Role of the Endocrine Pancreas in the Kalemic Response to Acute Metabolic Acidosis in Conscious Dogs

Horacio J. Adrogué, Zvi Chap, Toshihiko Ishida, and James B. Field
Department of Medicine, Baylor College of Medicine, Division of Nephrology, The Methodist Hospital, and Diabetes Research Center, St. Luke's Episcopal Hospital, Division of Endocrinology and Metabolism, Houston, Texas 77030

Abstract

Metabolic acidosis due to organic acids infusion fails to elicit hyperkalemia. Although plasma potassium levels may rise, the increase is smaller than in mineral acid acidosis. The mechanisms responsible for the different effects of organic acid acidosis and mineral acid acidosis remain undefined, although dissimilar hormonal responses by the pancreas may explain the phenomena. To test this hypothesis, beta-hydroxybutyric acid (7 meq/kg) or hydrochloric acid (3 meq/kg) was infused over 30 min into conscious dogs (n = 12) with chronically implanted catheters in the portal, hepatic, and systemic circulation, and flow probes were placed around the portal vein and hepatic artery. Acid infusion studies in two groups of anesthetized dogs were also done to assess the urinary excretion of potassium (n = 14), and to evaluate the effects of acute suppression of renal electrolyte excretion on plasma potassium and on the release/uptake of potassium in peripheral tissues of the hindleg (n = 17). Ketoacid infusion caused hypokalemia and a significant increase in portal vein plasma insulin, from the basal level of 27±4 μU/ml to a maximum of 84±22 μU/ml at 10 min, without changes in glucagon levels. By contrast, mineral acid acidosis of similar severity resulted in hyperkalemia and did not increase portal insulin levels but enhanced portal glucagon concentration from control values of 132±25 pg/ml to 251±39 pg/ml at 40 min. A significant decrease in plasma glucose levels due to suppression of hepatic release was observed during ketoacid infusion, while no changes were observed with mineral acid infusion. Plasma flows in the portal vein and hepatic artery remained unchanged from control values in both acid infusion studies. Differences in renal potassium excretion were ruled out as determinants of the disparate kalemic responses to organic acid infusion compared with HCl acidosis. Evaluation of the arteriovenous potassium difference across the hindleg during ketoacid infusion demonstrates that peripheral uptake of potassium is unlikely to be responsible for the observed hypokalemia. Although the tissue responsible for the different kalemic responses could not be defined with certainty, the data are compatible with an hepatic role in response to alterations in the portal vein insulin and/or glucagon levels in both acid infusion studies. We propose that cellular uptake of potassium is enhanced by hyperinsulinemia in ketoacid infusion, and release of potassium results from increased glucagon levels in HCl acidosis. Whether the changes in plasma potassium that other types of organic acid acidosis produce are accounted for by a similar hormonal mechanism remains to be determined.

Introduction

Cellular shifts of potassium, instead of changes in its body stores, are considered to be responsible for the altered levels of plasma potassium during acute acid-base disturbances (1–4). Fluxes of potassium among the fluid compartments were previously thought to be solely determined by changes in acidity. Other factors, however, have been identified as modulators of the plasma potassium response (5); prominent among them is the nature of the anion escorting the protons responsible for acidemia (6–9). In sharp contrast with mineral acid acidosis that consistently leads to hyperkalemia, acute organic acid acidosis is not associated with a significant increase in plasma potassium (10). Differences in the ability of the anion to penetrate the intracellular compartment have been cited as being responsible for the disparate kalemic responses to acidemia (11, 12). However, organic acid acidosis not only failed to elicit hyperkalemia but induced hypokalemia (9, 13). The decrease in plasma potassium, therefore, cannot be explained by the currently held hypothesis.

Several hormones, especially those of pancreatic origin, play a major role in internal potassium balance (14, 15). Thus, it is conceivable that the diverse kalemic response to mineral and nonmineral acids may be mediated by differences in hormonal levels. Oster and co-workers evaluated this possibility by measuring insulin and glucagon in peripheral blood (10) and found comparable plasma levels in both forms of metabolic acidosis. However, since the hepatic extraction of insulin is quite different from that of glucagon (16), the levels of these hormones in peripheral blood may not accurately reflect their portal vein concentration or the magnitude of pancreatic secretion.

Since neither the mechanisms involved, nor the tissue responsible for the different kalemic responses to mineral and organic acid acidosis is known, the present studies attempted to shed further light on both issues. Firstly, insulin and glucagon concentration were examined in both peripheral blood and in the portal circulation, and blood flow in the splanchnic bed was measured, so that absolute levels of pancreatic hormonal secretion and hepatic extraction of these hormones could be estimated. Secondly, the potassium balance across the liver and in the peripheral tissues of the hindleg was evaluated in both types of acidosis. Thirdly, acid infusion studies were performed in animals with intact renal function and after acute ureteral obstruction to evaluate the effects of differences in external potassium balance. We infused ketoacid as a model of organic acid acidosis because the various forms

Correspondence and reprint requests should be addressed to Dr. Horacio J. Adrogué, Division of Nephrology, The Methodist Hospital, Houston TX 77030.

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of ketoacidosis (fasting, diabetic, and alcoholic) represent the most frequently observed organic acid acidoses in humans. The results led us to propose that the endocrine pancreas plays a major role in the different potassium responses to the infusion of organic acids (ketoacids) compared with mineral acids.

Methods

Acute infusion studies with either beta-hydroxybutyric acid (racemic form, Sigma Chemical Co., St. Louis, MO) or hydrochloric acid were carried out on adult male and female mongrel dogs ranging in weight between 13 and 27 kg. The acid infusion was performed either on conscious dogs or on dogs under general anesthesia. The studies done on dogs under general anesthesia were compared with previous studies, all of which were carried out on anesthetized animals, and enabled evaluation of animals with acutely interrupted renal function.

Conscious dogs (n = 12). The acid infusion studies were done on conscious unrestrained dogs, after at least a 2-wk period of recovery from preparatory surgery. Only animals who appeared in healthy condition, with good appetites and normal stools, were used. Seven dogs received beta-hydroxybutyric acid, and five were infused with hydrochloric acid.

Preparatory surgery was performed after an overnight fast, under general anesthesia with intravenous sodium pentobarbital (30 mg/kg body wt). After a midline incision, pulsed range-gated ultrasonic Doppler flow probes were placed around the portal vein and the hepatic artery, and catheters positioned in the portal, left common hepatic vein, and the carotid artery, as described previously (15). Catheters were flushed with heparin sodium (50 U/ml), and the ends were closed with short stainless wire plugs. Postoperatively, the catheters were flushed with 2 ml heparinized saline (50 U/ml) daily to assure their patency.

Acute experimental protocol. On the day of the acute experiment, after an overnight fast, two control blood samples, drawn 10 min apart for acid-base and electrolyte composition, glucose, insulin, and glucagon, were obtained from the portal vein, hepatic vein, and carotid artery simultaneously. Phasic and mean aortic blood pressure were measured with a pressure transducer (P^{23} db, Statham Instruments, Inc., Oxnard, CA) connected to the arterial catheter, and continual measurements of portal vein and hepatic artery blood flows were obtained during the entire protocol.

Acids, in 1-N solutions, were administered intravenously with an infusion pump over a 30-min period. Since our aim was to compare the effects of acute metabolic acidoses of similar severities, which were due to ketoacids and mineral acids, on the plasma potassium level, the dose of each acid was accordingly adjusted. Based on preliminary investigations, we administered a total dose of 3.0 and 7.0 meq/kg body wt of hydrochloric acid and beta-hydroxybutyric acid, respectively. After control observations, blood samples were simultaneously taken from the three vessels at 10, 20, 30, 40, and 60 min from the start of the acid infusion (time zero). Saline was given intravenously as replacement for blood loss. Rectal temperature was measured at the time of blood sampling. Throughout the protocol, all animals remained quiet, alert, and easy to manage.

Anesthetized dogs (n = 31). On the morning of the study, after an overnight fast, general anesthesia was induced with pentobarbital (30 mg/kg body wt, i.v.), and constant ventilation attained with a respirator (Harvard Apparatus Co., Inc., The Ealing Corp., S. Natick, MA). Additional small amounts of pentobarbital were administered when necessary. Catheters were placed in the femoral vein for acid infusion and in the femoral artery of the same leg for blood sampling and blood pressure monitoring with the transducer.

Group I (n = 17). The possible role of peripheral tissues in the different kalemic responses to acid infusion was evaluated in twelve dogs; for this purpose a venous catheter for blood sampling was inserted percutaneously in the contralateral limb to evaluate the arteriovenous potassium difference. Seven of these animals were infused with beta-hydroxybutyric acid and five with hydrochloric acid, with samples obtained every 5 min for the initial 20 min, then every 10 min up to 1 h, and at 90 and 120 min after the beginning of the infusion.

Bilateral ureteral ligation through a mid-abdominal incision was performed in five dogs; after a 60-min equilibration period after surgery, they received a beta-hydroxybutyric acid infusion (7 meq/kg). Blood samples were taken every 10 min for 1 h, and at 90 and 120 min after the beginning of the infusion.

Group II (n = 14). Bilateral ureteral catheterization through a mid-abdominal incision was performed to assess the urinary excretion of sodium and potassium. Ten dogs received acid infusion with either HCl (3 meq/kg, $n = 5$) or beta-hydroxybutyric acid (7 meq/kg, $n = 5$). Four dogs received a saline infusion (0.9% NaCl, 7 ml/kg). Urine was collected during one control (−30 min–0) and seven experimental (0–10, 10–20, 20–30, 30–45, 45–60, 60–90, and 90–120 min) periods. Blood samples were obtained at the initiation of each period of urine collection.

Analytical methods. The blood pH and PCO_2 were measured anaerobically at 38°C with a digital acid-base analyzer (model PHM 72; Radiometer America Inc., Westlake, OH). Plasma bicarbonate concentration was calculated from the Henderson-Hasselbalch equation. pH, pK′, and solubility coefficient of CO_2 were corrected for temperature; pK′ was also corrected for pH (17–19). The concentrations of sodium, potassium, chloride, and phosphate were determined as in previous studies (20). Plasma glucose was measured by a glucose autoanalyzer (Beckman Instruments, Inc., Fullerton, CA), using a glucose oxidase method. Plasma immunoreactive insulin was assayed with dextran-coated charcoal (21) and glucagon with Unger's 30,000-mol-wt antibody (22). Blood flow was measured with an ultrasonic range-gated pulsed Doppler flow meter (23, 24).

Calculations. Blood samples for the determination of plasma glucose, insulin, glucagon, and electrolytes were collected in chilled tubes containing 500 U Trasylol (FBA Pharmaceutical, Inc., New York) and 1.2 mg EDTA/ml of blood. The blood flow measurements were corrected to plasma flow based on hematocrits. The flux of potassium, phosphate, glucose, and hormones in each vessel was determined by multiplying plasma flow by plasma concentration. Hepatic vein plasma flow was taken as the sum of the plasma flows in the portal vein and hepatic artery. The amount of potassium, phosphate, glucose, and hormones presented to the liver was the sum of the amount from the portal vein and hepatic artery; the amount leaving the liver was the product of hepatic vein concentration and hepatic vein plasma flow. The net hepatic balance of glucose and potassium was calculated as the difference between the amount of each substance leaving the liver minus the amount presented to the liver per minute. The net hepatic glucose balance is expressed per kilogram body weight. A positive balance represents net hepatic output, while a negative balance represents net hepatic uptake.

The data are presented as mean values±SEM. The control (basal) value was the mean±SEM of the two values obtained at −10 and 0 min. The paired t test was used for statistical analysis of the fluctuation from the basal value within a group. Differences in mean values between groups were detected by the unpaired t test. P values < 0.05 were considered to be significant.

Results

Administration of mineral and nonmineral acid to conscious and anesthetized dogs did not result in significant changes in blood pressure; mean levels ranged between 90 and 118 mmHg. Control plasma flows were 343±52 and 330±50 ml/min in the portal vein, and 414±56 and 419±55 ml/min in the hepatic vein, in the beta-hydroxybutyric acid and HCl groups, respectively. No significant changes from control were observed in plasma flows in any of the three vessels during

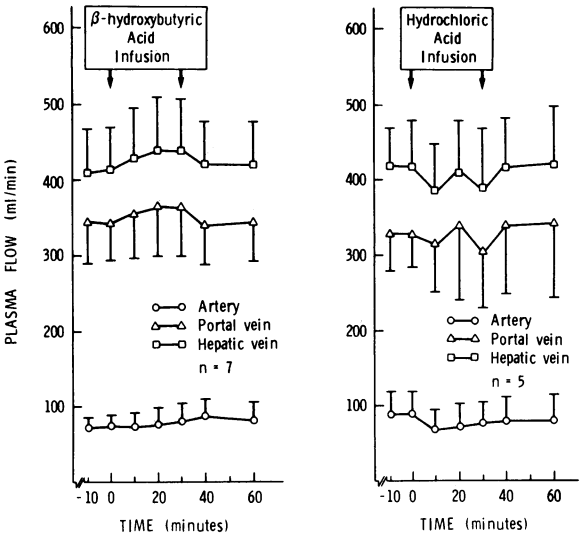

Figure 1. Plasma flows in hepatic artery, portal vein, and hepatic vein, during beta-hydroxybutyric acid (*left*) and hydrochloric acid (*right*) infusion studies in conscious dogs. Each symbol indicates mean value±SE. No significant changes from control were observed in any group. The arrows in each panel indicate the beginning and end of the acid infusion.

the acid infusion or after it, in either organic or mineral acid experiments (Fig. 1).

Acid-base and electrolyte composition. Acid infusion of either moiety, in both conscious and anesthetized dogs, significantly decreased blood pH and plasma bicarbonate, which reached the lowest level at 30 min, the end of the acid loading. The severity of the metabolic acidosis was of similar magnitude in both acid moieties in conscious dogs (Tables I and II). In the postinfusion period, however, the recovery of plasma bicarbonate and pH toward control was significantly less in the mineral acid group. Hypocapnia secondary to acidemia was found in all studies in conscious dogs. PCO_2 and plasma bicarbonate were significantly higher in portal and hepatic vein compared with the artery in all samples in both HCl and ketoacid infusion studies. In addition, blood pH values were lower in portal and hepatic vein compared with values in the artery in all samples from mineral acid studies and in control and postinfusion samples from beta-hydroxybutyric acid studies. By contrast, during the infusion of beta-hydroxybutyric acid, splanchnic conversion of infused ketoacid into plasma bicarbonate resulted in no decline of blood pH in portal and hepatic vein compared with the arterial pH.

Plasma unmeasured anion concentration (defined as the sodium concentration minus the sum of chloride and bicar-

*Table I. Changes in Acid-Base Parameters and Plasma Electrolytes After Administration of Beta-Hydroxybutyric Acid to Conscious Dogs**

	pH	$PaCO_2$	$[HCO_3]p$	[Na]p	[Cl]p	[P]p	Unmeasured anions‡
		mmHg	*meq/liter*	*meq/liter*	*meq/liter*	*mmol/liter*	*meq/liter*
Control							
Artery	7.44±0.01	34±2.1	20.2±2.3	148±1.4	116±1.5	1.9±0.2	12±3.2
Portal vein	7.42±0.01‖	38±2.8‖	21.6±2.7‖	149±1.4	116±1.3	1.8±0.1	12±3.6
Hepatic vein	7.41±0.01‖	40±2.7‖¶	22.2±2.6‖	149±1.5	115±1.1	1.8±0.1	12±3.8
10 min							
Artery	7.35±0.02§	32±2.2	15.8±2.2§	148±1.6	117±1.5	1.7±0.1	15±2.8§
Portal vein	7.34±0.02§‖	35±2.8§‖	17.3±2.4§‖	149±1.9	116±1.2	1.7±0.1	15±3.3§
Hepatic vein	7.34±0.01§‖	38±2.4‖	18.0±2.5§‖	149±2.0	117±1.7	1.7±0.1§	14±3.3
20 min							
Artery	7.29±0.02§	30±2.1§	13.0±2.0§	147±1.8§	116±1.0	1.7±0.1	18±3.2§
Portal vein	7.30±0.02§	33±2.6§‖	14.5±2.3§‖	147±2.3	115±0.9	1.8±0.1	18±3.8
Hepatic vein	7.29±0.02§	35±2.2§‖	14.4±2.2§‖	149±1.8	116±1.1	1.8±0.1	18±3.4§
30 min							
Artery	7.25±0.03§	28±2.4§	11.3±2.1§	147±2.2	116±1.0	1.7±0.1	20±3.3§
Portal vein	7.25±0.03§	31±2.7§‖	12.1±2.1§‖	148±2.1	116±1.0	1.9±0.1	20±3.0§
Hepatic vein	7.25±0.03§	34±2.7§‖¶	13.4±2.2§‖¶	149±1.9	117±1.9	1.9±0.1	19±2.2§
40 min							
Artery	7.34±0.02§	31±2.6	14.8±2.3§	148±1.7	117±1.2	1.7±0.1	16±3.0§
Portal vein	7.31±0.02§‖	33±2.6‖	15.1±2.2§	148±1.6	116±1.2	1.9±0.1	17±3.2§
Hepatic vein	7.32±0.02§‖	35±2.4§‖¶	15.9±2.1§‖¶	149±1.9	117±2.1	1.8±0.1	16±3.2
60 min							
Artery	7.37±0.01§	31±2.9	16.2±2.5§	148±1.5	118±1.5	1.6±0.1	14±2.9
Portal vein	7.35±0.01§‖	35±3.3‖	17.3±2.6§‖	148±1.9	117±1.0	1.7±0.2	14±3.6
Hepatic vein	7.35±0.01§‖	36±2.7‖	17.6±2.4§‖	148±1.6	117±1.5	1.8±0.1	14±3.3

* Values presented are the means±1 SE, $n = 7$. ‡ $[Na]p - ([HCO_3]p + [Cl]p)$. § $P < 0.05$ vs. control. ‖ $P < 0.05$ vs. simultaneous arterial sample. ¶ $P < 0.05$ vs. simultaneous portal vein sample.

*Table II. Changes in Acid-Base Parameters and Plasma Electrolytes After Administration of Hydrochloric Acid to Conscious Dogs**

	pH	$PaCO_2$	$[HCO_3]p$	[Na]p	[Cl]p	[P]p	Unmeasured anions‡
		mmHg	*meq/liter*	*meq/liter*	*meq/liter*	*mmol/liter*	*meq/liter*
Control							
Artery	7.43±0.01	32±2.0	20.6±2.0	150±0.7	116±1.1	1.8±0.1	13±2.9
Portal vein	7.40±0.01‖	39±2.2‖	23.0±1.8‖	150±0.7	115±1.3	1.9±0.2	12±2.7
Hepatic vein	7.40±0.01‖	41±2.0‖¶	24.8±2.4‖¶	150±1.2	114±1.1	1.9±0.2	11±2.4
10 min							
Artery	7.35±0.02§	30±2.1	16.4±2.0§	149±0.8	120±2.1§	1.8±0.1	13±2.3
Portal vein	7.32±0.02§‖	37±1.8‖	18.8±1.8§‖	150±0.9	119±2.3	1.8±0.2	12±1.4
Hepatic vein	7.30±0.02§‖	40±2.1‖¶	19.5±2.4§‖	150±1.1	119±2.0§	1.8±0.2	12±2.2
20 min							
Artery	7.27±0.04§	26±2.1§	12.2±1.9§	147±4.0	123±4.3	1.8±0.1	12±3.0
Portal vein	7.24±0.04§‖	32±1.8§‖	14.5±1.8§‖	148±1.0	123±3.1§	1.7±0.1	11±3.6
Hepatic vein	7.23±0.03§‖	35±2.1‖¶	14.6±2.1§‖	148±1.9	122±2.5§	1.7±0.1	11±3.0
30 min							
Artery	7.22±0.04§	23±2.0§	9.4±1.5§	147±1.9	126±3.4§	1.8±0.1	11±3.9
Portal vein	7.18±0.04§‖	31±2.3§‖	12.2±1.7§‖	148±1.7	125±3.9§	1.7±0.1	11±3.6
Hepatic vein	7.17±0.05§‖	35±2.7‖¶	12.9±2.1§‖¶	148±1.6	125±3.6§	1.7±0.1	10±3.8
40 min							
Artery	7.25±0.03§	26±2.2§	11.0±1.4§	148±1.7	126±3.2§	1.8±0.1	11±3.2
Portal vein	7.21±0.04§	32±1.9§‖	13.3±1.7§‖	149±1.5	125±3.5§	1.7±0.1	11±3.6
Hepatic vein	7.20±0.04§‖	36±1.8‖¶	14.0±2.0§‖¶	149±1.5	125±3.0§	1.7±0.1	10±2.8
60 min							
Artery	7.27±0.02§	25±2.3§	11.0±1.4§	148±2.0	126±3.6§	1.8±0.1	11±3.2
Portal vein	7.23±0.03§‖	32±1.6§‖	14.2±1.7§‖	149±1.1	125±3.0§	1.7±0.1	10±2.2
Hepatic vein	7.23±0.03§‖	35±1.4§‖¶	14.4±1.8§‖¶	149±1.1	126±3.9§	1.7±0.1	9±2.9

* Values presented are the means±1 SE, $n = 5$. ‡ [Na]p − ([HCO_3]p + [Cl]p). § $P < 0.05$ vs. control. ‖ $P < 0.05$ vs. simultaneous arterial sample. ¶ $P < 0.05$ vs. simultaneous portal vein sample.

bonate concentration) in the two infusion studies in conscious dogs is presented in Tables I and II. A progressive significant increase in plasma unmeasured anions was found during the infusion of the ketoacid, accompanied by a simultaneous decrease in plasma bicarbonate levels of comparable magnitude; the partial recovery of the bicarbonate level in the postinfusion period occurred in association with a decrease toward normalcy in the plasma unmeasured anions. On the other hand, the changes in plasma bicarbonate in the HCl experiments were accompanied by reciprocal significant changes of comparable magnitude in the chloride levels, so that the plasma unmeasured anions remained unchanged throughout the experiments. No significant changes in the plasma level of the other measured electrolytes, except potassium, were found in the infusion studies in conscious dogs. The phosphate delivery to and from the liver was unchanged from control in the HCl studies. However, in the organic acid experiments, while phosphate delivery to the liver remained unchanged, a significant increase in phosphate delivery from the liver was observed at 20 and 30 min.

Control acid-base values were slightly, but significantly different in anesthetized dogs compared with conscious animals. Table III presents data on plasma composition in group 1 of anesthetized dogs that shows that blood pH was lower and PCO_2 higher in this group than in conscious animals during control. The changes in acid-base parameters after the acid infusion were not significantly different among the various sets of anesthetized dogs within each group and between groups 1 and 2, and compared with the studies in conscious animals (Tables I–III). Evaluation of the acid-base composition of peripheral venous blood during infusion of either organic or mineral acid disclosed a characteristic pattern; the slow equilibration of bicarbonate stores from peripheral tissues with intravascular bicarbonate, acutely titrated by intravenous infusion of acids, resulted in transiently higher pH values in venous blood than in arterial blood tested simultaneously.

Potassium. Fig. 2 depicts the potassium levels in the three vessels in both acid infusion experiments in conscious dogs. Significant hypokalemia was detected at every time of observation and in all three vessels in the beta-hydroxybutyric acid studies, whereas either no changes or an increase in plasma potassium occurred in response to mineral acid. The decrease in potassium levels during organic acid infusion was similar in all three vessels. In addition, comparison of the plasma potassium during the infusion with the two acid moieties demonstrated statistically significant differences at every time of observation and in the three vessels (Fig. 2). The significant changes in plasma potassium in both acid infusion studies occurred and were of greatest magnitude within 10 min of the initiation of infusions. Subsequent samples disclosed plasma

*Table III. Changes in Acid-Base Parameters and Plasma Potassium After Acid Infusion to Anesthetized Dogs**

	pH		PCO_2		$[HCO_3]p$		[K]p	
	Artery	Vein‡	Artery	Vein	Artery	Vein	Artery	Vein
			mmHg	*mmHg*	*meq/liter*	*meq/liter*	*meq/liter*	*meq/liter*
Control								
A	7.35±0.02		39±3.3		21.5±2.0		3.8±0.1	
B	7.37±0.01	7.35±0.01‖	37±2.1	45±1.8‖	21.4±1.3	24.6±0.9‖	3.7±0.3	3.6±0.2
C	7.40±0.02¶	7.37±0.02‖	39±1.6	45±2.1‖	23.7±0.9	25.6±0.7‖	3.8±0.1	3.8±0.1
5 min								
A								
B	7.27±0.02§	7.27±0.02§	35±2.7	39±1.9§	16.8±1.1§	19.0±1.4§	3.3±0.2§	3.4±0.2§‖
C	7.29±0.03§	7.30±0.02§	46±2.9**	48±2.3**	21.4±0.6**	23.2±0.7‖**	3.7±0.1	3.7±0.1
10 min								
A	7.23±0.04§		33±3.3§		13.6±1.5§		3.2±0.1§	
B	7.25±0.02§	7.26±0.02§	39±2.2	43±2.3‖	16.1±1.1§	18.5±1.5§‖	2.9±0.2§	3.2±0.2§‖
C	7.26±0.03§	7.26±0.02§	45±2.8¶	51±3.5‖	19.7±0.9§¶	22.7±1.0‖	3.8±0.1¶**	3.8±0.1**
15 min								
A								
B	7.22±0.02§	7.23±0.02§	40±2.6	44±2.8‖	16.0±1.2§	17.7±1.3§‖	2.8±0.2§	3.0±0.2§‖
C	7.24±0.03§	7.25±0.02§	42±1.4	49±2.9‖	18.0±1.0§	21.0±1.2§‖	3.8±0.1**	3.8±0.1**
20 min								
A	7.21±0.06§		32±3.5§		12.7±1.4§		3.2±0.1§	
B	7.21±0.02§	7.21±0.02§	38±2.3	44±2.5‖	14.9±1.1§	16.6±1.2§‖	2.8±0.2§	3.0±0.2§‖
C	7.22±0.03§	7.22±0.02§	39±1.7	49±1.5‖	15.8±0.8§	19.5±1.0§‖	3.9±0.1¶**	4.0±0.1‖**
30 min								
A	7.18±0.06§		31±3.3§		11.4±1.6§		3.1±0.1§	
B	7.13±0.03§	7.12±0.03§	29±2.2§	36±1.9§‖	9.7±1.0§	11.5±0.6§‖	2.7±0.1§¶	2.8±0.1§‖
C	7.20±0.02§	7.18±0.03§‖	35±0.6	51±2.1‖**	13.5±0.8§**	17.4±1.1§‖**	4.0±0.1§¶**	4.0±0.1§‖
40 min								
A	7.27±0.05§		29±2.7§		13.2±1.5§		3.5±0.1	
B	7.25±0.02§	7.22±0.02§‖	31±2.2§	31±1.5§	13.4±1.1§	12.3±0.8§	2.9±0.2§¶	2.9±0.2§
C	7.23±0.03§	7.18±0.02§‖	38±2.1¶	48±3.9‖**	15.5±0.7§	17.6±1.0§‖**	4.1±0.1¶**	4.2±0.1§**
50 min								
A	7.30±0.04		31±3.2§		14.8±1.9§		3.6±0.1	
B	7.28±0.02§	7.25±0.01§‖	30±2.4§	32±2.0§	14.2±1.4§	13.8±0.8§	2.9±0.2§¶	3.0±0.2§
C	7.25±0.02§	7.19±0.03§‖	38±1.7**	50±4.4‖**	16.3±0.8§	18.8±1.2§‖**	3.8±0.1**	4.2±0.1§‖**
60 min								
A	7.31±0.03§		32±3.9§		15.7±2.1§		3.6±0.2	
B	7.31±0.01§	7.27±0.01§	34±2.4	32±1.7§	16.7±1.3§	14.7±0.9§	3.0±0.2§¶	3.0±0.2§
C	7.26±0.02§	7.20±0.02§‖**	38±1.6	52±3.9‖**	16.7±0.8§	19.7±1.2§‖**	3.8±0.1**	4.3±0.1§‖**
90 min								
A	7.33±0.02		29±3.5§		15.1±2.0§		3.9±0.2	
B	7.35±0.02	7.31±0.02§‖	31±2.9	33±2.5§	17.3±1.8§	16.2±1.3	3.1±0.2¶	3.2±0.2§
C	7.27±0.02§¶**	7.20±0.02§‖**	36±2.6	46±3.5‖**	16.3±1.3§	17.6±1.3§	4.0±0.1**	4.4±0.1§‖**
120 min								
A	7.37±0.02		26±2.8§		15.9±2.0§		4.2±0.3§	
B	7.39±0.01§	7.35±0.02‖	29±2.1§	29±2.2§	17.3±1.4§	16.0±1.2	3.2±0.2¶	3.3±0.2§
C	7.28±0.03§¶**	7.20±0.02§‖**	36±3.5¶	47±5.0‖	16.5±1.5§	17.8±1.7§	4.1±0.1**	4.5±0.2§‖**

A, β-hydroxybutyric acid infusion plus ureteral ligation ($n = 5$). B, β-hydroxybutyric acid infusion with intact renal function ($n = 7$). C, HCl infusion with intact renal function ($n = 5$). * Values presented are the means±1 SE. ‡ Peripheral vein, from the hindleg. § $P < 0.05$ vs. control. ‖ $P < 0.05$ vs. simultaneous arterial sample. ¶ $P < 0.05$ vs. simultaneous sample, group A. ** $P < 0.05$ vs. simultaneous sample, group B.

potassium values with either identical deviation or closer to pre-acid-infusion values. The delivery of potassium to the liver or from it, remained unaltered during the HCl infusion; by contrast, a significant decrease in potassium delivery to and from the liver was observed at various points of observation in the ketoacid studies (Fig. 3). However, the amounts of potassium reaching the liver at different times were not significantly different from the amounts of potassium leaving the liver in any acid infusion groups studied. Fig. 4 depicts the hepatic potassium balance in both acid infusion studies in conscious dogs; no significant changes from control were detected within any experimental group or between them. The pattern of plasma potassium changes in anesthetized dogs infused with organic acid was not different from that observed in conscious dogs (Table III and Fig. 1). A significant decrease in plasma potassium was observed during the organic acid

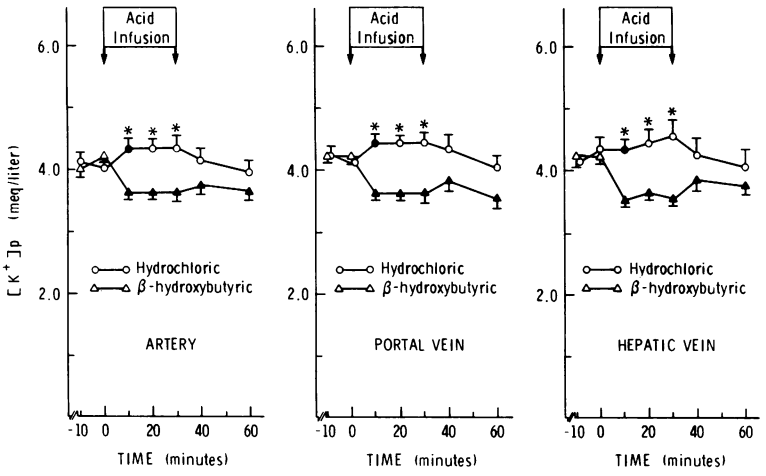

Figure 2. Changes in plasma potassium concentration in an artery, the portal vein, and the hepatic vein, during beta-hydroxybutyric acid (n = 7) and hydrochloric acid (n = 5) infusion studies in conscious dogs. Each symbol indicates mean value±SE. Black symbols indicate a significant change from the basal level ($P < 0.05$). Asterisks indicate significant differences from values obtained in the same vessel and at the same time in the other group of acid infusion studies ($P < 0.05$). The arrows in each panel indicate the beginning and end of the acid infusion.

infusion in anesthetized animals with intact renal function and with bilateral ureteral ligation; a progressive recovery toward normal concentration was observed thereafter. The hypokalemia was somewhat less severe in dogs with bilateral ureteral ligation compared with those with intact kidneys. The changes in plasma potassium during acid infusion in anesthetized dogs with intact renal function (group 1, depicted in Table III, and group 2, not shown), were of similar magnitude; no significant differences in the changes in plasma potassium were detected among the various sets of acid infusion studies in groups I and II. Saline infusion did not significantly alter plasma potassium concentration.

Fig. 5 depicts the urinary excretion of sodium and potassium in the three sets of anesthetized dogs (group II). Potassium excretion remained unaltered along the entire protocol of HCl acidosis, while a significant decrease in sodium excretion was evident at various times. The changes in the urinary excretion of sodium during HCl acidosis are consistent with stimulation of aldosterone secretion (25). By contrast, sodium excretion was not significantly altered during organic acid infusion. Potassium excretion did not significantly change from control during beta-hydroxybutyric acid infusion, except at 30 min when a significant increase was detected. Saline infusion significantly increased sodium excretion without significantly altering potassium excretion.

Table III presents the acid-base composition and potassium values in arterial and venous blood from the hindleg in anesthetized dogs infused with either mineral acid or beta-hydroxybutyric acid. Plasma potassium from the peripheral vein was not different from that from arterial blood in the control state in any group of acid studies. As can be seen, although organic acid infusion resulted in hypokalemia and HCl-induced hyperkalemia, the plasma potassium from the peripheral vein was either equal to or greater than the simultaneous arterial value in both groups of acid infusion studies. In the postinfusion period, significant exit of potassium from the peripheral tissues was evident in the mineral acid studies.

Insulin. Infusion of beta-hydroxybutyric acid resulted in a significant increase in portal vein insulin levels from the basal level of 27±4 μU/ml to a maximum of 84±22 μU/ml at 10 min (Fig. 6, upper panel). Insulin levels in arterial blood also significantly increased at 10, 20, and 30 min; however, the rise in arterial insulin levels was modest compared with the increase in portal vein values. Insulin levels returned to basal in the postinfusion period. By contrast, no significant changes in

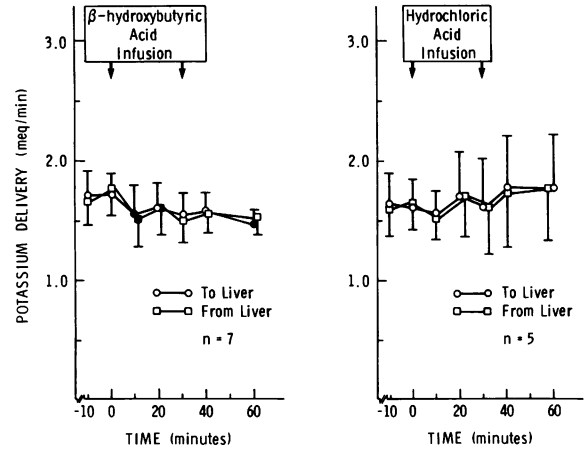

Figure 3. Potassium delivery to and from the liver (meq/min) during beta-hydroxybutyric acid (*left*) and hydrochloric acid (*right*) infusion studies in conscious dogs. Each symbol indicates mean value±SE. Black symbols indicate a significant change from the basal level ($P < 0.05$). The arrows in each panel indicate the beginning and end of the acid infusion.

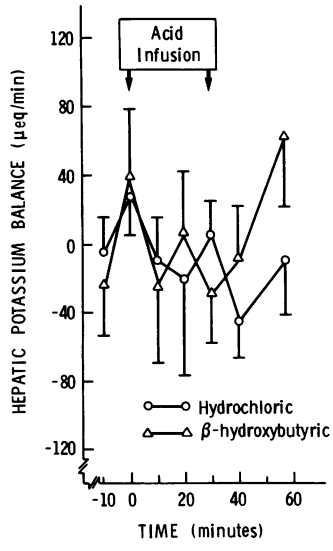

Figure 4. Hepatic potassium balance (μeq/min) during beta-hydroxybutyric acid (n = 7) and hydrochloric acid (n = 5) infusion studies in conscious dogs. Each symbol indicates mean value±SE. No significant changes from control were observed in any group. The arrows indicate the beginning and end of the acid infusion.

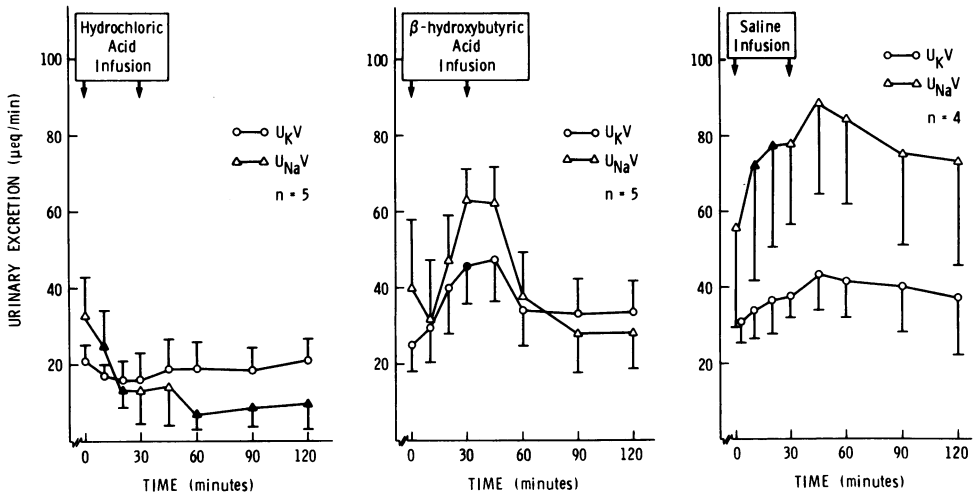

Figure 5. Urinary excretion of sodium and potassium during hydrochloric acid (*left*), beta-hydroxybutyric acid (*middle*), and normal saline (*right*) infusion studies in anesthetized dogs. Each symbol indicates mean value±SE. Black symbols indicate a significant change from the basal level ($P < 0.05$). The arrows in each panel indicate the beginning and end of the acid infusion.

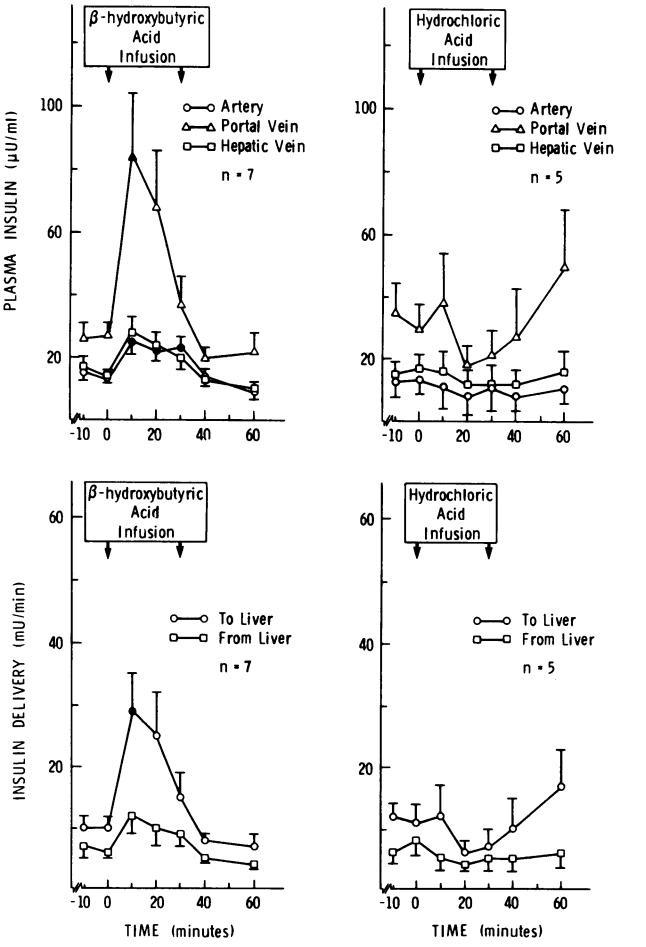

Figure 6. The upper panel presents the changes in plasma insulin concentration in an artery, the portal vein, and the hepatic vein during beta-hydroxybutyric acid (*left*) and hydrochloric acid (*right*) infusion studies in conscious dogs. The lower panel depicts the insulin delivery to and from the liver (milliunits per minute) during beta-hydroxybutyric acid (*left*) and hydrochloric acid (*right*) infusion studies in conscious dogs. Each symbol indicates mean value±SE. Black symbols indicate a significant change from the basal level ($P < 0.05$). The arrows in each panel indicate the beginning and end of the acid infusion.

insulin levels in any vessel were observed during or after the administration of hydrochloric acid. Fig. 6, lower panel, shows that a significant increase in the insulin delivery to the liver was exclusively observed during the infusion of organic acid. The sizable hepatic extraction of insulin was responsible for only small changes in peripheral plasma insulin in the ketoacid infusion studies. Significant changes in the hepatic extraction of insulin were not observed in any group of acid infusion studies. Since we infused the racemic form of beta-hydroxybutyric acid, the observed stimulation of insulin secretion could be due to either L(+), D(−), or both forms of the acid.

Glucagon. During the basal state, plasma glucagon levels in the artery, portal, and hepatic veins were not significantly different in the two groups of acid infusion studies (Fig. 7, upper panel). Mild suppression of glucagon levels, although not statistically significant, was observed in the three vessels during beta-hydroxybutyric acid infusion; in the postinfusion period, plasma glucagon returned to values equal to control. In contrast, administration of mineral acid resulted in a progressive increase in plasma glucagon levels, which reached statistical significance at 20 and 40 min in the portal vein, and at 60 min in the hepatic vein. The delivery of glucagon to and from the liver, depicted in Fig. 7, lower panel, demonstrates the relatively small hepatic extraction of glucagon (26) compared with the extraction of insulin. The HCl infusion studies revealed significant increases in glucagon delivery to the liver at 40 and 60 min; by contrast, significant changes in the delivery of glucagon to the liver were not observed in the ketoacid infusion studies. The hepatic extraction of glucagon did not significantly change in any group of acid infusion studies.

Glucose. During the control period, the hepatic vein plasma glucose significantly exceeded the arterial and portal vein levels in both acid infusion studies (Fig. 8). Infusion of beta-hydroxybutyric acid significantly decreased glucose levels from control levels in all three vessels, whereas no decrease was observed during HCl acidosis; in fact, the glucose level in the portal vein at 20 min increased significantly over the control level in HCl acidosis (Fig. 8, upper panel). Suppression of hepatic release of glucose was observed at 30, 40, and 60 min, in the beta-hydroxybutyric acid studies, whereas glucose output re-

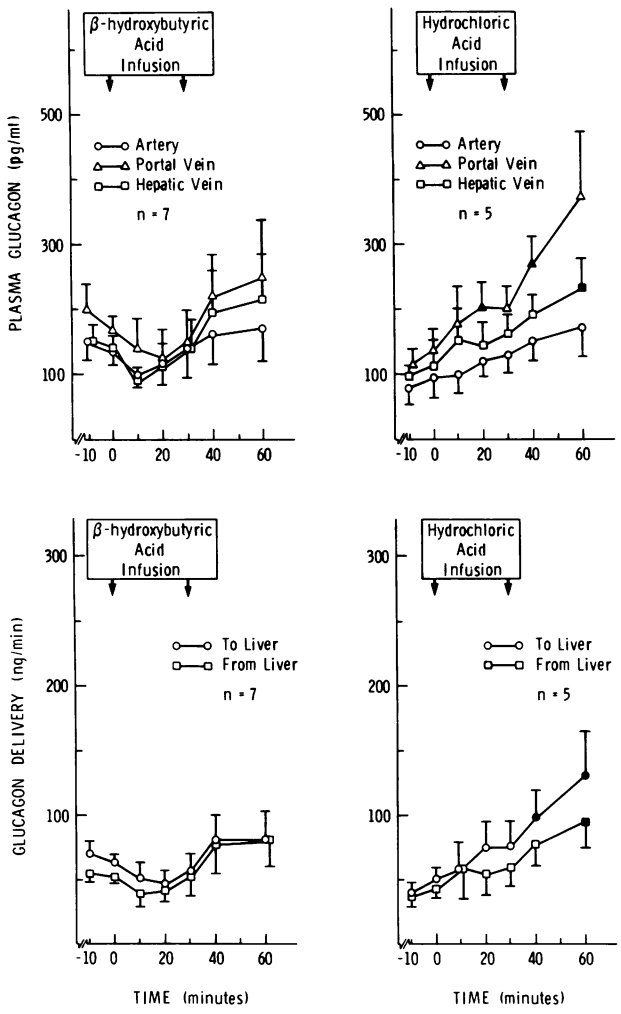

Figure 7. The upper panel depicts the changes in plasma glucagon levels in an artery, the portal vein, and the hepatic vein during beta-hydroxybutyric acid (*left*) and hydrochloric acid (*right*) infusion studies in conscious dogs. The lower panel presents the glucagon delivery to and from the liver (nanograms per minute) during beta-hydroxybutyric acid (*left*) and hydrochloric acid (*right*) infusion studies in conscious dogs. Each symbol indicates mean value±SE. Black symbols indicate a significant change from the basal level ($P < 0.05$). The arrows in each panel indicate the beginning and end of the acid infusion.

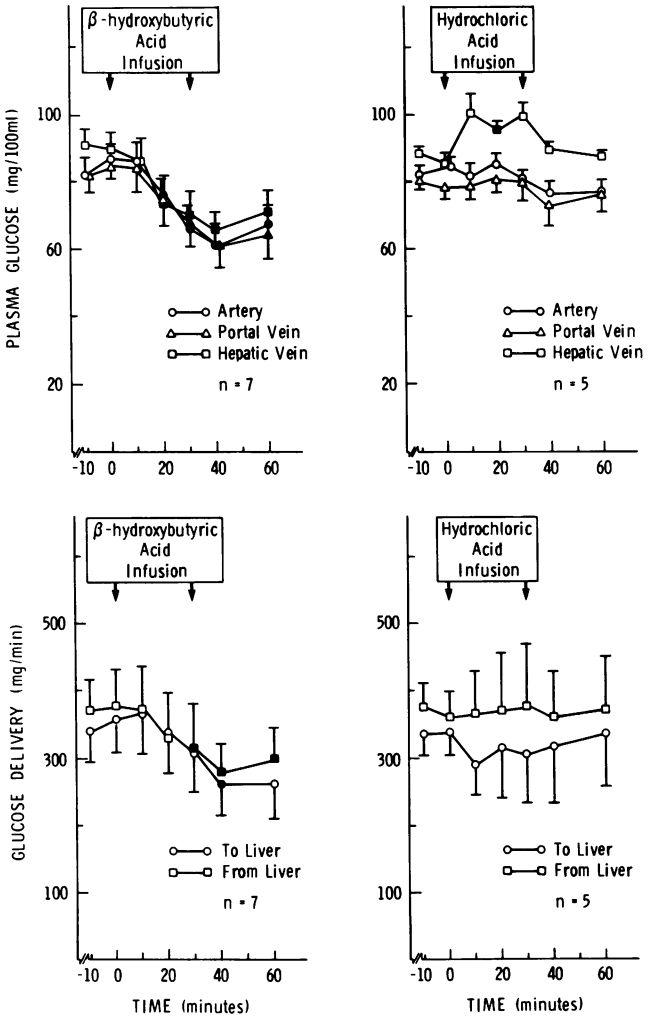

Figure 8. The upper panel depicts the changes in plasma glucose concentration in an artery, the portal vein, and the hepatic vein during beta-hydroxybutyric acid (*left*) and hydrochloric acid (*right*) infusion studies in conscious dogs. The lower panel presents the glucose delivery to and from the liver (milligrams per minute) during beta-hydroxybutyric acid (*left*) and hydrochloric acid (*right*) infusion studies in conscious dogs. Each symbol indicates mean value±SE. Black symbols indicate a significant change from the basal level ($P < 0.05$). The arrows in each panel indicate the beginning and end of the acid infusion.

mained unchanged with the infusion of HCl (Fig. 8, lower panel). A comparison of the hepatic glucose balance in both acid infusion studies is depicted in Fig. 9; while no differences in the hepatic glucose balance were found between control values of mineral acid and organic acid acidosis, statistically significant differences were obtained in all observations made during the infusion period. In the postinfusion period, the hepatic glucose balance was not significantly different in the two groups of studies.

Discussion

This study discloses a heretofore unrecognized role for the major glucoregulatory hormones in the kalemic response to acute metabolic acidosis. Similar degrees of acidemia resulting from ketoacid and mineral acid infusion induced opposite

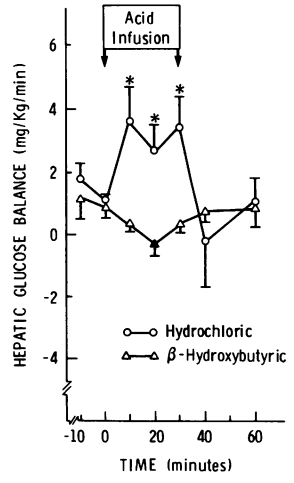

Figure 9. Hepatic glucose balance (milligrams per kilogram per minute) during beta-hydroxybutyric acid and hydrochloric acid infusion studies in conscious dogs. Each symbol indicates mean value±SE. Black symbols indicate a significant change from the basal level ($P < 0.05$). Asterisks indicate significant differences from values obtained at the same time in the other group of acid infusion studies ($P < 0.05$). The arrows indicate the beginning and end of the acid infusion.

kalemic responses; ketoacid infusion resulted in hypokalemia, and mineral acid infusion caused hyperkalemia. Concomitant with the alteration in plasma potassium, significant changes in hormonal levels were detected. A severalfold increase in plasma insulin levels in the portal vein was found during the infusion of beta-hydroxybutyric acid. Yet, only a very modest, but significant increase in insulin levels in peripheral blood was observed. The liver's large capacity for insulin extraction was responsible for the observed difference in plasma levels (16). Thus, the previously reported unchanged insulin levels during organic acid infusion (10) are compatible with the mild stimulation of insulin release that was undetected because plasma levels in the portal vein were not evaluated. The increased insulin secretion associated with organic acid infusion was sufficient to suppress hepatic glucose production, resulting in a decrease in the plasma glucose concentration (27, 28).

Since hydrochloric acid infusion did not significantly increase plasma insulin levels, the lack of alterations in both the plasma glucose and the release of glucose from the liver were not unexpected. A progressive increase in glucagon levels in the three vessels resulting from mineral acid infusion, which persisted throughout the entire acute protocol, was observed. The increase in glucagon secretion was associated with an enhanced hepatic vein glucose concentration that was significant at 20 min. In all three vessels the administration of ketoacid, by contrast, decreased plasma glucagon, which was completely recovered to baseline once the infusion was completed. These changes, however, did not reach statistical significance. The ketoacid-induced reduction in glucagon levels occurred simultaneously with hypoglycemia; the latter should have induced a rise in plasma glucagon. Thus, our results may be explained by suggesting that a composite direct effect of ketones enhanced insulin and suppressed glucagon secretion.

What is the nature of the stimuli for hormonal response to different acid moieties? Systemic acidity may alter plasma levels of various hormones (25, 29). Thus, we produced metabolic acidosis of similar severity in both groups of acid infusion studies. Yet, different hormonal responses were found, suggesting a specific role for the anion accompanying the acid infusion (27, 28). Since insulin is primarily a fuel-storage hormone, nutrients are known to stimulate its secretion, and their insulinotropic potency is correlated with their rate of oxidation by the pancreatic islets (30). Moreover, beta-hydroxybutyrate and acetoacetate are oxidized by pancreatic islets and may enhance the insulin release by glucose infusion in humans (30). Thus, we believe our data indicate that ketones, probably acting as nutrients, stimulated insulin secretion; by contrast, the infusion of hydrochloric acid, which does not have an anion for islet oxidation, failed to enhance insulin release.

The mechanisms responsible for the enhanced glucagon levels during HCl acidosis are less evident. Since comparable deviation in systemic acidity during organic acid infusion did not elicit a stimulatory effect, it might be argued that it was not the result of acidemia per se. A stronger inhibitory stimulus resulting from the infusion of ketoacid (see above), however, may overcome an enhanced glucagon response triggered by acidemia. Support for this potential mechanism arises from the different acid-base patterns observed in both types of metabolic acidosis after the acid infusion was terminated; while recovery of the deranged acid-base status was minimal in HCl acidosis, significant correction toward normalcy was observed in organic acid acidosis. Thus, persistent acidemia in the postinfusion period in HCl acidosis, may be responsible for the progressive elevation of plasma glucagon at 40 and 60 min.

What is the tissue responsible for the different kalemic responses to acid infusion? Is there a causal relationship between hormonal and electrolyte alterations? Previous studies have proposed that differences in potassium excretion could not account for dissimilar plasma potassium responses to mineral and nonmineral acids (10). The present study confirms this thesis since hypokalemia was observed in animals infused with beta-hydroxybutyric acid having intact renal function and bilateral ureteral ligation. Furthermore, the maximal and opposite deviations of plasma potassium in both acid infusion studies occurred 10 min after the initiation of the infusion, when urinary potassium excretion remained the same as control (Fig. 5). Potassium excretion during the organic acid studies significantly increased only at the end of the infusion (30 min), but plasma potassium at that time had not decreased from the values observed at 10 min. Thus, the alterations in plasma potassium are due to changes in the internal rather than the external potassium balance. While previous studies have focused on the H^+/K^+ exchange in skeletal muscle during acid-base disturbances, limited information is available on the possible role of the liver, which comprises the second major reservoir of body potassium that can be rapidly altered in response to humoral factors, particularly the high levels of pancreatic hormones in the portal circulation (31–33). The present study evaluated the uptake/release of potassium by the liver in both organic and mineral acid infusion studies and found no significant changes in net hepatic potassium balance (Fig. 4). It should be noted, however, that the estimated change in total potassium content in plasma and extracellular fluid that accounted for the observed deviations in plasma potassium was only ~0.5–1.0 and 2–4 meq, respectively, while the potassium delivery to the liver during the acid infusion studies was ~50 meq. Thus, changes in the net hepatic potassium balance would have been difficult to detect even if samples were drawn at shorter intervals. The observed stimulation of insulin secretion during ketoacid infusion and the alterations in glucose homeostasis indicative of metabolic effects of hyperinsulinemia on the liver lead us to believe that potassium uptake probably occurred in the liver (31, 33). Furthermore, this tissue was the only one exposed to high insulin levels, since the portal circulation was the only vascular bed in which insulin plasma levels increased substantially. Although potassium uptake in muscle and adipose tissue is also enhanced by insulin, the plasma insulin levels required for that effect (34) are significantly higher (25–40 μU/ml) than the peripheral insulin values found in this study. Additionally, our evaluation of the exchange of potassium in the hindleg under the current experimental conditions tended to exclude the changes in the uptake of potassium in the peripheral tissues during ketoacid infusion (Table III).

While the hyperinsulinemia of ketoacid infusion is considered to be responsible for the hypokalemia, the increased glucagon levels in HCl acidosis may be responsible for the observed early rise in plasma potassium. Glucagon may induce an increased potassium output from the liver, but this effect is usually transient because of the glucagon-induced stimulation of plasma insulin levels; if an impaired insulin secretion is present, an elevation in plasma glucagon levels may result in

hyperkalemia (15). Since changes in insulin levels were not detected in our studies while plasma glucagon was significantly increased, the hormonal imbalance may have caused hyperkalemia. The observed significant increase in plasma glucose in the hepatic vein at 20 min is compatible with this hypothesis. We believe, however, that potassium output from skeletal muscle (Table III) may fully explain the persistent increase in plasma levels usually present in acute mineral acid acidosis of prolonged (several hours) duration (5).

While this study showed a decrease in plasma potassium during ketoacid infusion of short duration, others (10) have found complete recovery to normalcy or even minimal hyperkalemia after several hours of infusion. We believe that in prolonged acid infusions, the initial insulin-induced hepatic uptake of potassium may fade away, and possibly only a mild stimulatory effect on potassium uptake by the skeletal muscle will persist, which in turn may be overcome by the acidemia-induced exit of skeletal muscle potassium.

Because we have only used beta-hydroxybutyric acid in the evaluation of the differential kalemic responses to organic versus mineral acids, caution should be exercised in applying the present results to other organic acids. Nonmineral acids other than ketoacids, although they may not act as physiologic regulators of insulin release, when infused in amounts that result in moderately severe metabolic acidosis, may enhance insulin secretion leading to hypokalemia. Only additional studies with other organic acids will provide the answers.

Since the adrenergic nervous system represents, in addition to insulin, the other major regulator of the internal potassium balance (35), changes in sympathetic activity may have played some role in the different kalemic responses to mineral acid compared with organic acid acidosis. Further studies are required to evaluate this possibility. However, with the exception of acute respiratory acidosis, there is no evidence for a significant role of the adrenergic system in the electrolyte changes observed during acute acid-base disturbances (5).

The results of this investigation may shed some light on the complex interrelationship between serum potassium and organic acid acidosis in humans. Patients presenting with metabolic acidosis due to alcoholic ketosis and lactic acidosis postseizures or postpulmonary edema have been reported to have normal serum potassium; all these patients are considered to have no major abnormalities in the endocrine pancreas. In harmony with the results presented in this study, we postulate that the organic acid acidemia may stimulate insulin release, preventing the development of hyperkalemia. By contrast, higher serum potassium values are found in patients with metabolic acidosis due to diabetic ketoacidosis and with phenformin-associated acidosis; both groups of patients are characterized by major impairments in either plasma levels or physiologic effects of insulin.

In summary, the present study confirms the previous observation that different kalemic responses occur as a result of the exogenous administration of mineral compared with organic acids. Changes in the internal potassium balance resulting from a different hormonal response by the endocrine pancreas acting upon the liver seem to account for the early changes in plasma potassium concentration observed during acute metabolic acidosis due to HCl and ketoacids infusion.

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