^+, mM	—	—	—	0.17 ± 0.03
V-A NH_4^+ × RBF, *μmoles/100 g body wt · hr*	—	—	—	40 ± 7
Renal NH_4^+ production, *μmoles/100 g body wt · hr*	—	—	—	103 ± 8

[a] Values are means ± SEM of 7 rats.

[b] D-β-Hydroxybutyrate (pH 4.4 or 5.0) was infused at a rate of 350 μmoles/kg · min during period 2 and at the same or half (175 μmoles/kg · min) the rate during periods 3 and 4.

[c] NH_4^+ refers to total ammonia (NH_3 + NH_4^+).

and the arterial blood concentration of ketone bodies (β-hydroxybutyrate and acetoacetate) was 6.0 mM.

Ammonia production from glutamine by renal cortical slices. Roxe et al [3] have reported significant inhibition of ammonia production by β-hydroxybutyrate (and lactate) in kidney slices (from untreated rats) incubated with glutamine. In contrast, in the present study we found that elevation of blood β-hydroxybutyrate concentration did not inhibit renal ammonia production or glutamine extraction in vivo when tested in rats made acidotic with ammonium chloride or in diabetic ketoacidosis. In an attempt to resolve the discrepancy between our in vivo results with those obtained previously in vitro, we tested the effects of sodium DL-β-hydroxybutyrate (BHB) on ammonia production from L-glutamine by cortical slices prepared from kidneys of untreated, ammonium-chloride-induced acidotic and diabetic ketoacidotic rats. Slices were incubated with a physiologic concentration (0.6 mM) of L-glutamine and a concentration of BHB (4 mM DL-BHB) found in diabetic ketoacidotic rats and in ammonium-chloride-induced acidotic rats infused with BHB. As shown in Table 5, BHB inhibited ammonia production approximately 60% in slices prepared from normal rats. This percent inhibition is similar (57%) to that reported by Roxe et al [3] in experiments with renal cortical slices incubated with 5.0 mM BHB and 0.6 mM L-glutamine. As shown in Table 5, we found that the percent inhibition by BHB, but not the absolute magnitude of inhibition, was reduced when the ketone body was tested on cortical slices prepared from kidneys of rats made acidotic by ammonium chloride. Both percent and absolute inhibition were nearly completely abolished in cortical slices from diabetic ketoacidotic rats. Thus, previous exposure of rats to diabetic ketoacidosis eliminates the inhibitory effect of BHB on ammonia production. Ammonium chloride acidosis reduces the percent inhibition but does not eliminate it. The rate of ammonia production in slices incubated in the presence of BHB is still significantly lower ($P < 0.01$) than the rate observed in the absence of the inhibitor.

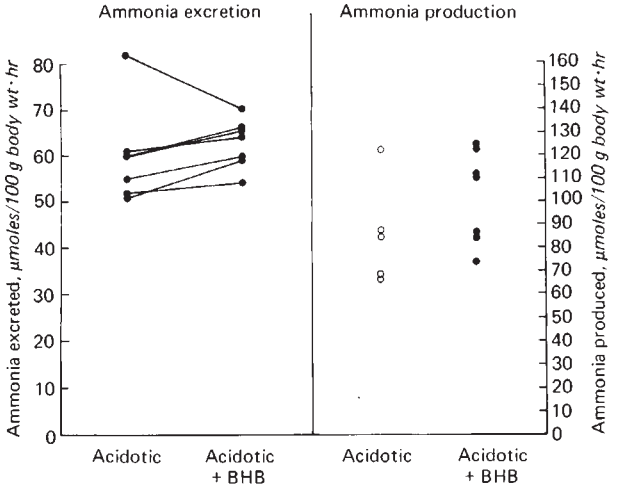

Fig. 3. *Lack of effect of* DL-β-*hydroxybutyrate (BHB) infusion on renal ammonia excretion and production in NH_4Cl-acidotic rats.* Points shown on left side of figure represent values obtained in individual rats before and after infusion of BHB (collection periods 1 and 4, Fig. 1). *Open circles* on right side of figure are values obtained in individual NH_4Cl-acidotic rats (Table 2); *closed circles* are values obtained in individual NH_4Cl-acidotic rats infused with DL-β-hydroxybutyrate (period 4, Fig. 1).

Discussion

The results obtained in this study show that diabetic ketoacidosis is a strong stimulus of renal ammonia production in the rat. Ammonia production rose approximately sevenfold during the 3-day peri-

Table 4. Arterial concentrations and renal arteriovenous concentration differences of glutamine and β-hydroxybutyrate (BHB) in acidotic rats infused with sodium DL-β-hydroxybutyrate (BHB)[a]

Group	Plasma concentrations			Blood concentrations	
	Arterial glutamine *μmoles/ml*		A-V difference in glutamine *μmoles/ml*	Arterial D-BHB *μmoles/ml*	A-V difference in D-BHB *μmoles/ml*
	Period 1	Period 4	Period 4	Period 4	Period 4
NH_4Cl acidotic	0.56 ± 0.01 (7)	0.57 ± 0.06 (11)	0.16 ± 0.2[c]	—	—
NH_4Cl acidotic infused with BHB[b]	0.76 ± 0.03 (10)	0.77 ± 0.06 (10)	0.16 ± 0.03[c] (10)	4.17 ± 0.26	1.38 ± 0.18[c]

[a] Values are means ± SEM, with the numbers of animals tested shown in parentheses. Periods refer to time periods shown in Table 3.
[b] DL-β-hydroxybutyrate (pH, 5.0) was infused at a rate of 350 μmoles/kg·min during the last 90 min of the experiment (periods 2 to 4).
[c] Significantly different from zero ($P << 0.001$) when tested by paired-data analysis

Table 5. Effect of sodium DL-β-hydroxybutyrate (BHB) on ammonia production from L-glutamine by renal cortical slices[a]

Previous animal treatment	Additions to incubation medium	Ammonia production *μmoles/100 mg slice·hr*	Percent inhibition by BHB
None	L-glutamine (0.6 mM)	0.64 ± 0.08 (13)	
	L-glutamine (0.6 mM) + BHB (4.0 mM)	0.25 ± 0.05[b] (13)	61
NH_4Cl acidosis (2 days)	L-glutamine (0.6 mM)	1.82 ± 0.7 (9)	
	L-glutamine (0.6 mM) + BHB (4.0 mM)	1.39 ± 0.09[b] (9)	24
Diabetic ketoacidosis (3 days)	L-glutamine (0.6 mM)	1.38 ± 0.14 (7)	
	L-glutamine (0.6 mM) + BHB (4.0 mM)	1.18 ± 0.17 (7)	14

[a] Values are means ± SEM with the numbers of animals from which kidney slices were prepared shown in parentheses. Slices were incubated in Krebs-Ringer bicarbonate medium gassed with 95% oxygen and 5% carbon dioxide for 1 hour at 37° C.
[b] Significantly different from control group without BHB ($P < 0.01$).

od in which the diabetic animals were taken off insulin injections. Blackshear and Alberti [5] have shown that rats made diabetic by streptozotocin become ketoacidotic between days 1 and 2 after insulin withdrawal. Thus, the large increase in renal ammonia production occurs during a 2-day period of ketoacidosis. These results are consistent with the observation of Atchley et al [1] who found a fourfold to fivefold increase in renal ammonia excretion in a diabetic patient from whom insulin was withdrawn for 3 days.

The specific stimuli of renal ammonia production in diabetic ketoacidosis are not known. It is possible that the ketone bodies (β-hydroxybutyrate and acetoacetate) themselves are the stimuli. Note in Table 1, however, that plasma carbon dioxide was depressed approximately 11 mM (26.4 vs. 15.5) in diabetic rats off insulin for 3 days, whereas the total β-hydroxybutyrate and acetoacetate concentrations were elevated only 6 mM. Increased production of other endogenous acids, such as sulfuric acid, that arise as a result of increased protein degradation (muscle wasting) in uncontrolled diabetes [12] also contributed to the severity of the metabolic acidosis and the stimulation of renal ammonia production. Atchley et al [1] found that sulfate excretion increased nearly 70% and that total nitrogen excretion almost doubled in their diabetic, ketoacidotic patients. These observations are consistent with the idea that endogenous acids derived from increased protein breakdown must make an important contribution to the stimulation of renal ammonia production in diabetic ketoacidosis.

The response of renal ammonia production to ketoacidosis in the diabetic rat is inconsistent with the reported inhibitory action of β-hydroxybutyrate and acetoacetate on renal ammonia production in the intact kidney of the acidotic dog [2] and in renal cortical slices of the rat [3] and the dog [4]. The simplest explanation for this inconsistency is that the renal ammonia producing system becomes refractory to the inhibitory action of ketone bodies during diabetic acidosis. To support this idea, we attempted to demonstrate that the ketone body β-hydroxybutyrate infused into nondiabetic, acidotic rats inhibited renal ammonia production and glutamine

extraction. β-hydroxybutyrate infusion, however, had no detectable effect on either renal ammonia excretion and production or on arteriovenous plasma glutamine concentration differences in nondiabetic acidotic rats. The lack of effect is somewhat puzzling in view of the inhibitory action of ketone bodies on these parameters in vivo in the dog and on ammonia production from glutamine by rat and dog kidney preparations in vitro. The experiments performed in this study on the effects of β-hydroxybutyrate on renal ammonia production from glutamine in rat kidney slices, however, help to explain the different results obtained in vivo and in vitro in the rat.

In agreement with the previous observations of Roxe et al [3], our observations were that β-hydroxybutyrate added to the incubation medium markedly inhibited (by approximately 60%) the production of ammonia from glutamine by renal cortical slices from normal rats. Previous induction of ammonium chloride for 2 days or diabetic ketoacidosis for 3 days, however, modified the inhibitory effects of the ketone body on renal ammonia production. The inhibitory effect of the ketone body was nearly completely eliminated in renal cortical slices from ketoacidotic rats, and the percent inhibition by β-hydroxybutyrate was markedly reduced (from 61% down to 24%) in slices from rats treated with ammonium chloride. Thus, in some way, in vivo metabolic acidosis changed the inhibitory effects of β-hydroxybutyrate on the glutamine deamidating/deaminating system, as measured in vitro.

The manner in which acidosis modifies the inhibitory effect of β-hydroxybutyrate on ammonia production is not clear. Two possibilities are: *(1)* that ammonia is generated from glutamine by the same pathways in renal cortical slices from normal and acidotic animals but that the sensitivity of the pathways to inhibition by β-hydroxybutyrate is reduced by acidosis; or *(2)* that the relative contributions of different pathways to ammonia formation are not the same in slices from normal vs. acidotic rats and that the altered sensitivity to β-hydroxybutyrate inhibition reflects these changes. With regard to the second possibility, Schoolworth, Nazar, and LaNoue have recently reported [13] that in rat renal mitochondria isolated from normal rats, ammonia is produced from glutamine mainly by deamidation; deamination of glutamate formed from glutamine contributes only a minor fraction to ammonia formation, because most of the glutamate is metabolized via transamination. In contrast in mitochondria from acidotic rats, glutamate deamination is augmented and contributes almost as much as glutamate deamidation to ammonia formation. If the augmented glutamate deamination is relatively insensitive to β-hydroxybutyrate inhibition or leads to a decrease in the sensitivity of glutamine deamidation to inhibition, this could account for the acidosis-induced modification of β-hydroxybutyrate inhibition. A difference in relative importance of renal glutamyl transferase and glutaminase in ammonia formation in normal vs. acidotic rats [14] might also contribute to the change in sensitivity of renal ammoniagenesis to BHB inhibition.

Lemieux et al [4] proposed that the oxidative metabolism of the ketone bodies is the cause of their inhibitory action on renal ammonia production. β-hydroxybutyrate oxidation may compete for NAD^+ with oxidation of other metabolic intermediates. For example, elevation of ketone bodies could inhibit oxidation of glutamate in renal mitochondria by keeping more of the nucleotide in the reduced form (NADH), thereby elevating the concentration of this glutaminase inhibitor [8] in the mitochondria. Acidosis could conceivably decrease the inhibitory effects of ketone bodies by increasing the ratio of NAD^+ to NADH within the mitochondrial matrix; this might counteract the effect of ketone body oxidation on the NAD^+/NADH ratio. Indeed Preuss [15] has reported that the NAD^+/NADH ratio in rat renal mitochondria rises almost twofold during metabolic acidosis. Neither Hems and Brosnan [15] nor Goldstein and Harley-DeWitt [16], however, have been able to confirm this observation. Thus, it is still uncertain whether the NAD^+/NADH ratio does change during in vivo alterations in acid-base balance. In any case, it is clear that until more information is available on the effects of ketone bodies on the NAD^+/NADH ratio, as well as their effects on the many other metabolic intermediates affecting renal glutamine deamidation/deamination, it is not possible to either explain the inhibitory action of ketone bodies on renal ammonia production or to understand how metabolic acidosis alters the sensitivity of the kidney to this inhibition.

The failure to detect inhibition of renal ammonia production by β-hydroxybutyrate in rats treated with ammonium chloride in vivo when inhibition by the ketone body, albeit modest, is clearly demonstrable in vitro may be due to a difference in sensitivity of the techniques used in the two assays. It is conceivable that the complexities of the method used to determine renal ammonia production in vivo make it relatively more difficult to detect small

changes in renal ammonia production compared to the simpler, better controlled in vitro assay. Indeed, β-hydroxybutyrate may have produced a modest, undetected inhibition of renal ammonia production in vivo in the rat, similar in nature but not in magnitude to that observed in the dog. If so, then differences between the dog and rat in this regard may be more quantitative than qualitative.

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