Influence of aldosterone on renal ammonia production

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WELBOURNE, TOMAS C., AND DANIELE FRANCOEUR. *Influence of aldosterone on renal ammonia production.* Am. J. Physiol. 233(1): E56–E60, 1977 or Am. J. Physiol.: Endocrinol. Metab. Gastrointest. Physiol. 2(1): E56–E60, 1977. — Administering D-aldosterone, 7 $\mu g\ 100\ g^{-1}$, to rats results in a marked rise in ammonium excretion and metabolic alkalosis. Increased ammonium excretion is not related to either a significant elevation in potassium excretion nor to hypokalemia. Consequently, potassium depletion does not appear to be the causative factor in the aldosterone-stimulated ammonium excretion. Isolated kidneys from aldosterone-treated rats, perfused with 1 mM L-glutamine, produced twice as much ammonia from glutamine as did controls. Ammonia production per glutamine extracted increased from 1.33 ± 0.07 in control to 1.79 ± 0.08 in kidneys from hormone-treated rats, suggesting stimulation of the mitochondrial glutaminase I–glutamate dehydrogenase pathway; this was supported by a proportional rise in production of glucose and CO_2, end products of glutamine's carbon skeleton. Consequently, aldosterone-stimulated renal ammonia production, by specifically activating the mitochondrial pathway, leads to the elimination of hydrogen ions in the form of urinary ammonium excretion and an ensuing metabolic alkalosis.

glutamine metabolism; renal function

THE ADRENAL GLANDS APPEAR TO PLAY THE KEY ROLE in the renal ammonia production response to acid loading (19, 24, 32, 33, 35). This response involves the corticosterone-mediated activation of the mitochondrial glutaminase I–glutamate dehydrogenase pathway resulting in 2 molecules of ammonia produced per glutamine entering this pathway (18, 29, 33, 34). In the absence of adrenals, the response to acid loading is markedly blunted (4, 17, 19, 23, 32, 35, 38) and glutamine fails to penetrate the mitochondria (32, 35). This defect can be corrected by administering exogenous glucocorticoids (4, 17, 24, 32, 35, 38) and the response can be restored within 30 min (32). Noteworthy, aldosterone, the predominant mineralocorticoid in the rat, is remarkably effective in restoring the renal response in adrenalectomized, acid-loaded rats (4, 17). The mechanism by which aldosterone stimulates renal ammonia production has not been evaluated in the rat. Results from the present work show that aldosterone stimulates ammonia production by activating the mitochondrial glutaminase I pathway by an apparently direct mechanism.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing between 350 and 450 g were employed in this investigation. Rats were maintained, one per cage, in specially designed metabolic cages for 24-h urine collections. Food, Purina rat chow, and water were provided ad libitum. Preservation of 24 urine collections was ensured by the addition of 0.5 ml of concentrated HCl (10). Analysis of 24-h urine collections for sodium and potassium was by flame photometry; measurement of urine ammonium has previously been described (31). Rats were made potassium depleted by being fed a low potassium diet over a 2-wk period; depletion was estimated by measurement of plasma potassium concentration determined by flame photometry. Blood acid-base balance was estimated from plasma total CO_2 measured manometrically (15), and arterial blood pH measured on an Orion pH meter.

Aldosterone monoacetate (Sigma Chemical Co., St. Louis, Mo.) was suspended in corn oil and administered by intramuscular injection at a daily dose of 7 $\mu g\ 100\ g^{-1}$ body wt in 0.5 ml corn oil; control rats received 0.5 ml corn oil.

Injections were given once a day over a 2-day period except for kidney perfusion studies in which case aldosterone was administered once, 12 h prior to isolation and perfusion of the kidney. Blood samples for determination of total CO_2 and potassium concentration were obtained at the time of perfusion.

Perfused kidneys. Twelve hours after aldosterone or corn oil injection, rats were anesthesized with sodium pentobarbital, 30 mg kg^{-1} ip, and the kidneys isolated and perfused as described (30). The perfusion, containing Dextran T-110 in place of albumin, was filtered through a 3-μm Millipore filter prior to use. L-Glutamine at an initial 1 mM concentration was the sole exogenous substrate employed; tracer amounts of uniformily labeled L-[^{14}C]glutamine (New England Nuclear Corp., Boston, Mass.) (sp act. 127 $\mu Ci/\mu mol$) were added just prior to perfusing. Radioactive glutamine was checked for purity by thin layer chromatography and scanned on a Packard radiochromatogram scanner; radioactivity was undetected in the glutamate or 5-oxoproline fraction. In order to retain metabolically produced $^{14}CO_2$ from glutamine, the perfusion circuit was converted to a closed system by the modification described by Bowman (28). During the 60-min perfusion period, samples were taken at 15-min intervals and perfusate

concentrations of glutamine L-[^{14}C]glutamine, ammonia, glutamate, glucose, and $^{14}CO_2$ changed in a linear fashion over the perfusion time course. Partial pressure of perfusate oxygen was always above 250 mmHg at the end of the perfusion period.

Analysis. Perfusate concentrations of ammonium, glutamine, and glutamate were determined as previously described (30, 34); glucose was measured using a modification of the glucose oxidase method (12). To analyze perfusate $^{14}CO_2$, anaerobically drawn aliquots, 0.1 ml, of perfusate were placed in the outer well of a microdiffusion chamber. Hyamine hydroxide was placed in the center well of the microdiffusion chamber, and the reaction was begun by placing phosphoric acid, pH 5.0, in the outer well, covering, and mixing it, followed by a 90-min incubation period. After incubation, the hyamine hydroxide was transferred to counting vials containing Aquasol (New England Nuclear) and radioactivity was determined by scintillation spectrometry.

Radioactive glutamine and glutamate were isolated from the perfusate on thin layer chromatograms (Kodak cellulose sheets no. 6064) with *n*-butanol, pyridine, and H_2O solvent (1:1:1). Ninhydrin spray was employed to localize the spots with glutamine, glutamate, and 5-oxoproline standards run simultaneously in each experiment; R_Fs for glutamine, glutamate, and 5-oxoproline were 0.33, 0.50, and 0.58, respectively.

Calculations. Glutamine uptake was derived for both enzymatically determined glutamine as described (14) and from isotopic glutamine as follows

$$\text{glutamine}_{t_0},\ \mu\text{mol} \times \frac{\text{glutamine}_{t_0},\ \text{cpm} - \text{glutamine}_{t_{60}},\ \text{cpm}}{\text{glutamine}_{t_0},\ \text{cpm}} = \text{glutamine},\ \mu\text{mol}/60\ \text{min} = \text{glutamine uptake},\ \mu\text{mol min}^{-1}$$

Glutamate was determined in an analogous fashion. Ammonia and glucose production rates were calculated from enzymatically measured concentrations (5, 16), whereas conversion of [^{14}C]glutamine to $^{14}CO_2$ is given below

$$\text{glutamine}_{t_0},\ \mu\text{mol} \times \frac{^{14}CO_{2\ t_{60}},\ \text{cpm} - {}^{14}CO_{2\ t_0},\ \text{cpm}}{[^{14}\text{C}]\text{glutamine}_{t_0},\ \text{cpm}} = \text{glutamine},\ \mu\text{mol, converted to } CO_2/60\ \text{min}$$

All measurements of substrate and product concentrations were performed in duplicate. Results following aldosterone administration are considered significantly different from the sham-treated controls at the 0.05 percentile level by the Student *t* test analysis.

RESULTS

A single injection of aldosterone increases ($P < 0.05$) 24-h ammonium excretion, whereas continuing the aldosterone for a second 24-h period elevates the excretion levels threefold greater than controls (Fig. 1). In addition, aldosterone in a dose as small as 2.5 μg 100 grams^{-1} increases ammonium excretion from 297 ± 25 to 407 ± 51 μmol 24 h^{-1} ($P < 0.05$). Figure 2 illustrates the relationship between ammonium, sodium, and potassium excretion. The elevation in ammonium excretion during the first 24 h does not correspond with increased potassium excretion,[1] a result which suggests

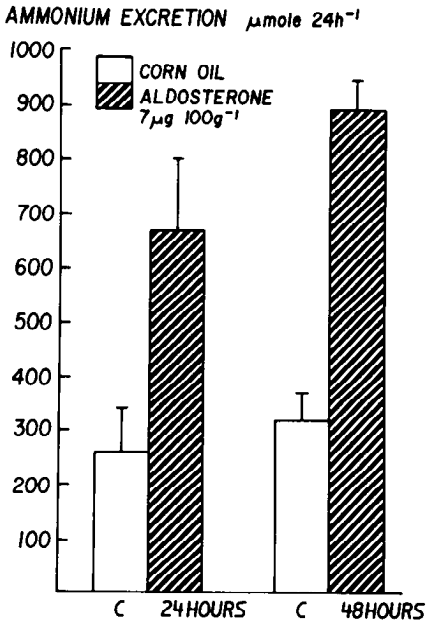

FIG. 1. Twenty-four-hour ammonium excretion by 6 control and 6 aldosterone-treated rats. Aldosterone was administered as described in METHODS. Results are means and SE.

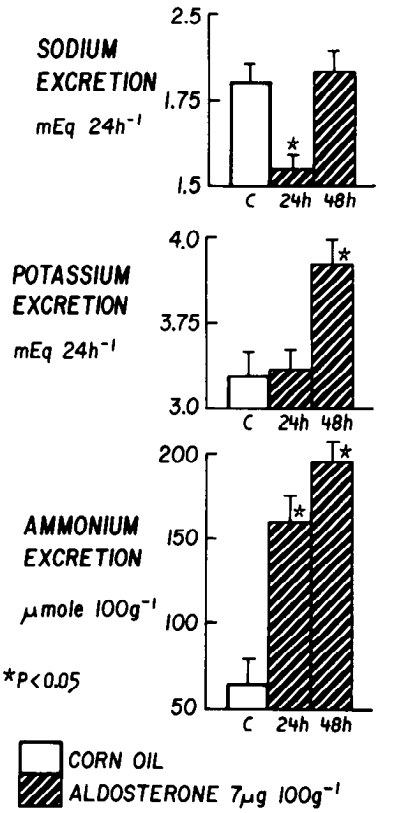

FIG. 2. Twenty-four-hour sodium, potassium, and ammonium excretion from control and aldosterone-administered rats. Each group was composed of 6 rats; results are means and SE.

[1] Although an antinatriuresis is characteristic of aldosterone, a kaliuresis is much more variable. In agreement with the present findings, other investigations have shown potassium excretion to be unchanged after similar doses of aldosterone (9, 27, 36) or synthetic mineralocorticoids (26).

that potassium depletion is not a factor in the ammonium-excretion response. The characteristic sodium effect retention of aldosterone is shown on the left; an increased ammonium excretion rate, secondary to sodium-for-hydrogen ion exchange, is an unlikely explanation because 48-h sodium excretion has returned to control values, whereas ammonium remains elevated. The rise in potassium on the second day reflects "carbohydrate activity" of aldosterone (2), which might be caused by aldosterone occupying glucocorticoid receptors (8). In spite of these effects of aldosterone on sodium and potassium excretion, the increase in ammonium excretion is independent of, and appears to be a basic expression of, an effect of the hormone upon renal metabolism.

As the result of aldosterone treatment, plasma-electrolyte and acid-base balance represents a pattern different from that seen in either control or potassium-depleted rats (Table 1). The significant rise in ammonium excretion occurs without a reduction in plasma potassium concentration, a finding consistent with the normal urinary potassium excretion (Fig. 2). As a consequence of excess H^+ elimination as ammonium, the rats developed a metabolic alkalosis, plasma HCO_3^- 32.8 ± 1.0 mmol (Table 1).

In contrast, maintaining rats on a potassium-deficient diet for 14 days results in hypokalemia without a concomitant metabolic alkalosis. Interestingly, this degree of potassium depletion had little effect on ammonium excretion because excretion rose only 40%, a finding in agreement with others (5). In fact such a small rise can be attributed to the significant elevation in plasma glutamine concentration (Table 1). Nevertheless, aldosterone-treated rats exhibit a twofold increase in ammonium excretion without either an elevation in potassium excretion or hypokalemia; potassium depletion itself cannot account, quantitatively, for this response.

Renal metabolism after aldosterone treatment is profoundly altered (Table 2). Qualitative changes in perfusate [^{14}C]glutamine during a typical experiment are shown in Fig. 3. Glutamine is extracted, accounting for the gradual decrease in the ninhydrin spot (R_F = 0.33), whereas ammonia, not shown, and a small amount of glutamate is returned. Table 2 shows glutamine uptake and ammonia, glucose, and $^{14}CO_2$ production rates by kidneys from control and aldosterone-treated rats. In the control kidneys, ammonia produced per glutamine extracted was 1.33, a result consistent with predominant glutamine utilization via the cytoplasmic γ-glutamyltransferase pathway (29, 33, 34). In addition, the carbon end products of the mitochondrial pathway, CO_2 and glucose, accounted for only $^{33}/_{125} \times 100$ or 26.4% of the glutamine carbon atoms extracted. Consequently,

TABLE 1. *Plasma electrolytes and acid-base balance in control, aldosterone-treated, and potassium-depleted rats*

	Na, meq/liter	K, meq/liter	HCO_3^-, mmol/liter	pH, μm	Glutamine, μmol/ml	Urine Ammonia, μmol/day
Control (5)	136±3	4.3±0.6	25.8±2.3	7.35±0.04	0.58±0.06	312±42
Aldosterone (5)	138±6	4.2±0.4	32.8*±1.0	7.51*±0.07	0.62±0.06	680*±115
K Depleted (5)	138±5	3.2*±0.4	26.5±1.1		0.74*±0.06	442*±72

Values are means ± SE. Numbers in parentheses are numbers of rats in each group. Aldosterone, 7 μg 100 g^{-1}, was administered 12 h prior to measurements. Potassium-depleted rats were maintained on low K diet for 2-wk. * Significantly different from control ($P < .05$).

TABLE 2. *Glutamine metabolism in isolated kidneys from aldosterone-treated rats*

	Ammonia Production	Glutamine Uptake, μmol	Glucose Production, h^{-1} kd^{-1}	$^{14}CO_2$ Production
Control (8)	34.2±2.7	25.7±4.2	3.42±0.60	4.02±1.60
Aldosterone (8)	68.3±2.0	38.4±2.0	10.20±0.85	12.0±2.4
P value	$P < .001$	$P < .01$	$P < .01$	$P < .01$

Values are means ± SE. Kidneys were perfused with 1 mM L-glutamine and tracer [^{14}C]glutamine. Aldosterone, dissolved in corn oil, was administered im for 12 h (7 μg 100 g^{-1}); controls received corn oil.

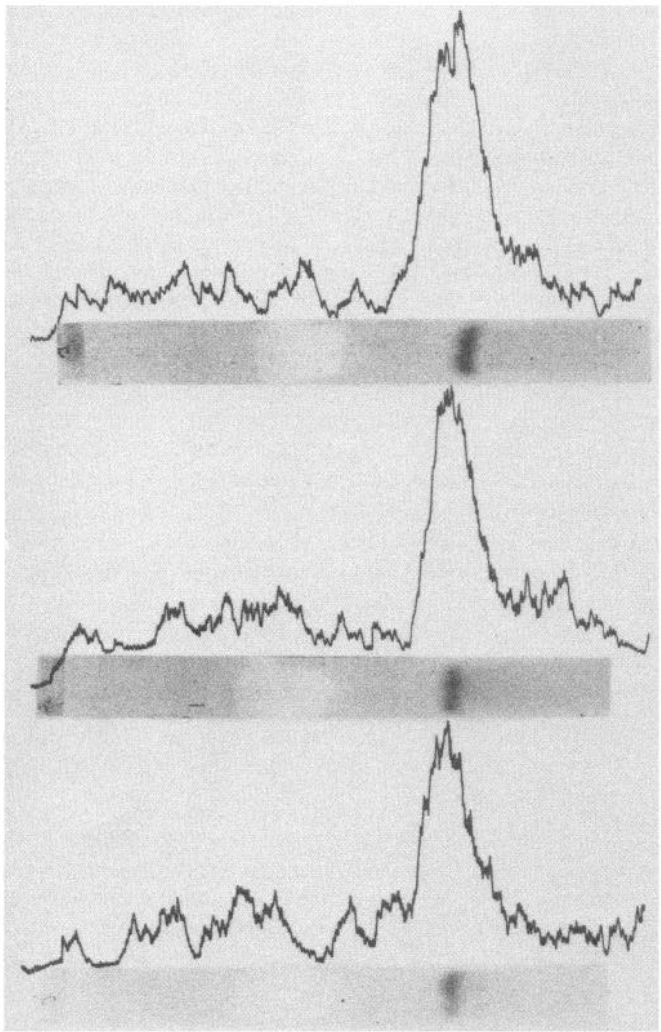

FIG. 3. Typical radiochromatogram of perfusion media containing glutamine, 1 mM, and ^{14}C-μ-glutamine sampled at 2 min (top), 30 min (middle), and 60 min (bottom) after beginning of perfusion. Glutamine, sole ninhydrin spot with R_F = 0.33, was extracted with an ammonia produced to glutamine uptake ratio of 1.33. Remainder of glutamine, both nitrogen and carbon atoms, was not released into perfusate.

the normal rat kidney utilizes less than 30% of the glutamine extracted via the mitochondrial pathway. In contrast, aldosterone administration stimulates ammonia production from glutamine twofold, 34 vs. 68 μmol h^{-1}, a result that is consistent with the twofold increase in ammonium excretion shown in Fig. 1. Glutamine uptake was significantly increased, whereas the ammonia produced per glutamine uptake ratio rose from 1.33 to 1.77 ($P < 0.01$) consonant with activation of the mitochondrial pathway. Glutamate production, 16.2 ± 3.2 μmol h^{-1}, remained unchanged 15.0 ± 5.6 μmol h^{-1}, whereas the bulk of glutamine nitrogen is released as ammonia by the combined action of glutaminase I-glutamate dehyrogenase. Glucose production and production of $^{14}CO_2$ from glutamine, the carbon end products of glutamine catabolism, both increased significantly confirming the accelerated flow of glutamine molecules through the mitochondrial pathway.

DISCUSSION

These results indicate that aldosterone is a potent stimulant of ammonia production by the kidney (Table 2). Because glutamine is the major precursor of endogenous ammonia formation (1, 20), it is not surprising that kidneys from aldosterone-treated rats showed an increase in glutamine uptake (Table 2). Glutamine taken up by hormone-treated kidneys could, in theory, be utilized by either one of the two parallel glutamine-utilizing pathways, that is, by either cytoplasmic γ-glutamyltransferase, the dominant pathway in nonacidotic kidneys (18, 29, 33, 34), or by the mitochondrial pathway. It is clear from the data that aldosterone activates preferentially the mitochondrial pathway. This conclusion is based on the following facts: 1) the ammonia produced per glutamine taken up rises toward 2.0, a ratio expected if glutamine were deamidated and deaminated inside the mitochondria (the ratio is less than 2.0 because of the mixed activities of both the mitochondrial and cytoplasmic pathways); and 2) the carbon end products, $^{14}CO_2$ and glucose, are derived from the mitochondrial pathway and these increase with aldosterone-stimulated glutamine uptake into the mitochondrial pathway.

The mechanism by which aldosterone stimulates the mitochondrial pathway is apparently independent of potassium depletion. This is supported by two lines of evidence. First, no evidence for potassium depletion was obtained during the initial 24 h (Table 1, Fig. 2). Second, although potassium depletion is capable of increasing ammonium excretion (Table 1 and refs. 1 and 4), quantitatively the response, roughly an increase of 40%, is not comparable to that observed, some 100%. One must conclude that potassium depletion is not the factor responsible for the increased ammonia production.

Another possible mechanism by which aldosterone could act is through induction of protein synthesis associated with enzymatic activity of one or more enzymes of the mitochondrial pathway (8). However, Edelman and associates (13) have shown that aldosterone stimulates acid excretion by the dog kidney and that this response is not blocked by actinomycin D, a result which suggests that a second pathway exists by which aldosterone exhibits its renal effect on acid excretion. A direct effect of aldosterone upon mitochondrial membranes would not be inconsistent with the above study in the dog. This possibility as a second pathway becomes even more attractive in light of the association of injected tracer amounts of aldosterone with mitochondrial membranes (7) of proximal tubule cells (37); physiological amounts of aldosterone exert effects on mitochondrial function that are not inconsistent with alterations in membrane permeability (3, 14). Interestingly, the proximal tubule mitochondrial population can be activated during acidosis to levels 10- to 20-fold greater than control (6, 18, 33, 35) and this is associated with an increase in mitochondrial permeability (33). Consequently, aldosterone activation of the mitochondrial pathway is similar to that observed during chronic acidosis.

This leads to the question as to a possible role of aldosterone in activating the mitochondrial pathway in the response to acidosis. It is known that eliminating the adrenals results in a blunted response to acid loading (4, 17, 19, 23, 32, 35, 38) because of an inability of acidosis itself to activate the mitochondrial pathway (32, 38). Administering exogenous glucocorticoids restores (4, 17, 32), and even increases, ammonia production (4); in fact, glucocorticoid administration is capable of stimulating the mitochondrial pathway to levels observed in acidosis (33), whereas acidosis has no direct stimulatory effect on renal ammonia production (1, 11, 21, 22). Endogenous glucocorticoid levels are elevated by acidosis in the rat, and this response correlates with activation of the mitochondrial pathway (35). However, inasmuch as acidosis stimulates the adrenals via the pituitary (35), it is possible that both glucocorticoid (corticosterone) and mineralocorticoid (aldosterone) release rates are stimulated. In support of this, metabolic acidosis stimulates release of aldosterone in the rat (25), and aldosterone is a far more potent stimulator of renal ammonia production than most glucocorticoids (4). Furthermore, in the dog, acute metabolic acidosis results in a rise in the K^+/Na^+ excretion level suggesting mineralocorticoid activity (38), whereas no similar effect is seen in the rat (unpublished observation). Regardless of the actual physiological role of aldosterone in the response to acidosis, this hormone can activate the mitochondrial glutaminase I pathway by a mechanism that has implications for acid-base regulation as well as for the second pathway of aldosterone action in the kidney.

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