Metabolism of the renal medulla

BERNANKE, DAVID, AND FRANKLIN H. EPSTEIN. Metabolism of the renal medulla. Am. J. Physiol. 208(3): 541–545. 1965.—Slices of the inner medulla of kidneys removed from hydroperic dogs were incubated with glucose and succinate under aerobic and anaerobic conditions. When theoretical maximum ATP generation was calculated from oxygen uptake and lactate production, the calculated energy production from oxidative degradation of glucose was twice that from glycolysis. Hypertonic solutions of NaCl and urea, in concentrations comparable to those present in vivo, depressed both lactate production from glucose and oxygen uptake in succinate to the same extent. Studies of CO2 generation by medullary slices from labeled glucose in 100% O2 showed a Cr/C& ratio of 3.7, compared to 1.7 for cortex. It is suggested that oxidative metabolism may play a critical role in the renal medulla of intact animals in providing energy for active transport of sodium and thereby facilitating the process by which urine is concentrated.

The unusual structural and environmental characteristics of the renal medulla have stimulated studies to determine whether the medulla has distinctive metabolic features. Its low rate of blood flow in vivo (10), the histological deficiency of mitochondria (15), the comparatively low rate of oxygen utilization by slices of renal medulla, and the high rate of glycolysis in vitro (4, 8, 11, 20) have all been interpreted to indicate that the medulla depends largely on anaerobic metabolism to furnish energy for its activities.

The present experiments in vitro emphasize, by contrast, the critical role that oxidative metabolism may play in providing energy for the renal medulla.

METHODS

Mongrel dogs kept on a high-protein diet were killed by an intravenous injection of pentobarbital. Except where indicated in the ensuing text, food and water were removed from the cage at least 12 hr previously. The kidneys were removed immediately and placed in chilled physiological saline and the white inner medulla was rapidly dissected out in toto. Longitudinal slices of medulla 0.3–0.4 mm thick were then prepared with a Stadie-Riggs microtome, blotted, weighed on a torsion balance, and placed in Warburg vessels for study by standard manometric techniques at 37°C in a Braun-Warburg apparatus. A shaking rate of 160/min was used and incubation was carried out for 1 hr. Krebs-Ringer phosphate buffer (2.8 ml) was used in all experiments except where stated below. The concentration of substrate was 10 mM/liter. Lactic acid was determined chemically by the Barker-Summerson method (1). The dry weight of representative slices before incubation was determined by weighing before and after desiccation at 95°C for 48 hr.

Experiments utilizing C14-labeled glucose were performed in the same general manner except that incubations were conducted in rubber-capped 25-ml Erlenmeyer flasks with suspended glass wells. A Dubnoff shaker at 37°C and a rate of 110 oscillations/min was used in these experiments. Seventy-two thousand counts/min of radioactive glucose (New England Nuclear Corp.) were added to each flask so that the specific activity of glucose in the incubation medium was 2.5 X 103 count/min per µM. After incubation, 0.5 ml Hyamine was placed in the suspended well to trap CO2; 1 ml 3 N H2SO4 was added to the flask and shaking was continued for 30 min. The contents of the center well were transferred to 12 ml of fluor solution (4 g PPO and 50 mg POPOP/1,000 ml toluene) and counted in a Packard liquid-scintillation counter.

RESULTS

O2 consumption, lactate production, and theoretical ATP yield by slices of medulla in varying concentrations of oxygen (Table 1, Fig. 1 and 2). Slices of medulla showed a significant uptake of oxygen in both glucose (avg. 0.07 µM O2/mg

Received for publication 29 July 1964.
1 This study was aided by Public Health Service Grants H-834 and AM-6397, and by grants from the Connecticut and New Haven Heart Associations.
3 Recipient of a Public Health Service Career Research Award.
lactate production increases substantially when oxygen is removed. Even a low-oxygen tension in the medulla is not sufficient to stimulate lactate production, but it increased by approximately 50% in pure nitrogen. The addition of vasopressin (Pitressin, Parke, Davis) in concentrations of 1–7 pressor U/liter medium to medullary slices from dogs undergoing water diuresis did not alter oxygen uptake with succinate or lactate production from glucose in nitrogen.

**Effect of feeding, hydration, and vasopressin (Table 3).** Four dogs were fasted overnight, as in the preceding experiments, but permitted to drink water. One or two hours before sacrifice, they were given an amount of water by stomach tube equal to 5% of their body weight. The urine of most animals was dilute with an osmolality of less than 300 milliosmoles/kg at the time the kidneys were removed. Hydration and water diuresis did not alter O₂ uptake in succinate, anaerobic glycolysis, or the production of C₄O₂ from glucose labeled in the C₄ or C₅ positions.

On the other hand, when nine animals were permitted to eat and drink up to the time of the experiment, and were also hydrated by stomach tube as above, QO₂ in succinate increased significantly, as did anaerobic glycolysis.

The theoretical estimate of high-energy phosphate bond production by medullary slices is presented in Fig. 2. Oxygen utilization in succinate is assumed to yield four high-energy phosphate bonds per molecule of oxygen. In glucose, six such bonds are theoretically produced per molecule of oxygen used. Each mole of lactate produced accounts for one molecule of oxygen used. Each mole of lactate produced is theoretically as effective in 5% O₂ as succinate. Glucose as substrate in 5% O₂ clearly supports a higher calculated level of production of high-energy phosphate bonds than does glucose in nitrogen, though lactate production increases substantially when oxygen is removed. Even a low-oxygen tension in the medulla is therefore likely to contribute significantly to the energy available from glucose for the work of the medulla.

**Effect of hypertonicity (Table 2, Fig. 3).** Increasing the tonicity of the medium with either NaCl or urea reduced oxygen consumption when either succinate or glucose was the substrate. Both aerobic and anaerobic production of lactate from glucose were depressed by hypertonicity. The proportion of energy theoretically derived from oxygen utilization on the one hand and lactate production on the other was not appreciably altered. In these hypertonic media glucose metabolism in an atmosphere of 100% or 5% O₂ was again theoretically more efficient than glucose metabolism in nitrogen. Extrapolating to the situation in vivo, where high concentrations of sodium and urea bathe the cells of the renal medulla, these data further suggest the importance of oxidative metabolism to medullary tissue.
TABLE 2. Effect of hypertonicity on metabolism of slices of renal medulla

<table>
<thead>
<tr>
<th>Solute Added to Isotonic KRP Medium per Liter</th>
<th>Atmospheric O₂ %</th>
<th>O₂ Uptake in Succinate, µM/mg DW per hr</th>
<th>O₂ Uptake in Glucose, µM/mg DW per hr</th>
<th>Lactate Formed, µM/mg DW per hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl, 150 mM</td>
<td>100</td>
<td>.217±.01 (33)</td>
<td>.069±.007 (36)</td>
<td>.143±.01 (36)</td>
</tr>
<tr>
<td>NaCl, 300 mM</td>
<td>100</td>
<td>.164±.008 (9)*</td>
<td>.040±.015 (3)</td>
<td>.130±.02 (3)</td>
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<tr>
<td>NaCl, 450 mM</td>
<td>100</td>
<td>.194±.02 (5)†</td>
<td>.036±.004 (12)*</td>
<td>.089±.01 (12)*</td>
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<tr>
<td>Urea, 600 mM</td>
<td>100</td>
<td>.180±.01 (8)*</td>
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<tr>
<td>NaCl, 450 mM</td>
<td>5</td>
<td>.048±.009 (11)</td>
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<tr>
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<td>.030±.005 (5)</td>
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<tr>
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<tr>
<td>Urea, 600 mM</td>
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<td>.175±.008 (12)*</td>
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<tr>
<td>Urea, 900 mM</td>
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<td>.137±.019 (15)*</td>
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<tr>
<td>Mannitol, 900 mM</td>
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<td>.139±.016 (14)*</td>
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<td></td>
<td></td>
<td>.107±.009 (15)*</td>
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<tr>
<td></td>
<td></td>
<td>.145±.01 (14)*</td>
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<td></td>
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</tbody>
</table>

Values are means ± SE. Number of observations are in parentheses. KRP = Krebs-Ringer phosphate. DW = dry weight. * P < 0.01 compared to isotonic medium. † P < 0.05 compared to isotonic medium.

TABLE 3. Effect of fasting and hydration on the metabolism of succinate and glucose by slices of renal medulla

<table>
<thead>
<tr>
<th>Condition</th>
<th>O₂ Uptake in Succinate, µM/mg DW per hr</th>
<th>Aerobic Lactate Formation, µM/mg DW per hr</th>
<th>Anaerobic Lactate Formation, µM/mg DW per hr</th>
<th>C14O₂ count/min per mg DW per hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted and dehydrated</td>
<td>.217±.01 (23)</td>
<td>.145±.05 (36)</td>
<td>.219±.05 (33)</td>
<td>15.2±2.1 (12)</td>
</tr>
<tr>
<td>Fasted and hydrated</td>
<td>.233±.038 (12)</td>
<td>.183±.037 (10)*</td>
<td>.215±.044 (8)</td>
<td>13.7±3.8 (10)</td>
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<tr>
<td>Fed and hydrated</td>
<td>.238±.021 (49)*</td>
<td>.171±.038 (14)*</td>
<td>.333±.034 (13)*</td>
<td>3.5±0.7 (10)</td>
</tr>
</tbody>
</table>

Values are means ± SD. DW = dry weight. Number of observations are in parentheses. * P < 0.01, compared with fasted and dehydrated.

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**DISCUSSION**

It has been suggested many times that the unique anatomical and physiological characteristics of the renal medulla have distinctive metabolic correlates. The inner medulla of the dog, making up about 10% of the weight of the kidney, receives about 1% of the renal blood flow, or 20–30 ml/100 g tissue per min (19). This is only 1/50–1/30 of the blood flow to renal cortex. Oxygen utilization by the intact medulla has been estimated as 0.3–0.4 ml/100 g per min, or 3–5% of the oxygen uptake of cortex (10). The oxygen tension of the urine, presumably reflecting that of the papilla, is considerably lower than that of renal venous blood (14). Cells lining medullary tubules contain fewer mitochondria than do cortex.
tical cells (15) and the yield of mitochondrial material obtained by disruption of cells and centrifugation is approximately 15 times greater in cortex than in medulla (7). Respiratory enzymes necessary for oxidative metabolism are less plentiful in medulla than in cortex; for example, no succinic dehydrogenase can be demonstrated at all in the inner medulla by histological staining techniques (18). The unusually large capacity of medullary slices for anaerobic glycolysis and the persistent formation of large amounts of lactate from glucose in the presence of oxygen (4, 8, 11, 20) have suggested to many that the renal medulla relies largely on anaerobic rather than aerobic pathways for its metabolic needs (5, 8, 11).

Kean et al. (8) proposed that the dependence of the medulla on glycolysis was compounded by its normally hypertonic environment, since, when they progressively increased the tonicity of the medium with NaCl, the oxidation of succinate by slices of dog medulla was decreased before anaerobic glycolysis diminished. Lee, Vance, and Cahill (11), on the other hand, reported that the addition of sodium and sucrose, but not urea, decreased the oxidation of glucose and decreased lactate production in slices of rabbit medulla incubated aerobically.

The present studies suggest that, because of the greater efficiency of oxidative metabolism, oxidative pathways might furnish approximately twice as much energy as glycolysis to the medulla of the dog. Similar conclusions have suggested (15) that the renal medulla relies largely on anaerobic rather than aerobic pathways for its metabolic needs (5, 8, 11).

The improvement in both oxygen uptake and lactate production seen in the medulla of fed, hydrated dogs is interesting and deserves further study. It could not be ascribed to water diuresis alone, since it was not apparent in starved dogs given water. Feeding and starvation certainly influence the metabolism of other tissues, and changes in the protein, fat, and carbohydrate composition of the diet may alter the pattern of metabolism of succinate and glucose in the renal medulla (unpublished observations). Another explanation may lie with the increased solute output in the urine of fed dogs, since osmotic diuresis has been shown to increase the oxygen to the medulla were reduced, this might then be expected to interfere with active reabsorption of sodium and the production of a concentrated urine.

Certain experiments in intact animals support this hypothesis. While the blood flow per gram of renal medulla is small compared to cortex, it is 30% of that to the heart and 50% of the blood flow to brain (19). Measurements of glucose and lactate in blood from the vasa recta of hamsters indicate that only 25% of the glucose which disappears from blood as it traverses the medulla is accounted for by the formation of lactate (16). The intraluminal electrical potential of the ascending thin limb of Henle’s loop, normally negative, disappears when the tubule is perfused with cyanide, which blocks oxidative metabolism, as well as with iodoacetate, which interferes with glycolysis (21). Occlusion of the ureter in dogs undergoing mannitol diuresis greatly decreases medullary blood flow and oxygen tension (9), reduces the concentration of sodium in the renal papilla (6), and results in an impairment of concentrating ability which persists for a variable time after the obstruction is relieved (6). The defect in urinary concentrating ability that characterizes patients with sickle-cell anemia may be associated with a diminution in medullary blood flow; it is greatly ameliorated during osmotic diuresis, which increases the delivery of oxygen to the medulla (15).

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content of lactate of the papilla of hamsters (2) and rats (17), presumably by increasing glycolysis.

The much higher C\textsubscript{3}/C\textsubscript{6} ratio of CO\textsubscript{2} generated from glucose by medulla as compared with cortex might be accounted for by the different rates of glycolysis in these tissues, since the medulla metabolizes much glucose to lactate rather than to carbon dioxide. The relatively high rate of CO\textsubscript{2} production from Cl-labeled glucose in medulla also suggests a significant role in this tissue for the phosphogluconate shunt pathway. The Cl/C\textsubscript{6} ratio of 3.7 in these experiments was much higher than the value of 1.3 found by Lee, Vance, and Cahill in the inner medulla of rabbits (11). Persistence of C\textsubscript{14}O\textsubscript{2} generation from C\textsubscript{14} glucose in poorly oxygenated slices of medulla further suggests that medullary slices have the ability to utilize the shunt under circumstances of oxygen deprivation, perhaps in the synthesis of lipids or other constituents of cell membranes. The relatively high content of TPN diaphorase found in the thin limbs and collecting ducts of the inner medulla (18) may be related to the activity of the shunt pathway in these structures.

We are grateful to Lorraine Pozika, Corinne Wilson, and Margaret Davis for technical assistance.

REFERENCES