Oxygen consumption, glycolysis, and sodium reabsorption in the hypothyroid rat kidney

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Hypothyroid rats. Hypothyroidism was produced by the intraperitoneal injection of 1 mc of sodium radio iodide (131I) (E. R. Squibb & Sons, New York City), as previously described (13). These animals were studied either 10 weeks or 8 months after the administration of 131I.

Respirometry studies. Metabolic studies were performed on one group of control (n = 13) and hypothyroid (n = 15) rats allowed food and water ad libitum until the time of study (nondiuretic rats). Studies were also performed on a second group of rats after the administration of 2,500 ϋEq of sodium per 100 g body wt. Local anesthesia with 1% xylazine, a catheter was placed in the femoral vein of awake restrained rats and 5% saline infused at a rate of 150 μL/min in three control and two hypothyroid rats. Because of the technical difficulties with intravenous infusions in awake rats, two additional control and three hypothyroid rats (all unanesthetized) were studied after the intraperitoneal injection of 2,500 ϋEq of sodium (5% NaCl) per 100 g. The route of saline administration did not alter the results and, therefore, these saline-loaded animals are reported together.

Animals were sacrificed by decapitation and both kidneys removed in less than 1 min and placed in iced buffer (0-4 C), without substrate. After the capsule was stripped, cortical slices were made with a Stadie-Riggs microtome (Arthur H. Thomas Co., Philadelphia, Pa.). The outside slice was always discarded, as well as any slice greater than 0.3 mm in thickness. A sufficient number of cortical slices was usually obtained from one animal for two independent determinations. Coronal sections were then made on the remaining kidney until a clear demarcation appeared between the white medulla, red medulla, and cortex. The cortical tissue was dissected away from the red medulla and slices were then made of the red and white medulla. Later the red and white medulla were separated. In most cases it was necessary to pool tissue from both kidneys in any one animal to obtain an adequate amount of tissue for one determination each of red and white medulla. At no time was the kidney tissue out of the iced buffer for more than 2 min, and the entire procedure was completed in less than 25 min from the time the animal was sacrificed.

The tissue was immediately placed in a conventional Warburg vessel—cortex and red medulla in 15-ml flasks with 1.5 ml of buffer and inner medulla in 7-ml flasks with 0.75 ml of buffer. The flasks were attached to an all glass Gilson differential respirometer-model G-14 (Gilson Medical Electronics, Inc., Middleton, Wis.) and the system...
FIG. 1. Oxygen consumption ($Q_{O_2}$) and anaerobic glycolysis ($Q_{CO_2}$) in renal tissue of nondiuretic control rats. $Q_{O_2}$ and $Q_{CO_2}$ were determined in cortical slices, red medullary slices (outer medulla), and white medullary slices (inner medulla). $CO_2$ liberation is used as a relative, but not absolute, measure of anaerobic lactic acid production.

was evacuated and filled with the appropriate gas. Following a 15-min equilibration period at 30°C with a shaking rate of 125/min, readings were obtained every 10 min for the next hour. At the conclusion of the experiment the tissue was removed and dried at 100°C until a constant weight was obtained. All results are expressed in microliters of oxygen consumed per milligram of dry tissue weight per hour (aerobic studies) or microliters of CO$_2$ evolved$^1$ per milligram of dry tissue weight per hour (anaerobic studies) ($WV$).

Krebs-Ringer phosphate buffer, pH 7.4, with 10 mM sodium succinate was used for oxygen consumption studies. The center well of the flask contained 20% KOH, and the system was gassed with 100% oxygen. Krebs-Ringer bicarbonate buffer, pH 7.4, with 10 mM glucose was used for anaerobic studies. A stick of yellow phosphorus was placed in the center well and the system was gassed with CO$_2$-N$_2$ (5%–95%).

Clearance studies. After 24-hr dehydration, control (n = 5) and hypothyroid (n = 5) rats were anesthetized with sodium pentobarbitol (5 mg and 3.5 mg/100 g body wt, respectively). Animals were prepared as previously described (13). A prime and sustaining infusion of inulin was begun, and following a 1-hr equilibration 5% saline was infused at a rate of 110–230 μl/min. One clearance period of 5–8 min duration was performed in each animal. The clearance of inulin, plasma sodium, and sodium excretion were determined after 2,500 μEq of sodium per 100 g body wt had been infused.

In a second group of control (n = 4) and hypothyroid (n = 4) rats anesthetized as above, each animal was weighed to the nearest gram, and both kidneys were removed, stripped of all fat, blotted on filter paper, and weighed to the nearest milligram. The ratio of kidney weight to body weight was determined in each animal.

The clearance of inulin and tubular sodium reabsorption was calculated in the standard manner.

FIG. 2. Anaerobic glycolysis in nondiuretic control and hypothyroid rats.

Aerobic and anaerobic metabolism in nondiuretic control rats. In Fig. 1 the rates of oxygen consumption ($Q_{O_2}$) and anaerobic glycolysis ($Q_{CO_2}$) are compared in three different sections of the kidney. These three zones were chosen because each is clearly demarcated in the fresh kidney slice and each provides a sample of different nephron segments. The cortical slices contain primarily proximal tubules, distal tubules, and glomeruli; the red medulla (outer medulla) contains mostly thick ascending and descending limbs of Henle’s loop and collecting ducts, and the white medulla (inner medulla) is composed of thin ascending and descending limbs of Henle’s loop with collecting ducts (33).

In the nondiuretic control rat $Q_{O_2}$ (μl/mg dry wt per hr) is greater in the cortex than in the outer medulla ($P < 0.001$) and greater in the outer medulla than in the inner medulla ($P < 0.001$). $Q_{CO_2}$ (μl/mg dry wt per hr) is greater in both the inner and outer medulla than the cortex ($P < 0.001$). However, the difference in $Q_{CO_2}$ between the inner and outer medulla was not statistically significant ($P > 0.40$).

Anaerobic glycolysis in nondiuretic control and hypothyroid rats. Anaerobic studies were performed in hypothyroid rats 8 months after $^{131}$I administration and the results are presented in Fig. 2. Control data are also presented for comparison. $Q_{CO_2}$ of cortical slices was not significantly altered in the hypothyroid group of rats ($P > 0.80$). Although the mean $Q_{CO_2}$ of tissue from the outer medulla was 14% lower in the hypothyroid group of animals, this difference was not statistically significant ($P > 0.50$). $Q_{CO_2}$ of tissue from the inner medulla of the hypothyroid rat was reduced by 19% and this difference was statistically significant ($P < 0.05$).

Oxygen consumption in nondiuretic control and hyperthyroid rats. Results of studies in two different groups of hyperthyroid rats are presented in Fig. 3. One group was studied 10 weeks after $^{131}$I administration and another group 8 months after $^{131}$I administration. Control data are included for com-

$^1$ Carbon dioxide evolved is an indirect measurement of anaerobic glycolysis and depends on lactic acid production by the kidney with subsequent acidification of the bicarbonate buffer and CO$_2$ evolution. It assumes that significant amounts of other acids are not produced.
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FIG. 3. Oxygen consumption in nondiuretic control and hypothyroid rats. Hypothyroid animals were studied 10 weeks and 8 months after $^{131}$I administration.

Comparison. $Q_{O_2}$ of cortical slices was decreased by 24% in both groups of hypothyroid animals when each one was compared to the control group ($P < 0.001$). There was no statistical difference in $Q_{O_2}$ of cortical slices when the 10-week and 8-month hypothyroid animals were compared to each other ($P > 0.70$). $Q_{O_2}$ of tissue from the outer medulla was not significantly different when 10-week or 8-month hypothyroid animals were compared to controls ($P > 0.40$ and $P > 0.05$, respectively). Likewise, $Q_{O_2}$ of tissue from the inner medulla of 10-week or 8-month hypothyroid animals was not significantly different from that of the control group ($P > 0.20$ and $P > 0.50$, respectively). When the $Q_{O_2}$ was compared between the two groups of hypothyroid animals in either the outer or inner medullary tissue, the differences were not statistically significant ($P > 0.20$ and $P > 0.05$, respectively).

Oxygen consumption and sodium reabsorption following saline expansion. Tubular sodium reabsorption ($T_{Na}$) and $Q_{O_2}$ were compared in control and hypothyroid rats after the animals were loaded with 5% saline. At that point in the diuresis when 2,500 $µ$Eq of sodium per 100 g body wt had been infused, $T_{Na}$ was calculated in each animal. Since the hypothyroid rat has a smaller kidney mass per unit of body weight (26), it is not valid to relate $T_{Na}$ to body weight. Therefore, it seemed more appropriate to express $T_{Na}$ as a function of kidney mass. At the conclusion of a hypertonic saline diuresis, however, the kidney is swollen and its weight is not an accurate reflection of kidney mass. In an attempt to overcome this problem, the ratio of kidney weight to body weight (%), it is not valid to relate $T_{Na}$ to body weight. Therefore, it seemed more appropriate to express $T_{Na}$ as a function of kidney mass. At the conclusion of a hypertonic saline diuresis, however, the kidney is swollen and its weight is not an accurate reflection of kidney mass. In an attempt to overcome this problem, the ratio of kidney weight to body weight was determined in a group of nondiuretic control and hypothyroid rats. The kidney weight-to-body weight ratio expressed in milligrams of kidney per 100 g body wt was 787 ± 19 in eight control rats and 542 ± 7 in four hypothyroid rats ($P < 0.001$). These ratios were then used to convert $T_{Na}$ per 100 g body wt (as determined in the intact animal) to $T_{Na}$ per gram of kidney weight. This procedure seems justified for two reasons. First, the ratio of kidney weight to body weight was very constant in animals from each group as evidenced by the small amount of variation from the mean. Second, the two control groups are very similar in body size, and there was no statistical difference in mean body weights of the two hypothyroid groups. Also the two hypothyroid groups were both studied at the same time interval after $^{131}$I administration.

In Fig. 4 the results of $T_{Na}$ (µEq/min per g of kidney) and $Q_{O_2}$ are compared in control and hypothyroid animals after 2,500 $µ$Eq of sodium per 100 g had been administered to each animal. $T_{Na}$ per gram of kidney tissue was 27% lower in the hypothyroid than control rats at the peak of the saline diuresis ($P < 0.01$). $Q_{O_2}$ of cortical slices prepared from animals at the same point in the saline diuresis was 30% lower in the hypothyroid than control rats ($P > 0.001$).

In control animals saline expansion tended to increase $Q_{O_2}$ of cortical tissue (29.3 ± 9.97 vs. 31.5 ± 7.77), but the differences were not statistically significant ($P > 0.20$). Saline loading had no effect on $Q_{O_2}$ of cortical tissue from the hypothyroid rat (22.4 ± 0.49 vs. 22.0 ± 1.11, $P > 0.80$). $Q_{O_2}$ of tissue from the red and white medulla was not significantly altered by saline expansion in animals from either the control or hypothyroid groups.

DISCUSSION

The observations reported here on aerobic and anaerobic metabolism in the control rat demonstrate that the cortex has a greater rate of aerobic metabolism than the inner medulla has. In contrast, the inner medulla of the rat kidney has a greater rate of glycolysis than the cortex. The outer medulla seems to be a metabolic transition zone between the cortex and inner medulla, with the capacity for appreciable rates of aerobic metabolism and anaerobic glycolysis. The present findings in control rats are basically similar to those previously reported in other species (14, 16, 17, 32), with the exception of the glycolytic capacity observed in the outer medulla. In the present study, performed on rats, the capacity for anaerobic glycolysis was as great in the outer medulla as in the inner medulla. In contrast, Winters (32) has reported that the rate of anaerobic
glycolysis in the outer medulla of the rabbit kidney is much lower than in the inner medulla. Lee and Peter (17), however, have found that the outer medulla of the rabbit kidney has a significant amount of anaerobic glycolysis as well as aerobic metabolism. The present group of experiments in rats cannot resolve this discrepancy in the rabbit data, but the results of this study are more in accord with those of Lee and Peter (17) in suggesting that the outer medulla of the rat kidney is a metabolic transition zone with appreciable rates of aerobic and anaerobic metabolism.

Anaerobic metabolism is thought to be important for the energy supply of the inner medulla because of the small numbers and small size of the mitochondria (31), the countercurrent medullary blood flow (27), and low-oxygen tension (2, 18) found in this region of the kidney. When the capacity for anaerobic glycolysis was evaluated in the hypothyroid rat, a 19% reduction in $Q_{\text{o2}}$ was found in tissue from the inner medulla. The technique used in the present study did not detect any reduction in the capacity for oxygen consumption of medullary tissue from the hypothyroid animal. Clearance and micropuncture studies with metabolic inhibitors have suggested that inner medullary sodium transport and solute-free water reabsorption are dependent, at least in part, on glycolytic metabolism (12, 19, 28, 30). The previous findings of a limit in solute-free water reabsorption and an associated increase in sodium excretion in a group of hypothyroid rats similar to the above animals (13) suggest that the reduction in the capacity for anaerobic glycolysis of the inner medulla of the hypothyroid rat may be of physiological significance.

The capacity for oxygen consumption in cortical tissue prepared from the hypothyroid rat was reduced by 24%. This magnitude of reduction in $Q_{\text{o2}}$ occurred as early as 10 weeks after $^{131}$I administration and persisted without change for at least 8 months. These results are in agreement with previous reports of $Q_{\text{o2}}$ in renal cortex from surgically thyroidectomized rats (3, 22). It is of interest to note, however, that one previous study on rats injected 2-8 months before study with $^{131}$I failed to demonstrate any changes in the number, ultrastructure, or histochemistry of mitochondria from the renal cortex (6).

Since active sodium transport in the proximal tubule is largely dependent on aerobic metabolism (5, 28, 31) and because proximal tubules constitute the bulk of cortical tissue (1), any alteration in aerobic metabolism of cortical tissue is potentially significant to active sodium transport in the proximal tubule. In the hypothyroid rat, the $Q_{\text{o2}}$ of tissue slices prepared from the renal cortex was decreased by 24%. The previous report on a similar group of hypothyroid rats concluded that fractional sodium reabsorption may have decreased more rapidly in the proximal tubule of the hypothyroid than control rats in response to a saline infusion (13). A recent micropuncture study has confirmed that fractional and absolute water reabsorption are decreased in the proximal tubule of the hypothyroid rat (21). The earlier demonstration of a reduction in glomerular filtration rate and contraction of the extracellular fluid volume in the hypothyroid rat cannot explain the observed decrease in proximal sodium reabsorption and further suggests that some factor is operating in the hypothyroid rat to oppose these stimuli which commonly increase proximal sodium reabsorption (13). The observed decrease in $Q_{\text{o2}}$ of cortical tissue from the hypothyroid rat could be such a factor since it would provide an explanation for the decrease in sodium reabsorption within the proximal tubule of the hypothyroid rat.

If the decrement in $Q_{\text{o2}}$ of cortical tissue from the hypothyroid rat is related to the exaggerated natriuresis seen in these animals, then a positive correlation should be demonstrable between the reduction in oxygen consumption and sodium reabsorption (7, 15, 29). When these parameters were measured in control and hypothyroid rats at the peak of a saline diuresis, $T_{\text{Na}}$ was 27% lower and $Q_{\text{o2}}$ 30% lower in the hypothyroid than control rat. Although the relative reductions in $T_{\text{Na}}$ and $Q_{\text{o2}}$ were quite similar with the present methodology, a quantitative comparison between these two is hazardous since $T_{\text{Na}}$ was measured in vivo and $Q_{\text{o2}}$ in vitro. A qualitative comparison does seem valid, however, since in vivo studies performed on animals during a hyperosmolar saline diuresis have demonstrated a tight relationship between $T_{\text{Na}}$ and oxygen consumption (7) and in vitro studies with renal cortical tissue slices have also demonstrated a close relationship between $Q_{\text{o2}}$ and sodium transport (15, 29). Therefore, the present group of experiments demonstrates a positive correlation between the reduction in oxygen consumption and the reduction in tubular sodium reabsorption in the hypothyroid rat kidney. Because thyroid hormones are felt to have a direct effect on oxidative metabolism at the cellular level (25) and because the hypothyroid state results in a reduction in oxygen consumption of a number of tissues not primarily concerned with sodium transport (3, 22), it is postulated that the initial change in the hypothyroid kidney is a reduction in energy supply and oxygen consumption and that the decrease in sodium reabsorption is secondary to the reduction in energy supply.

The present studies have demonstrated that an insufficiency of thyroid hormone in the rat results in a reduction in the primary source of energy supply for the renal cortex, aerobic metabolism, and a decrease in one of the major sources of energy supply for the inner medulla, anaerobic glycolysis. The reduction in oxygen consumption was correlated with a reduction in tubular sodium reabsorption. It was postulated that the exaggerated natriuretic response of the hypothyroid rat is a consequence of an insufficient supply of energy for active sodium transport, either through a reduction in aerobic metabolism and/or glycolytic metabolism.

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REFERENCES