Increased mitochondrial activity in renal proximal tubule cells from young spontaneously hypertensive rats

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Renal proximal tubule cells from spontaneously hypertensive rats (SHR), compared with normotensive Wistar–Kyoto rats (WKY), have increased oxidative stress. The contribution of mitochondrial oxidative phosphorylation to the subsequent hypertensive phenotype remains unclear. We found that renal proximal tubule cells from SHR, relative to WKY, had significantly higher basal oxygen consumption rates, adenosine triphosphate synthesis-linked oxygen consumption rates, and maximum and reserve respiration. These bioenergetic parameters indicated increased mitochondrial function in renal proximal tubule cells from SHR compared with WKY. Pyruvate dehydrogenase complex activity was consistently higher in both renal proximal tubule cells and cortical homogenates from SHR than those from WKY. Treatment for 6 days with dichloroacetate, an inhibitor of pyruvate dehydrogenase kinase, significantly increased renal pyruvate dehydrogenase complex activity and systolic blood pressure in 3-week-old WKY and SHR. Therefore, mitochondrial oxidative phosphorylation is higher in renal proximal tubule cells from SHR compared with WKY. Thus, the pyruvate dehydrogenase complex is a determinant of increased mitochondrial metabolism that could be a causal contributor to the hypertension in SHR.

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Essential hypertension is a heterogeneous disorder in which both genetics and environmental factors contribute to increased cardiovascular and renal morbidity and mortality.[1,2] The importance of the renal proximal tubule in the pathogenesis of hypertension, especially polygenic or essential, has recently been appreciated.[1,3] Selective deletion in the renal proximal tubule of aromatic amino-acid decarboxylase, the enzyme that converts L-3,4-dihydroxyphenylalanine to dopamine, causes salt-sensitive hypertension in mice.[4] Conversely, selective deletion of angiotensin type I_A receptor in the renal proximal tubule reduces sodium transport in this nephron segment, decreases sodium balance, and protects against angiotensin II- and salt-induced hypertension in mice.[5]

Renal proximal tubule cells and cortical homogenates from spontaneously hypertensive rats (SHR), compared with normotensive Wistar–Kyoto rats (WKY), have increased oxidative stress,[6,7] which may contribute to the increased renal tubular sodium reabsorption.[1,3,6] The association of the increased activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase with hypertension is well established,[6,8] but the importance of renal mitochondria-derived reactive oxygen species in hypertension has only recently been recognized.[9–11] Zhang *et al.*[9] have reported that the mitochondrial respiratory chain is a major source of oxidative stress in mice with deoxycorticosterone acetate salt-induced hypertension. In stroke-prone SHR, the mitochondria-targeted antioxidant MitoQ10 protects against the development of hypertension.[10] The above reports suggest that mitochondrial reactive oxygen species are important in the development of hypertension.

The major function of mitochondria is to produce adenosine triphosphate (ATP) to supply energy for various cellular functions, with reactive oxygen species as by-products. The level of mitochondrial ATP production is often reflected by the mitochondrial oxygen consumption

rate (OCR).[12] Previous studies have shown that renal proximal tubules from SHR have increased OCR compared with normotensive WKY;[13] however, the molecular mechanisms underlying this difference between WKY and SHR are not well understood.

The mitochondrial pyruvate dehydrogenase complex catalyzes the decarboxylation of pyruvate to acetyl-coenzyme A.[14] The reaction is regulated by reversible phosphorylation of the E1 subunit via pyruvate dehydrogenase kinase and dephosphorylation via pyruvate dehydrogenase phosphatase.[15] The activity of pyruvate dehydrogenase kinase is inhibited by pyruvate and its analogs. Dichloroacetate (DCA), a pyruvate analog and competitive inhibitor of pyruvate dehydrogenase kinase, is used widely to increase pyruvate dehydrogenase complex activity.[14]

To explore the potential molecular mechanisms involved in the increased OCR and mitochondrial function in the SHR, we identified that increased activity of the pyruvate dehydrogenase complex contributes to the pathogenesis of hypertension in this animal model.

RESULTS

Characterization of renal proximal tubule cells in primary culture

We isolated proximal tubules from rat kidneys. The purity of renal proximal tubule cells in primary culture was determined by western blot and immunofluorescence. Contaminating renal cells derived from nephron segments distal to the proximal tubule were detected by antibodies against Na^+–K^+–$2Cl^-$ cotransporter 2 (slc12a1), Na^+–Cl^- cotransporter (slc12a3), and epithelial sodium channel (Scnn1) (Supplementary Figure S1a online). Immunofluorescence staining with sodium glucose-linked transporter 2 (slc5a2) (Supplementary Figure S1b online) showed that 92.3–96.0% of cells were positive for this renal proximal tubule cell marker. Although these cells were not 100% renal proximal tubule in origin, we refer to them as renal proximal tubule cells in primary culture for the purpose of this study.

Bioenergetic profiles of renal proximal tubule cells from WKY and SHR

Using the Seahorse Bioscience XF24 Extracellular Flux Analyzer, we studied the OCR response to several mitochondrial inhibitors,[16,17] including oligomycin (ATP synthase inhibitor), carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP, uncoupler), rotenone (Complex I inhibitor), and antimycin A (Complex III inhibitor) in renal proximal tubule cells isolated from WKY and SHR (Supplementary Figure S2 online).

Renal proximal tubule cells in primary culture from SHR, compared with normotensive WKY, displayed a distinct bioenergetic profile (Figure 1a). Basal OCR was significantly higher in SHR than in WKY (223.7 ± 20.9 vs. 151.0 ± 6.6 pmol/min per 20,000 cells, $n = 8$–9, $P < 0.001$) (Figure 1b). ATP synthesis–linked OCR was also greater in SHR than in WKY ($75.6 \pm 7.5\%$ vs. $60.1 \pm 4.4\%$ of basal OCR, $n = 9$, $P < 0.01$) (Figure 1c). FCCP increased OCR in cells from both rat strains, but the increase was greater in SHR than in WKY (418.9 ± 21.4 vs. 252.6 ± 18.1 pmol/min per 20,000 cells, $n = 8$–9, $P < 0.001$) (Figure 1d). Similar to the maximum respiration, the reserve respiration was greater in SHR than in WKY ($88.2 \pm 13.6\%$ vs. $67.5 \pm 14.2\%$ of basal OCR, $n = 8$–9, $P < 0.05$) (Figure 1e). Proton leak-linked OCR tended to be lower in SHR than in WKY ($10.3 \pm 7.9\%$ vs. $19.1 \pm 11.4\%$ of basal OCR, $n = 8$–9, $P = 0.08$) (Figure 1f) but non-mitochondrial respiration was similar (37.4 ± 20.6 vs. 31.5 ± 13.1 pmol/min per 20,000 cells, $n = 8$–9, $P > 0.05$) in the two rat strains (Figure 1g). These results suggest that renal proximal tubule cells in primary culture from SHR have higher mitochondrial function than those from WKY. Similar distinct bioenergetics profile also exists in immortalized cells[18] from SHR, compared with WKY, with higher basal OCR, ATP synthesis-linked OCR, maximum respiration, and reserve respiration (data not shown). Of note, the mitochondrial inhibitors, in the concentrations used, had no effect on cell viability (Supplementary Figure S3 online).

Glycolysis in renal proximal tubule cells from WKY and SHR

Both primary (Figure 2a) and immortalized (data now shown) renal proximal tubule cells from SHR, relative to WKY, had higher basal extracellular acidification rate (ECAR) (9.29 ± 1.5 vs. 2.94 ± 0.7 mpH/min per 20,000 primary cells, $n = 12$–16, $P < 0.001$), indicating greater anaerobic glycolysis. Inhibition of mitochondrial function by oligomycin, FCCP, and a combination of rotenone and antimycin A increased ECAR in primary (Figure 2a) and immortalized (data now shown) renal proximal tubule cells from both rat strains, but the increase was greater in SHR than in WKY, indicating the potential for increased compensatory anaerobic glycolysis in SHR.

Although ECAR is reflective of the level of glycolysis, any metabolic acid secreted from cells contributes to the values of ECAR.[16] Therefore, we measured lactate production in whole-kidney homogenates from WKY and SHR. As shown in Figure 2b, lactate production was higher in SHR than in WKY, indicating higher glycolysis in former than in the latter rat strain.

ATP level in renal proximal tubule cells from WKY and SHR

Because the foregoing data suggested higher mitochondrial function in renal proximal tubule cells from SHR than those from WKY, we next measured the intracellular ATP concentration. As shown in Figure 3, ATP levels were significantly higher in renal proximal tubule cells from SHR than those from WKY.

Mitochondrial characterization in renal proximal tubule cells from WKY and SHR

We next examined mitochondrial abundance in renal proximal tubule cells by both fluorescence confocal (Figures 4a–f) and transmission electron (Figures 4g–j) microscopy. Renal proximal tubule cells in primary culture, which were