Influence of potassium on renal ammonia production

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TANNEN, RICHARD L., AND JOHN McGILL. Influence of potassium on renal ammonia production. Am. J. Physiol. 231(4): 1178-1184. 1976.—The influence of potassium homeostasis on ammonia production was investigated with both cortical and medullary slices from rat kidney. Renal cortical slices from rats depleted of potassium by dietary restriction produced 31% more NH3 than slices from pair-fed controls. A high-potassium diet for 1 wk diminished ammonia production in cortical slices by 5% in comparison with rats pair fed a normal diet (161 vs. 169 μmol/90 min per g wet wt; P < 0.05). Pair feeding did not introduce an experimental artifact, since animals ingesting similar K+ diets showed no difference in NH3 production. In contrast to cortex, NH3 production by outer medullary slices from K+-depleted animals was similar to pair-fed controls. Medulla from potassium-loaded rats exhibited an impressive inhibition in NH3 production averaging 36%. These striking differences between cortex and medulla suggest that specific alterations in potassium homeostasis influence NH3 production selectively at different tubular sites. In vitro manipulation of K+ homeostasis produced by varying bathing media K+ from 0 to 144 mM, with concomitant changes in intracellular K+ from 30 to 130 mM, had no detectable influence on NH3 production by cortical slices. Hence altered cortical ammoniagenesis is not the direct result of acute changes in extracellular or intracellular cortical fluid K+ or in the transcellular gradient for K+. Although the specific cellular mechanisms whereby K+ alters ammoniagenesis remains undefined, the observation that K+ loading diminishes while K+ depletion enhances NH3 production supports the supposition that K+ and NH3, are linked in a physiologic control system.

renal cortex; renal medulla; gluconeogenesis; potassium depletion; potassium adaptation

RENAL AMMONIA PRODUCTION and potassium metabolism appear to be intimately related in a fashion suggesting a homeostatic regulatory system (33, 35, 36). Studies in intact animals and humans, as well as investigations of renal metabolism in vitro, indicate that potassium deficiency increases renal ammonia production (8, 15, 22, 26, 33). Furthermore, a primary increase in renal ammonia production is accompanied by a decrease in potassium excretion (35). Finally, in vivo studies of rats, dogs, and men have suggested that chronic ingestion of a high-potassium diet may inhibit renal ammoniagenesis (19, 20, 36); however, this finding has not been confirmed directly (19).

The present studies of renal slice metabolism were undertaken to determine whether sustained ingestion of a high-potassium diet suppresses renal ammonia production and to explore further the mechanism whereby alterations in potassium homeostasis influence ammoniagenesis. The data indicate that ammoniagenesis is suppressed by a high potassium intake, that cortex and outer medulla respond in different fashions to alterations of potassium homeostasis, and that ammoniagenesis by renal cortex is not influenced by in vitro alterations of extracellular and intracellular potassium concentration.

METHODS

Male Sprague-Dawley rats weighing 211-476 g were sacrificed by decapitation. The kidneys were removed, the capsule was stripped, and cortical or outer medullary slices were prepared with a Stadie-Riggs microtome. Slices of cortex with a total weight of 150 or 200 mg or slices of outer medulla weighing 50 or 100 mg were placed in 6.2 ml of medium in a 50-ml Erlenmeyer flask and incubated at 37°C for 90 min in a shaking water bath at 100 oscillations/min. In all paired experiments the weight of tissue utilized was comparable (±5 mg). The incubation medium, which was equilibrated with 95% O2-5% CO2, contained in mM: Na 139, K+ 4.8, Ca2+ 2.4, Mg2+ 1.1, Cl 123, P 1.1, HCO3− 24, SO3− 1.1, and glucose 9.7. In experiments where tissue spaces were determined 0.25 μCi of [14C]inulin was added to each flask.

In some experiments slices were preincubated at 0°C for 2 h in a potassium- (sodium-substituted) and glutamine-free medium otherwise identical to the standard incubation solution and then incubated in standard fashion.

At the termination of the incubation the medium was deaerated. An aliquot utilized for glucose determination was treated with zinc sulfate and barium hydroxide and the remaining medium analyzed directly for NII3 content. The tissue was dried overnight at 104°C and electrolytes and inulin content were determined on nitric acid extracts.

Analytical Procedures

Sodium and potassium were determined by flame photometry. Glucose was assayed with glucose oxidase (17). Ammonia was determined with an Orion Research Inc. ammonia electrode on specimens alkalized with NaOH (10, 34). Specimens for [14C]inulin determinations were added to Aquasol (New England Nuclear Corp.) and assayed with a liquid scintillation spectrometer. The electrolyte content of muscle was analyzed with nitric acid extracts of tissue.
Experimental Protocols

Alterations of potassium homeostasis in vivo. A low-Na+/K+ diet (Nutritional Biochemicals Corporation) supplemented with appropriate quantities of NaCl, KCl, and Alphacel was utilized for manipulations of potassium homeostasis in vivo. Pair-feeding techniques were employed with animals of similar initial weights. Ad libitum ingestion of tap water was permitted. Slice studies with paired animals were performed on the same day and the analytic procedures were carried out in parallel.

Potassium depletion (N = 16). Potassium depletion was induced by feeding a diet for 4 wk that contained no potassium and 0.05 mmol Na/g of diet. Pair-fed controls ingested a diet with a comparable sodium concentration and 0.06 mmol K/g. Rats depleted of potassium by this means had a mean muscle potassium concentration of 280 compared with 380 μmol/g dry wt in paired controls, a serum potassium of 2.4 compared with 3.8 mM. These changes are similar to those observed by Ching, Rogoff, and Gabuzda (9), who utilized an identical model of potassium depletion.

Potassium loading (N = 51). Potassium loading was accomplished by feeding for a minimum of 5 days a diet containing 0.9 mmol K and 0.05 mmol Na/g. Pair-fed controls ingested a diet containing comparable concentrations of sodium and 0.03 mmol K/g. Urinary potassium excretion measured in four rats for the last 2 days of the ingestion period averaged 1.4 mmol/kg per day with the normal diet and 41.2 mmol/kg per day with potassium loading.

Normal studies (N = 10). To rule out potential artifacts possibly induced by the pair-feeding techniques, slice studies utilizing renal tissue from animals given a diet for 1 wk containing 0.05 mmol Na and 0.03 mmol K/g were compared with controls ingesting the same diet.

Alterations of potassium content of incubation medium. Incubation of cortical slices in potassium concentrations of 0, 7.6, 11.7, 73, and 144 mM were compared in paired fashion with normal media (4.8 mM) with sodium used as a counterion to maintain isotonicity. These in vitro manipulations were carried out with preincubated slices as well as with the standard incubation technique.

Calculations

All in vitro and in vivo manipulations were performed and analyzed in paired fashion with a Student’s t test. No difference was detected with the ammonia electrode between distilled water and solutions containing 10 mM glutamine. Incubations of substrate blanks containing 9.7 mM glutamine but without renal tissue were carried out with each experiment and ammonia production averaged 3% of experimental production rates with cortical slices. In view of these results, and since all studies were paired, absolute ammonia production rates in relation to both initial wet and final dry weight without subtraction of the substrate blank are reported.

Total tissue water assayed at the termination of the incubation was determined by the difference between wet and dry weights, and the partition into intracellular and extracellular fluid (ICF, ECF) spaces performed assuming that the inulin space represented the ECF space. Tissue electrolytes were calculated per gram dry weight or per liter intracellular water, making appropriate correction for ECF contamination.

RESULTS

Data on ammonia and glucose production were analyzed in relation to the initial wet weight and the final dry weight of the incubated tissue. Since virtually all results were comparable with either method of analysis, the data are presented only in relation to initial wet weight. These instances in which a discrepancy exists between these two methods of analysis are indicated.

During the 15-mo period of these studies, significant variability was observed in ammonia production of cortical slices from normal animals incubated under standard conditions. Analyses of the data revealed several key factors that accounted, at least in part, for this variability. One is the amount of tissue incubated. Five studies comparing ammonia production by tissue slices weighing either 50 or 100 mg with slices weighing 200 mg demonstrated higher ammonia production per tissue weight (mean ± 16%) when less tissue was incubated. Furthermore, in earlier studies 200 mg of tissue were employed whereas in later studies 150 mg were used. Ammonia production averaged significantly less with 200 than with 150 mg of tissue (15 vs. 178 μmol/g per 90 min; P < 0.01). A similar observation has been reported previously in reference to cortical slice glucose production (23). A second variable appeared to be rat size (i.e., age), which significantly correlated inversely (r = 0.55, P < 0.01) with ammonia production. When tissue was analyzed at the conclusion of the incubation intracellular fluid content correlated positively (r = 0.85, P < 0.01) whereas intracellular potassium concentration (r = -0.73, P < 0.01) and potassium/g dry wt (r = -0.48, P < 0.01) correlated negatively with rat size. Each of these parameters correlated significantly with NH₃ production in the opposite fashion. These data suggest that slices from larger animals may be less viable, resulting in lower rates of ammonia production.

It should be emphasized, however, that variables affecting base-line ammonia production should have no effect on the experimental results reported here, since a paired design was used for all studies. In each paired experiment tissue from the same rat was used for in vitro manipulations or from rats of equal size for in vivo studies, and tissue of comparable weight was always incubated.

Alteration of Potassium Homeostasis In Vivo

Cortical slices. As shown in Fig. 1, potassium depletion resulted in a significant (P < 0.01) increase in ammonia production from a mean of 133 to 174 μmol/g per 90 min. In contrast, chronic potassium loading caused a small but significant (P < 0.05) decrease in
ammonia production from 169 to 161 µmol/g per 90 min. To rule out the possibility that the small decrease noted with potassium loading was an artifact of the pair-feeding techniques, animals ingesting the same diet were studied in a paired fashion. The results of these normal but paired studies were virtually identical, ammonia production by the experimental pair averaging 192 and the control 190 µmol/g per 90 min.

At the conclusion of incubation mean tissue slice potassium content was greater by 16 µmol/g dry wt in potassium depletion and lower by 9 µmol/g dry wt in potassium loading than in similarly incubated slices from pair-fed controls, but neither of these changes was significant statistically; virtually identical values were found in the paired normal studies. The water content of the experimental and control tissue was similar in all these studies and intracellular potassium concentration paralleled changes per gram dry weight.

Glucose production increased significantly from 13.7 to 14.8 µmol/g per 90 min (n = 10, P < 0.01) with potassium deficiency. A significant increase from 15.5 to 16.8 µmol/g per 90 min (n = 19, P < 0.001) was also noted with potassium loading, and virtually identical production rates (16.3 vs. 16.2 µmol/g per 90 min) were observed in the paired normal study.

Medullary slices. As shown in Fig. 2 ammonia production by outer medullary slices responds to alterations in potassium homeostasis in a fashion significantly different from cortical tissue. Studies of medulla from potassium deficient animals showed a minimal increase in mean ammonia production (149 vs. 141 µmol/g per 90 min; n = 10) that was not significant statistically; however, in each instance cortical tissue studied simultaneously demonstrated a striking increase in ammoniagenesis (213 vs. 156 µmol/g per 90 min; P < 0.01).

Medullary tissue from potassium-loaded rats demonstrated a substantial decrease in ammonia production averaging 104 µmol/g per 90 min in comparison with 136 µmol/g per 90 min for pair-fed controls (P < 0.01). In three clearly aberrant studies, shown as open circles in Fig. 2, ammonia production by medullary tissue from potassium-loaded animals appeared to increase markedly. The animals used in these three studies were all pair fed as a group, the studies were performed on successive days during the same week, and the rates of ammonia production by the potassium-loaded animals were the highest for medulla recorded in our laboratory; however, it has not been possible to identify a specific reason for these aberrant results. If mean medullary ammonia production is calculated excluding these three studies, it is decreased 36% by potassium loading (87 vs. 136 µmol/g per 90 min).

At the conclusion of the incubation renal tissue potassium content appeared to be greater in potassium-depleted compared with control tissue (280 vs. 240 µmol/g; n = 4, P < 0.025) and of comparable water content, whereas the potassium content of potassium-loaded tissue was unchanged (207 vs. 205 µmol/g; n = 17) but the
water content was slightly lower (85.2 vs. 86.2%; n = 23, P < 0.05).

Alterations of Potassium Concentration of Incubation Medium

Manipulations of potassium homeostasis in vitro were carried out by two means. In most instances the cortical tissue slices were incubated promptly in media of varying potassium concentrations. Incubation for 90 min in the normal potassium medium resulted in a decrease in renal slice potassium content from an initial mean value of 307 to 181 μmol/g dry wt and to an intracellular concentration of 68.6 mM. Preliminary studies indicated that the decrease in tissue potassium occurred within 5 min and that stable concentrations persisted thereafter until the incubation was terminated. In some studies, the fresh tissue was preincubated for 120 min at 0°C in a potassium-free medium. This leaching procedure resulted in a decline in tissue potassium content to an average of 130 μmol/g dry wt (K_i = 32.4 mM). Subsequent incubation for 90 min in normal potassium medium resulted in a final tissue potassium of 182 μmol/g dry wt (K_i = 68.2 mM), which is virtually identical to that found with nonleached tissue. Others have demonstrated that a new steady state is achieved by 30 min when the leached tissue is incubated in potassium-containing media (39). Since no differences were apparent between experiments with leached tissue and standard incubations the data are presented together.

As shown in Fig. 3, no significant changes in ammonia production were detected despite incubation in media with potassium concentrations varying from 0 to 144 mM. The concomitant changes in tissue potassium, shown in Fig. 4, ranged from an intracellular concentration of 30 mM in the 0-mM potassium studies to 130 mM in the 144-mM potassium studies compared with 70 mM in normal media. In the experiments in which either 0, 7.6, or 11.7 mM potassium was employed the water content of the tissue was unchanged compared with control incubations in 4.8 mM potassium; therefore K_i per gram dry weight is a reasonable reflection of changes in intracellular potassium concentration. It averaged 116 μmol/g in the 0-mM potassium, 191 in the 7.6 mM potassium, and 217 in the 11.7-mM potassium studies compared with a normal value of 170 μmol/g. In the studies utilizing 73 or 144 mM potassium in the media, tissue water content increased significantly, from 81.7 to 84.9% (n = 4, P < 0.025) in the 73-mM studies and from 81.7 to 89.2% (n = 5, P < 0.01) in the 144-mM studies. Furthermore, ECF contamination significantly influences K_i per gram dry weight when media potassium concentrations of this magnitude are used. Therefore in these studies K_i per gram dry weight cannot be used to reflect changes in tissue potassium concentration. The K_i values determined for the 144-mM study, as shown in Fig. 4, were approximately twice that of slices incubated in normal potassium concentrations.

As shown in Fig. 5, glucose production was lower than control at 0 mM potassium and greater than control at high media potassium concentrations. These changes were statistically significant at the 2.5 and 0.1% level, respectively, when analyzed in relation to initial wet weight of the slice. Although the mean changes were...
DISCUSSION

Utilizing a model of pure dietary-induced potassium depletion our data confirm previous studies indicating that potassium deficiency increases renal ammoniagenesis (22, 26). Although studies of intact humans, dogs, and rats have suggested that chronic potassium loading may suppress ammoniagenesis (19, 20, 36) this question has been investigated directly in only one study reported to date (19). In this study of renal cortical slice metabolism employing rats potassium loaded for 3 days, a statistically significant decline in ammonia production was not demonstrated. In our studies cortical slices from rats ingesting a high-potassium diet for 5 days or more demonstrated a statistically significant decrement of 5% in ammoniagenesis. In view of the small changes observed the possibility of an artifact resulting from the pair-feeding technique was considered and excluded by demonstrating similar rates of ammonia production when animals ingesting diets of comparable and normal potassium content were compared. The difference in animal preparation between our study and that reported by Kamm (19) may account for the apparent discrepant results. Alternatively the enhanced ability to detect small changes in the present study may be attributable to use of the ammonia electrode, which in the presence of high glutamine concentration has less inherent errors than titrimetric or colorimetric techniques. Although this change in renal cortical slice ammonia production is quite modest, it is consistent with the magnitude of decrease in ammoniagenesis suggested by our earlier studies of urinary ammonia excretion in humans (36). Nevertheless larger decrements in ammonia production, if detectable, would provide more convincing evidence for the suppressive effect of potassium loading.

In view of the observation that during potassium loading Na-K-ATPase is stimulated to a greater degree in outer medulla than cortex (31), we undertook a study of outer medullary slices. Potassium loading resulted in an impressive decrement in ammoniagenesis (36%) when outer medullary tissue slices were utilized. By contrast an increase in ammonia production was not apparent with outer medullary tissue from potassium-depleted animals. This altered pattern of ammoniagenesis with outer medullary in contrast to cortical tissue could reflect differences in the response of juxtamedullary compared with superficial nephrons, but in view of recent observations with states of altered acid-base metabolism a more likely possibility would appear to be a unique responsiveness of different tubule segments. It has been shown that inner medulla does not increase ammoniagenesis during chronic metabolic acidosis (16). Furthermore, Curthoys and Lowry (12) recently demonstrated that only proximal convoluted tubules, which are cortical in location, increase phosphate-dependent glutaminase during metabolic acidosis. Coupled with our observation that renal cortical mitochondria, which contain only phosphate-dependent glutaminase, can quantitatively account for the increase in ammoniagenesis during potassium depletion, it is tempting to speculate that potassium depletion may exert its effect mainly on proximal convoluted tubules (34). By contrast potassium loading presumably affects predominantly either straight proximal tubules, thick ascending limbs, or collecting ducts that are found in outer medulla (38). Both ascending limbs and collecting ducts have higher base line phosphate-dependent glutaminase activity than proximal convoluted tubules and therefore may be more susceptible to a suppressive stimulus. Straight proximal tubules, on the other hand, are the sole location of phosphate-independent glutaminase, an extramitochondrial enzyme, which it has recently been suggested is actually gamma-glutamyltranspeptidase (11, 37) or transpeptidase (27). An alternative possibility is that potassium loading alters NH₃ production by affecting this enzyme system. Finally, the possibility that concomitant changes in Na-K-ATPase might specifically alter potassium entry into tubule segments located in outer medulla, thereby selectively altering ammoniagenesis at this site, is also intriguing. These issues clearly require further exploration.

Since variations in potassium homeostasis in vivo altered ammoniagenesis, we investigated whether these effects could be replicated by in vitro manipulation of potassium homeostasis. As is apparent from Fig. 4, striking changes in renal potassium content, accompanied by changes in bath potassium concentrations from 0 to 144 mM, and marked changes in the transepithelial gradient for potassium exerted no detectable influence on renal ammonia production. Furthermore, it made no difference whether the tissue potassium was altered via an increase or decrease in potassium content at the time of incubation. In contrast to the lack of change in ammonia production, glucose production was diminished with
low-potassium and increased with high-potassium incubations, confirming earlier observations with glutamate as a substrate (14), and indicating that in vitro manipulation of potassium can influence certain cellular mechanisms. On the other hand, with respect to ammoniagenesis, these results suggest that in vitro manipulations either 1) do not alter potassium at the critical sites, 2) do not activate an effector mechanism that takes place in vivo, or 3) require an adaptation over time that is not realized with the in vitro approach.

Renal tissue contains both a diffusible and nondiffusible potassium pool, and the major changes in renal potassium content in the potassium-depleted state occur in the readily diffusible component (13, 22). This suggests that in vitro manipulations that also alter the readily diffusible pool should affect potassium at similar sites. In fact, tissue potassium content was modified to a greater extent during our in vitro studies than was apparent when slices from either potassium-loaded or potassium-depleted animals were incubated. Furthermore, the potassium concentration of extramitochondrial fluid does not influence ammoniagenesis by renal cortical mitochondria, and the potassium content of mitochondria isolated from potassium-depleted and normal animals does not differ (1, 34). Thus it appears unlikely that an inability to alter potassium content of critical sites is responsible for the changes in ammoniagenesis with in vitro maneuvers. Either of the other two possibilities suggested above would seem to be a more likely explanation.

A number of investigators have suggested that the increase in ammoniagenesis that accompanies potassium depletion may result from an accompanying intracellular acidosis (2–4, 22); however, direct measurements of renal tubular cell pH with potassium depletion induced in vivo are unavailable and the results of studies with in vitro potassium depletion of renal tissue are conflicting (7, 32). Our measurements of glucose production may indirectly provide some information in this respect, since in vitro diminution of medium pH, and presumably intracellular pH, elevates glucose production (5, 18, 21, 24–26, 28–30). In our studies incubation of renal cortex from normal rats in a potassium-free medium diminished gluconeogenesis, in direct contrast to the change anticipated if in vitro potassium depletion results in an intracellular acidosis. These results must be interpreted cautiously, however, since net glucose production is not necessarily a direct reflection of the rate of metabolic flow through phosphoenolpyruvate. Data concerning potassium loading and intracellular hydrogen ion concentration are unavailable. Therefore the relationship between renal cellular metabolism, potassium homeostasis, and hydrogen ion concentration remains unresolved.

Finally, regardless of the specific tubular site and mechanism for the metabolic alterations that accompany changes in potassium homeostasis, the existence of physiologic control mechanisms linking potassium and ammoniagenesis is becoming increasingly apparent. Mild, probably physiologic, degrees of potassium depletion result in potassium conservation and an increase in renal ammonium excretion (33), whereas chronic ingestion of a diet high in potassium content, a clearly physiologic event, results in a striking increase in urinary potassium and an apparent decrease in ammonium (36). Additionally, a primary increase in renal ammoniagenesis, induced by glutamine ingestion, is accompanied by a decrease in potassium excretion (35). In view of the present direct demonstration that potassium loading, as well as depletion, can alter ammoniagenesis, a linked control mechanism seems highly likely.

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REFERENCES


