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Acid-Base Alterations and Renal Gluconeogenesis: Effect of pH, Bicarbonate Concentration, and P_{CO_2} *

DONALD E. KAMM,† ROBERT E. FUISZ,‡ A. DAVID GOODMAN,§ AND GEORGE F. CAHILL, JR.||

(From the Elliott P. Joslin Research Laboratory in the Department of Medicine, Harvard Medical School, the Peter Bent Brigham Hospital, and the Diabetes Foundation, Inc., Boston, Mass.)

Summary. **In previous studies it was found that renal cortical slices from rats with induced metabolic acidosis have an increased capacity to produce glucose, whereas cortical slices from rats with metabolic alkalosis manifest decreased gluconeogenesis. To evaluate the relative influence of extracellular fluid pH, [HCO_3^-], and carbon dioxide tension on renal gluconeogenesis, we observed glucose production by cortex from rats with induced respiratory acidosis, and by cortex taken from normal animals and incubated in acid and alkaline media.**

We found glucose production to be increased in cortex from rats with respiratory acidosis, as is the case in metabolic acidosis. Glucose production by slices from normal rats was increased in media made acidic by reducing [HCO_3^-], and decreased in media made alkaline by raising [HCO_3^-]. These effects were evident whether the gluconeogenic substrate employed was glutamine, glutamate, α-ketoglutarate, or oxalacetate. Glucose production was also increased in media made acidic by raising CO_2 tension and decreased in media made alkaline by reducing CO_2 tension. These data indicate that both *in vivo* and *in vitro*, pH, rather than CO_2 tension or [HCO_3^-], is the most important acid-base variable affecting renal gluconeogenesis.

The findings suggest that a decrease in extracellular fluid pH enhances renal gluconeogenesis through direct stimulation of one of the rate-limiting reactions involved in the conversion of oxalacetate to glucose. We hypothesize that the resultant increase in the rate of removal of glutamate, a precursor of oxalacetate, may constitute an important step in the mechanism by which acidosis increases renal ammonia production.

Introduction

Recent studies have demonstrated that renal cortical slices from rats with induced metabolic acidosis have an increased capacity to produce glucose, and that slices from alkali-fed rats have a decreased gluconeogenic capacity (3). This is evident whether glutamine, glutamate, α-ketoglutarate, or oxalacetate is used as gluconeogenic sub-

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† This work done during tenure as a research fellow of the National Institutes of Health. Present address: University of Rochester School of Medicine and Dentistry and the Rochester General Hospital, Rochester, N. Y.

‡ Special fellow, National Institutes of Health.

§ This work done during tenure as a clinical trainee in endocrinology, National Institutes of Health. Present address: Albany Medical College, Albany, N. Y.

|| Investigator, Howard Hughes Medical Institute.

Address requests for reprints to Dr. George F. Cahill, Jr., 170 Pilgrim Rd., Boston, Mass. 02215.

strate; we therefore postulated that metabolic acidosis and alkalosis affect a rate-limiting reaction involved in the conversion of oxalacetate to glucose. Since both pH and [HCO_3^-] of extracellular fluid (ECF) are reduced in metabolic acidosis, the change in either of these variables could be responsible for the observed stimulation of renal gluconeogenesis. To evaluate this point, we have studied the gluconeogenic capacity of renal cortex taken from animals with respiratory acidosis, a state in which pH is reduced and [HCO_3^-] is normal or elevated. It has been found that respiratory acidosis produces a significant increase in renal gluconeogenic capacity, suggesting that pH, rather than [HCO_3^-] or [H_2CO_3], is the important acid-base determinant of renal gluconeogenesis.

Alteration of acid-base status *in vivo* may influence renal gluconeogenesis in two ways. First, it may influence renal gluconeogenic capacity indirectly, possibly through an extrarenal hormonal mechanism or by alterations in the plasma concentration of glucose precursors. Second, changes in the acid-base composition of extracellular fluid may have a direct effect on renal gluconeogenic capacity. To evaluate the latter possibility, we have studied the effects of variations in pH, [HCO_3^-], and carbon dioxide tension (P_{CO_2}) of the incubation medium on glucose production by renal slices. It has been found that changes in media pH influence gluconeogenesis in the same manner as do similar changes in extracellular pH *in vivo*. This would suggest that changes in extracellular fluid pH *in vivo* may well have a direct effect on renal gluconeogenesis.

Methods

Male Sprague–Dawley rats (Holtzman) weighing 200 to 350 g were used in all experiments. For studies requiring adrenalectomized animals, bilateral adrenalectomy was performed with the rats under ether anesthesia 4 days before initiation of the experimental protocol.

In vivo respiratory acidosis

Experimental animals were kept in plastic tents continuously flushed with a gas mixture composed of 20% O_2, 10% CO_2, and 70% N_2. Tents flushed with air were used for control animals. To exclude possible variations in caloric intake, we deprived both control and experimental animals of solid food and removed them from their tents twice a day for 10 to 15 minutes to be tube fed 10 ml of a 20% glucose solution. When adrenalectomized animals were used, this solution also contained 75 mM NaCl. Animals were treated in this manner for 48 hours, after which they were sacrificed. Renal cortical slices were prepared, and glucose production by the slices was determined as previously described (3). All incubations were carried out at pH 7.40 in Krebs-Ringer bicarbonate buffer containing 24 mM bicarbonate and 10 mM α-ketoglutarate.

In vitro studies

In all *in vitro* studies, renal cortical slices from normal rats on an ad libitum Purina chow diet were used. Slices from several animals were pooled, and gluconeogenesis was studied as previously outlined (3), with modifications to be described. In all studies 150-mg portions of sliced cortex from the same pool were incubated for 2 hours in several different media, in which the concentrations of [HCO_3^-], [H_2CO_3], and/or pH had been varied by altering the amount of $NaHCO_3$ added to the media and/or the CO_2 concentration in the gas phase. Media sodium concentration was maintained constant at 144 mEq per L by varying the concentration of NaCl.

Effect of changing bicarbonate concentration. To evaluate the effect of changes in pH *in vitro* produced by varying [HCO_3^-], we incubated slices in different media in which bicarbonate concentration was 12 (acidotic), 24 (control), or 48 mmoles per L (alkalotic). All flasks contained a single substrate (10 mM glutamine, glutamate, α-ketoglutarate, or oxalacetate), and the media were equilibrated before incubation with a gas mixture containing 95% O_2 and 5% CO_2.

Effect of changing P_{CO_2}. The influence of changes in pH *in vitro*, produced by varying P_{CO_2}, was studied by incubating slices in flasks containing the standard media ([HCO_3^-] 24 mM) that had been flushed with a gas mixture containing 10% (acidotic), 5% (control), or 2½% CO_2 (alkalotic) before incubation. All gas mixtures also contained 80% O_2 with the balance being N_2. Slices from the same pool were also incubated in media in which a similar degree of acidity or alkalinity had been produced by varying the [HCO_3^-] while keeping the [H_2CO_3] constant as described in the preceding section. All flasks contained 10 mM α-ketoglutarate as substrate. pH determinations of the media were made on samples obtained anaerobically 10 to 20 minutes before the end of incubation.

Isohydric studies

Studies were performed in which the effect of changes in P_{CO_2} and [HCO_3^-] on renal gluconeogenesis was determined, independent of variations in pH. Slices were incubated in five different media in which pH was held constant at 7.4, while P_{CO_2} and [HCO_3^-] were proportionally varied over a wide range (see Figure 1). All flasks contained 10 mM α-ketoglutarate as substrate. Media pH was determined before incubation and 10 to 20 minutes before the end of an incubation. The average of

TABLE I

Glucose production from ketoglutarate by renal coritical slices from rats with induced respiratory acidosis

Experiment	Control n	Control Glucose production	Control Plasma $[HCO_3^-]$	Respiratory acidosis n	Respiratory acidosis Glucose production	Respiratory acidosis Plasma $[HCO_3^-]$
		μmoles/g dry wt/90 min	*mEq/L*		*μmoles/g dry wt/90 min*	*mEq/L*
Intact rats	6	257 ± 19*	22.6 ± 1.7	7	351† ± 26	31.4† ± 1.8
Adrenalectomized rats						
Experiment A	4	221 + 14		5	267† ± 17	
Experiment B	4	205 ± 9	24.1 ± 2.3	4	246† ± 10	35.5† ± 0.66
A + B	8	213 ± 8		9	257‡ ± 6	

* Standard error.
† Significantly different from control, $p < 0.05$.
‡ Significantly different from control, $p < 0.01$.

these measurements was taken as the pH of the media during incubation.

Results

In vivo respiratory acidosis

The effect of *in vivo* respiratory acidosis on renal glucose production from α-ketoglutarate *in vitro* is summarized in Table I. Slices from animals with respiratory acidosis were found to produce significantly more glucose than slices from animals without respiratory acidosis. This was observed in both intact and adrenalectomized animals.

In vitro studies

Effect of changing bicarbonate concentration. Table II summarizes the influence of changes in media pH produced by varying $[HCO_3^-]$ ("metabolic" acidosis and alkalosis) on renal glucose production from glutamate, glutamine, α-ketoglutarate, or oxalacetate. Acidosis significantly increased glucose production from glutamine, glutamate, α-ketoglutarate, and oxalacetate; alkalosis significantly decreased glucose production from each of these substrates.

Effect of changing P_{CO_2}. The effects on gluconeogenesis of alterations in media pH produced by varying P_{CO_2} ("respiratory" acidosis and alkalosis) are summarized in Table III, and are compared to the effects of similar alterations of media pH produced by varying $[HCO_3^-]$ ("metabolic" acidosis and alkalosis). Respiratory acidosis significantly increased gluconeogenesis from α-ketoglutarate ($p < 0.001$), and respiratory alkalosis produced a significant decrease ($p < 0.001$). Despite similar degrees of alteration of media pH, glucose production in metabolic acidosis was significantly greater than in respiratory acidosis ($p < 0.05$). Also, metabolic alkalosis reduced gluconeogenesis more than did respiratory alkalosis ($p < 0.02$) despite similar elevations of pH.

Isohydric studies

The influence of proportional (isohydric) changes of media $[H_2CO_3]$ and $[HCO_3^-]$ on renal

TABLE II

The effect of changes in medium pH produced by varying medium $[HCO_3^-]$ on glucose production by renal cortical slices from normal rats

Substrate (10 mM)	Glucose production: Control (7.4) No. of flasks	Control (7.4) Mean ± SE	Acidotic (7.1) No. of flasks	Acidotic (7.1) Mean ± SE	Alkalotic (7.7) No. of flasks	Alkalotic (7.7) Mean ± SE
		μmoles/g dry wt/2 hours		*μmoles/g dry wt/2 hours*		*μmoles/g dry wt/2 hours*
Glutamine	53	78.6 ± 2.4	48	139* ± 5.0	28	60* ± 3.9
Glutamate	52	134 ± 5.1	44	150† ± 6.5	18	72* ± 5.0
α-Ketoglutarate	54	184 ± 7.7	51	246* ± 11.7	45	104* ± 6.0
Oxalacetate	55	100 ± 6.1	49	121* ± 5.6	23	72* ± 6.0

* Significantly different from control, $p < 0.01$.
† Significantly different from control, $p < 0.05$.

TABLE III

Comparison of the effects of changes in medium pH produced by varying [HCO_3^-] or P_{CO_2} on glucose production from α-ketoglutarate by renal cortical slices from normal rats

	No. of flasks	Glucose production	Observed pH
mm Hg, mEq/L		*μmoles/g dry wt/2 hours*	
Experiment 1			
Control (P_{CO_2} 40, [HCO_3^-] 24)	18	181 ± 7.7	7.43 ± 0.02
Metabolic acidosis (P_{CO_2} 40, [HCO_3^-] 12)	22	257* ± 10.0	7.12* ± 0.03
Respiratory Acidosis (P_{CO_2} 80, [HCO_3^-] 24)	22	226* ± 9.9	7.14* ± 0.02
Experiment 2			
Control (P_{CO_2} 40, [HCO_3^-] 24)	16	228 ± 17.0	7.48 ± 0.05
Metabolic alkalosis (P_{CO_2} 40, [HCO_3^-] 48)	22	76.5* ± 9.6	7.88* ± 0.04
Respiratory alkalosis (P_{CO_2} 20, [HCO_3^-] 24)	22	123* ± 9.9	7.81* ± 0.04

* Significantly different from control, $p < 0.01$.

glucose production from α-ketoglutarate is shown in Figure 1. Glucose production on the ordinate is plotted against bicarbonate concentration (lower scale) and carbonic acid concentration (upper scale) on the abscissa. Each point is the mean (± standard error) of 12 or 13 observations. The mean observed pH is shown for each group. In spite of similar media pH in all groups, gluconeogenesis increased as [HCO_3^-] and [H_2CO_3] were proportionally decreased. For instance, glucose production was significantly greater ($p < 0.01$) at [HCO_3^-] 14.5 mmoles per L and [H_2CO_3] 0.634 mmole per L (pH 7.41) than it was at [HCO_3^-] 50.2 and [H_2CO_3] 2.37 (pH 7.44).

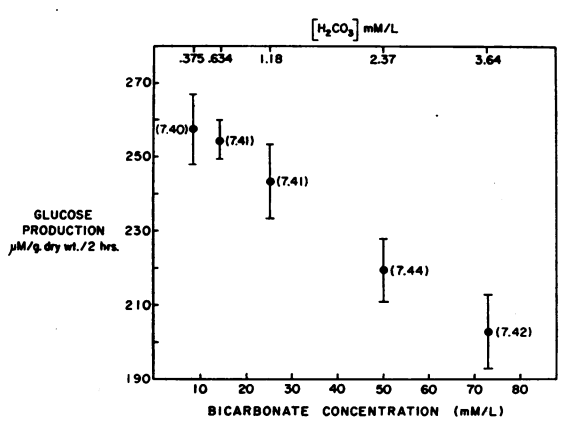

FIG. 1. THE EFFECT OF PROPORTIONAL (ISOHYDRIC) CHANGES IN MEDIUM [H_2CO_3] AND [HCO_3^-] ON GLUCOSE PRODUCTION FROM α-KETOGLUTARATE BY RENAL CORTICAL SLICES FROM NORMAL RATS. Figures in parentheses are the mean observed pH. Bars indicate ± 1 SEM.

Discussion

In previous studies renal cortex from rats with metabolic acidosis was found to have an increased capacity for glucose production. The present experiments demonstrate that this is also true for cortex from animals with respiratory acidosis, indicating that pH per se, rather than [HCO_3^-] or P_{CO_2}, is the most important acid-base variable of ECF affecting renal gluconeogenesis. The mechanism by which *in vivo* changes in ECF pH influence renal gluconeogenesis was not determined in these studies. Since changes in circulating hormones, plasma substrate concentrations, renal cortical blood flow, or other physiological variables may take place when extracellular pH is modified *in vivo*, the observed change in renal gluconeogenesis may be only indirectly related to pH. The effect of respiratory acidosis is not mediated through changes in adrenal cortical activity, since it is also demonstrable in adrenalectomized animals.

The changes in renal glucose production found when ECF pH was acutely varied *in vitro* were qualitatively similar to those noted during *in vivo* acidosis or alkalosis. The results of the *in vitro* experiments suggest a direct effect of acid-base status on renal gluconeogenesis. Since *in vitro* changes in ECF pH produced by varying [HCO_3^-] and *in vitro* changes in ECF pH produced by varying P_{CO_2} have similar effects on renal gluconeogenesis, these studies also suggest that ECF pH per se, rather than [HCO_3^-] or P_{CO_2}, is the im-

portant acid-base variable controlling renal glucose production.

The observation that renal cortical slices from acidotic animals produce more glucose from glutamine, glutamate, α-ketoglutarate, and oxalacetate than do slices from control animals (3) suggests that there is increased activity of one of the potentially rate-limiting reactions between oxalacetate and glucose. The mechanism of the presumed enhancement in enzyme activity is difficult to establish when ECF pH is varied *in vivo*. The enhanced deamidation and transamination of glutamine during acidosis *in vivo* (4–6) generate increased gluconeogenic substrate in the form of glutamate and α-ketoglutarate. This increase in glutamate and α-ketoglutarate might then, by sequential substrate induction, increase the activity of enzymatic reactions farther along the pathway to glucose. However, increased degradation of glutamine cannot be invoked to explain the results of our *in vitro* studies, in which glucose production from oxalacetate was increased when slices from normal animals were incubated in acidotic media in the absence of glutamine. These *in vitro* studies suggest that the enhanced renal gluconeogenesis in acidosis is due to direct stimulation of one of the enzymatic steps between oxalacetate and glucose, rather than to substrate induction of gluconeogenic enzymes secondary to increased degradation of glutamine.

An increase in enzyme activity may be due either to enhanced enzyme synthesis or to increased activation of existing enzyme. The enhanced activation of existing enzyme could be the result of increased conversion of inactive enzyme to an active form or of changes in the concentration of activators, cofactors, or inhibitors of the reaction. The present studies do not indicate whether the enhanced gluconeogenic capacity of renal cortex from acidotic animals is due to increased *in vivo* synthesis of a rate-limiting gluconeogenic enzyme, or to *in vivo* activation of existing enzyme. Neither is it known whether the increased glucose production by normal renal cortex incubated in acidotic media is due to enhanced *in vitro* enzyme synthesis or to *in vitro* activation of existing enzyme. It is possible that the stimulatory effect of *in vivo* acidosis on renal gluconeogenesis, which is not evident until the animal has been acidotic for more than 12 hours (3), is due to a gradual increase in enzyme synthesis; whereas the stimulatory effect of *in vitro* acidosis on renal gluconeogenesis, which is evident during a 2-hour incubation in acidotic medium, is due to rapid activation of previously existing enzyme.

Although changing ECF pH *in vitro* by varying $[HCO_3^-]$, and altering pH *in vitro* by modifying P_{CO_2}, had qualitatively similar effects on renal cortical glucose production, quantitative differences were observed. Changes in pH produced by varying $[HCO_3^-]$ resulted in greater increases during acidosis and greater decreases in glucose production during alkalosis than were noted after similar changes in pH produced by varying P_{CO_2}. When pH was held constant at 7.4 over a wide range of $[HCO_3^-]$ and P_{CO_2}, gluconeogenesis was found to vary independently of pH, increasing as P_{CO_2} and $[HCO_3^-]$ were proportionally decreased. A comparison of gluconeogenesis during metabolic acidosis with that during respiratory acidosis indicates that a proportional decrease in P_{CO_2} and $[HCO_3^-]$ isohydrically at pH 7.1 also enhances glucose production. Similarly the studies made during metabolic and respiratory alkalosis demonstrate that gluconeogenesis also increases when P_{CO_2} and $[HCO_3^-]$ are proportionally decreased at pH 7.8 (Table III).

In a bicarbonate buffer system there are three variables: pH, $[HCO^-_3]$, and P_{CO_2}. It is not possible to hold two of these variables constant while studying the effects of the third. Despite this, it seems clear from the present *in vitro* studies that renal cortical glucose production is at least in part controlled by media pH per se. The specific effects of P_{CO_2} or $[HCO_3^-]$, however, are poorly defined. Although renal glucose production is enhanced when $[HCO_3^-]$ is decreased and P_{CO_2} held constant, this increase may be secondary to the associated decrease in pH. Conversely, although renal gluconeogenesis is diminished by a fall in P_{CO_2} when $[HCO_3^-]$ is held constant, this decrease may be due to the associated rise in pH. The only definite statement than can be made concerning the effects of P_{CO_2} and $[HCO_3^-]$ is that renal glucose production increases when these parameters are proportionally decreased and pH is held constant.

The present experiments demonstrate that renal gluconeogenesis is stimulated by respiratory as well as metabolic acidosis. It has been suggested

that the increase in renal ammonium production known to occur during metabolic acidosis may be secondary to enhanced renal gluconeogenesis (3). Although the effects of respiratory acidosis on renal ammonium production are less well established, the studies of Aber and co-workers in man (7, 8) and Schwartz, Brackett, and Cohen in the dog (9), which demonstrate increased ammonium excretion during acute or chronic hypercapnia, are consistent with the hypothesis of an integral relation between renal gluconeogenesis and renal ammonia production in respiratory as well as metabolic acidosis. The recent demonstration by Aber, Morris, and Housley (10) of a correlation between *in vivo* renal glucose production and the bidirectional release of ammonium by the kidney in patients with chronic pulmonary disease lends further support to this hypothesis. The effect of hypercapnia on ammonium excretion in the rat was studied by Carter, Seldin, and Teng (11), who noted a significant increase in ammonium excretion during the first 2 days of hypercapnia. Thus, our *in vivo* studies, performed after 48 hours of hypercapnia, presumably were done with renal cortical slices from rats with enhanced ammonium excretion. Carter and his associates also noted a much smaller increase in ammonium excretion during respiratory acidosis as compared with metabolic acidosis, an observation consistent with the present *in vitro* studies in which reduction of pH by lowering media [HCO_3^-] regularly caused a greater stimulation of renal gluconeogenesis than did an equivalent reduction of pH achieved by raising P_{CO_2}.

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