ANG II causes insulin resistance and induces cardiac metabolic switch and inefficiency: a critical role of PDK4

Jun Mori,1,2,3 Osama Abo Alrob,1,2,3 Cory S. Wagg,1,2,3 Robert A. Harris,4 Gary D. Lopaschuk,1,2,3 and Gavin Y. Oudit1,5,6

1Department of Pediatrics, University of Alberta, Edmonton, Canada; 2Department of Pharmacology, University of Alberta, Edmonton, Canada; 3Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, Canada; 4Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana; 5Department of Physiology, University of Alberta, Edmonton, Canada; and 6Division of Cardiology, Department of Medicine, University of Alberta, Edmonton, Canada

Submitted 24 August 2012; accepted in final form 30 January 2013

Mori J, Alrob OA, Wagg CS, Harris RA, Lopaschuk GD, Oudit GY. ANG II causes insulin resistance and induces cardiac metabolic switch and inefficiency: a critical role of PDK4. Am J Physiol Heart Circ Physiol 304: H1103–H1113, 2013. First published February 8, 2013; doi:10.1152/ajpheart.00636.2012.—The renin-angiotensin system (RAS) may alter cardiac energy metabolism in heart failure. Angiotensin II (ANG II), the main effector of the RAS in heart failure, has emerged as an important regulator of cardiac hypertrophy and energy metabolism. We studied the metabolic perturbations and insulin response in an ANG II-induced hypertrophy model. Ex vivo heart perfusion showed that hearts from ANG II-treated mice had a lower response to insulin with significantly reduced rates of glucose oxidation in association with increased pyruvate dehydrogenase kinase 4 (PDK4) levels. Palmitate oxidation rates were significantly reduced in response to insulin in vehicle-treated hearts but remained unaltered in ANG II-treated hearts. Furthermore, phosphorylation of Akt was also less responsive to insulin in ANG II-treated wild-type (WT) mice, suggestive of insulin resistance. We evaluated the role of PDK4 in the ANG II-induced pathology and showed that deletion of PDK4 prevented ANG II-induced diastolic dysfunction and normalized glucose oxidation to basal levels. ANG II-induced reduction in the levels of the deacetylase, SIRT3, was associated with increased acetylation of pyruvate dehydrogenase (PDH) and a reduced PDH activity. In conclusion, our findings show that a combination of insulin resistance and decrease in PDH activity are involved in ANG II-induced reduction in glucose oxidation, resulting in cardiac inefficiency. ANG II reduces PDH activity via acetylation of PDH complex, as well as increased phosphorylation in response to increased PDK4 levels.

ANG II; cardiac metabolism; diastolic dysfunction; insulin resistance; sirtuin

THE RENIN-ANGIOTENSIN SYSTEM (RAS) is a well-known mediator of cardiac hypertrophy and heart failure, with RAS blockade being effective in treating heart failure (12, 33a). Insulin resistance is also an independent risk factor for heart failure development (13, 34), and activation of RAS in heart failure is known to be associated with insulin resistance (11, 39). The development of cardiac hypertrophy is also associated with insulin resistance (1, 26), which occurs due to disruptions in cardiac energy metabolism (20, 31). Perturbations in cardiac energy metabolism that occur as a result of coronary artery disease and/or hypertension can play a key role in the pathogenesis of heart failure (20, 24, 31). ANG II can also contribute to alterations in cardiac energy metabolism that may contribute to the severity of heart failure (21, 41). In support of this concept, the cardiac RAS is activated during the progression of diabetic cardiomyopathy (5, 30), whereas loss of ACE2 exacerbates diabetic cardiomyopathy (25). Combined, these observations suggest a possible link between ANG II, alterations in cardiac energy metabolism, development of cardiac insulin resistance, and the development of heart failure.

We studied the metabolic perturbations and the insulin response and their temporal profiles and the role of pyruvate dehydrogenase kinase 4 (PDK4) in an ANG II-induced hypertrophy model. ANG II treatment induced a marked cardiac insulin resistance and caused a metabolic switch from glucose oxidation to fatty acid β-oxidation that was accompanied by cardiac inefficiency. We also used mice deficient in PDK4 to show that overcoming these changes in glucose oxidation and insulin resistance can prevent the ANG II-induced development of diastolic dysfunction.

MATERIALS AND METHODS

Experimental animals and protocol. Osmotic minipumps (model 1002; Alza, Palo Alto, CA), containing ANG II or saline, were implanted subcutaneously at the dorsum of the neck of 9-wk-old male C57/B16 wild-type (WT) or homozygous PDK4 knockout (PDK4KO) mice, as reported previously (21, 41). PDK4KO mice, on a C57/B16 background, were used as previously described (18, 37). ANG II (1.5 mg·kg−1·day−1) or saline was administered to WT mice and PDK4KO mice via the osmotic minipumps for a 14-day period. Furthermore, only WT mice were treated with ANG II or saline for a 7-day period to assess the time course of ANG II effect. The investigation conforms to the Care and Use of Laboratory Animals published by the US National Institutes of Health [National Institutes of Health (NIH) Publication No. 85-23, revised 1996] and to the guidelines of the Canadian Council on Animal Care. All studies were approved by the University of Alberta Health Sciences Animal Welfare Committee.

Oral glucose tolerance test. Oral glucose tolerance test (OGTT) was performed to assess systemic insulin resistance, as described previously (2). Briefly, mice were bled from the tail for a baseline measurement (time 0), after 18 h fasting. D-glucose (2 g/kg) was delivered into the stomach through a gavage needle. Afterward, blood glucose was measured at 15, 30, 60, 90, and 120 min after glucose administration by Accu-Chek Aviva (Roche Diagnostics, Mannheim, Germany).

Echocardiography. Transthoracic echocardiography of cardiac systolic and diastolic function was performed and analyzed in a blinded manner with a Vevo 770 high resolution imaging system equipped...
with a 30-MHz transducer (RMV-707B; VisualSonics, Toronto, Canada), as described previously (21, 41).

Ex vivo working heart perfusion. After either 7 days or 14 days of treatment with ANG II or saline, mice were euthanized by pentobarbital sodium administered intraperitoneally. The hearts were isolated and cannulated via the aorta and the left atrium. After equilibration in the Langendorff mode, hearts were switched to the working mode. Isolated working hearts were perfused at a left atrial preload of 11.5 mmHg and an aortic afterload of 50 mmHg, as previously described (21, 40). During this perfusion, cardiac output (in ml/min) and pressures (in mmHg) produced by the working hearts were recorded

Isolated working hearts were perfused at a left atrial preload of 11.5 mmHg and an aortic afterload of 50 mmHg, as previously described (21, 40). During this perfusion, cardiac output (in ml/min) and pressures (in mmHg) produced by the working hearts were recorded to assess cardiac work. The perfusate contained 5 mM [U-14C]glucose, 1.2 mM [9,10-3H]palmitate, and 3% albumin. The palmitate was prebound to 3% fatty acid free bovine serum albumin. Two types of protocol for isolated working heart perfusion were performed. First, hearts were subjected to aerobic perfusion without insulin for first 30 min, then 100 μU/ml insulin was added to some hearts to investigate the response to insulin. In some perfusions, hearts were subjected to aerobic perfusion in the absence of insulin for the entire 60-min period. To assess the effect of PDK4 deletion on energy metabolism, hearts were subjected to aerobic perfusion with the presence of insulin (100 μU/ml) for a 40-min period. Rates of glucose oxidation and palmitate oxidation were determined by quantitative collection of 14CO2 and H2O from [U-14C]glucose and [9,10-3H]palmitate, respectively. At the end of aerobic perfusion (60 and 40 min, respectively), hearts were frozen by liquid N2 and stored at −80°C until used for subsequent biochemical analyses.

Calculation of TCA cycle activity and ATP production. Glucose-derived and palmitate-derived TCA cycle activities were calculated from the rate of acetyl-CoA production from glucose oxidation and palmitate oxidation, as previously reported (17). This was based on 2 and 8 mol acetyl-CoA produced per mole of glucose and palmitate oxidized. The rates of ATP production were also calculated from 31 mol ATP/mol of glucose oxidized and 105 mol ATP/mol of palmitate oxidized.

Western-blot analysis. Frozen ventricular heart tissue was homogenized in buffer containing 50 mM Tris-HCl (pH 8 at 4°C), 1 mM EDTA, 10% glycerol (in wt/vol), 0.02% Brij-35 (in wt/vol), 1 mM dithiothreitol, and 1:1000 protease inhibitors and 1:100 phosphatase inhibitors (Sigma). Protein was extracted, followed by quantification by a Bradford protein assay kit (Bio-Rad). Protein samples were subjected to 5%–10% SDS-PAGE and transferred onto a 0.45 μm nitrocellulose membrane (Bio-Rad). The membrane was incubated for blocking with 5% milk in Tris-Buffered Saline Tween-20 for 1 h and probed with either anti-phospho Akt (Ser473; Cell signaling), anti-phospho Akt (Thr308; Cell signaling), anti-total Akt (Cell signaling), anti-phospho AMPK (Cell signaling), anti-total AMPK (Cell signaling), anti-SIRT1 (Cell signaling), anti-phospho ACC (Millipore), anti-SIRT3 (Abcam), anti-SIRT6 (Sigma), anti-Nmnat2 (Santa Cruz), anti-voltage-dependent anion channels (Santa Cruz), or anti-β-actin (Santa Cruz). After probing with primary antibody, the membrane was washed with Tris-Buffered Saline Tween-20 and subsequently probed with a secondary antibody. Immunoblots were visualized with the enhanced chemiluminescence Western blot detection kit (Perkin Elmer) and quantified with ImageJ software (US NIH, Bethesda, MD).

Immunoprecipitation. A total of 100 μg of heart lysate was pre-cleared with 50 μl of protein A/G-agarose beads. Samples were centrifuged at 16,000 g for 5 min, and the supernatant subsequently incubated with 3 μg acetyl-k antibody overnight at 4°C. Each sample had 50 μl protein A/G-agarose beads added, followed by incubation for 4 h at 4°C. The solution was then washed three times using homogenization buffer containing 150 mM NaCl. After the final wash, the supernatant was removed. The sample buffer was added and boiled at 95°C for 5 min. Samples were loaded onto 10% gel for SDS-PAGE. The subsequent steps were then the same as described above for the Western blot analysis. Either anti-total Akt (Cell signaling) or anti-pyruvate dehydrogenase (PDH) (Cell signaling) was used as primary antibody.

Short-chain CoA analysis. Short-chain CoA analysis was performed by using a modified HPLC procedure, as previously reported (17). Frozen ventricular heart tissue was homogenized in 6% perchloric acid and centrifuged at 12,000 g for 5 min at 4°C. The resulting supernatant was subjected to UPLC for CoA ester separation and quantification (17).

Statistical analysis. All data are presented as means ± SE. Statistical analysis of the data was performed using the Student’s t-test or a one-way ANOVA followed by multiple comparison testing using Student Neuman-Keuls testing when appropriate using the SPSS Statistics 19 software. For OGTT, two-way ANOVA was performed. A probability value <0.05 was considered significant.

RESULTS

ANG II induces cardiac hypertrophy and insulin resistance. ANG II infusion induced systemic insulin resistance (Fig. 1A). Furthermore, ANG II infusion resulted in cardiac hypertrophy with a reduction in cardiac work in the isolated working hearts (Fig. 1, B and C) that occurred without changes in the rate-pressure product and developed pressure (Fig. 1, D and E). To assess cardiac insulin resistance in ANG II-induced hypertrophied hearts, the expressions of phosphorylation of Akt were determined. Akt is an important kinase in the insulin signaling pathway and ANG II can lead to dephosphorylation of Akt and impairment in insulin signaling (9, 16). We showed that ANG II was associated with whole-body insulin resistance as determined by the OGTT (Fig. 1A) with impaired phosphorylation of serine-473 (Fig. 1F) and threonine-308 (Fig. 1G) residues of Akt in response to insulin in ANG II-treated hearts. These results are consistent with insulin resistance in a setting of pathological hypertrophy and diastolic dysfunction.

Ex vivo working hearts were used to investigate the effect of ANG II-induced cardiac hypertrophy on energy metabolism. In the absence of insulin, the rates of glucose oxidation were almost similar between the two experimental groups (Fig. 2A). In response to insulin, only hearts from vehicle-treated WT mice showed a significant increase in glucose oxidation (265 ± 32 to 924 ± 121 nmol·g dry wt⁻¹·min⁻¹; P < 0.05; Fig. 2A). Fatty acids are a main energy substrate in the heart, and fatty acid β-oxidation is closely coupled with glucose oxidation via the Randle cycle (29). While the rate of palmitate oxidation was unaltered in the absence of insulin, the rate of palmitate oxidation significantly decreased to 48% in vehicle-treated-WT mice after adding insulin. This decrease in palmitate oxidation was blunted in the ANG II-treated hearts (Fig. 2B). Collectively, these data suggest that hearts from ANG II-treated WT mice have a lowered level of response to insulin, (i.e., insulin resistance), consistent with the reduction of glucose oxidation in hearts from ANG II-treated WT mice.

ANG II triggers a metabolic switch, accompanied by cardiac inefficiency. The contributions of glucose oxidation and palmitate oxidation to the TCA cycle activity are shown in Fig. 2, C and D, respectively. Glucose-derived TCA cycle acetyl CoA production in vehicle-treated WT mice was increased by 3.5-fold in response to insulin, whereas glucose-derived TCA cycle acetyl CoA production in ANG II-treated WT mice was unaltered (Fig. 2C). Palmitate-derived TCA cycle acetyl CoA production in vehicle-treated WT mice was significantly decreased after adding insulin, but not in ANG II-treated WT mice (Fig. 2D). Total ATP production in vehicle-treated WT
mice in the absence of insulin was slightly higher compared with ANG II-treated WT mice [WT + vehicle(−): 101 ± 5 μmol·g dry wt⁻¹·min⁻¹; WT mice + ANG II(−): 83 ± 10 μmol·g dry wt⁻¹·min⁻¹; Fig. 2E]. In the presence of insulin, total ATP production was similar between the two groups [WT + vehicle(+) : 71 ± 11 μmol·g dry wt⁻¹·min⁻¹; WT mice + ANG II(+) : 72 ± 7 μmol·g dry wt⁻¹·min⁻¹]. Importantly, the percentage of ATP derived from palmitate was greater in ANG II-treated WT hearts compared with vehicle-treated WT hearts (80% vs. 62%; Fig. 2F). Furthermore, cardiac work normalized to ATP production, as a measure of cardiac efficiency, was significantly lower in ANG II-treated WT mice in response to insulin (Fig. 2G). These results show that ANG II-induced cardiac hypertrophy switched cardiac metabolism from glucose to palmitate oxidation, which is associated with insulin resistance and a cardiac inefficiency.

**ANG II-induced hypertrophy is not associated with reduction in myocardial fatty acid β-oxidation.** As shown in Fig. 2, A and B, glucose oxidation rates are decreased in ANG II-treated WT mice, whereas the rate of palmitate oxidation rates remain unaltered. In other studies, however, it was shown that ANG II impairs fatty acid β-oxidation (27, 28). To determine why we observed unaltered rates of fatty acid oxidation, we did a molecular analysis of key enzymes involved in fatty acid β-oxidation, such as AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC), and malonyl CoA decarboxylase (MCD). AMPK phosphorylates and inactivates ACC, which converts acetyl CoA to malonyl CoA. Malonyl CoA, which degraded to acetyl CoA by MCD, inhibits carnitine palmitoyltransferase-1, a rate-limiting enzyme of mitochondrial fatty acid uptake. Phosphorylation of AMPK was elevated in ANG II-treated WT hearts (Fig. 3A) in close association with increased phosphorylation of ACC (which inhibits ACC activity; Fig. 3B). While the expression of MCD is not changed in ANG II-treated WT mice (Fig. 3C), short-chain CoA analysis showed malonyl CoA levels were decreased in ANG II-treated WT hearts (Fig. 3D). Collectively, this shows that ANG II treatment increases the phosphorylation of AMPK and
ACC, thereby decreasing malonyl CoA synthesis. This reduced synthesis, and maintained degradation, decreased malonyl CoA levels, leading to the relatively higher fatty acid oxidation rates in the ANG II-treated hearts. Short-chain CoA analysis also showed that succinyl CoA levels were significantly decreased in response to ANG II treatment (Table 1) possibly reflecting ANG II-induced impairment of mitochondrial function. This collection of biochemical changes can explain why ANG II did not lead to an overall impairment in fatty acid oxidation.

Deletion of PDK4 prevents the ANG II-induced diastolic dysfunction and partial restores depressed glucose oxidation rates. ANG II induces cardiac hypertrophy and diastolic dysfunction that is associated with a marked upregulation of PDK4 that may contribute to low glucose oxidation rates (21, 41). We therefore treated a group of mice in which cardiac PDK4 was deleted (PDK4KO mice) with ANG II to assess the role of PDK4 in ANG II-induced hypertrophy and diastolic dysfunction. M-mode imaging revealed increased wall thickness of the LV induced by ANG II in both WT mice and PDK4KO mice (Fig. 4A and Table 2). Interestingly, ANG II induced diastolic dysfunction in WT mice, characterized by increased A-wave amplitude with a reduction in the E/A ratio as well as tissue Doppler imaging showing reduced E’-wave amplitude resulting in elevation of E/E’ ratio (Fig. 4, B–E, and Table 2). While the magnitude of ANG II-induced hypertrophy was unaffected by loss of PDK4, unlike WT mice the PDK4KO mice did not develop diastolic dysfunction in response to ANG II (Fig. 4, B–E, and Table 2).

We confirmed that expression of PDK4 was selectively loss in the PDK4KO hearts (Fig. 5A), while the expression of PDK2 was unchanged (Fig. 5B). While ANG II reduces the rate of glucose oxidation in ANG II-treated WT mice, glucose oxidation rates in ANG II-treated PDK4KO mice were almost
similar compared with vehicle-treated WT mice and significantly greater than the WT + ANG II hearts (Fig. 5C). Palmitate oxidation rates were not significantly decreased in PDK4KO mice in response to ANG II, similar to our findings in WT mice (Fig. 5D). Phosphorylation of serine-473 residues of Akt was significantly decreased in response to ANG II in both WT and PDK4KO hearts (Fig. 5E), whereas the phosphorylation of threonine-308 residue of Akt was significantly increased in response to ANG II in PDK4KO mice (Fig. 5F), consistent with a protective effect against diastolic dysfunction via stimulation of insulin signaling. However, the significant decrease in glucose oxidation in the PDK4KO hearts suggests that alternative mechanism(s) regulate ANG II-mediated control of glucose oxidation in the heart.

Table 1. Acetyl CoA and free CoA content, acetyl CoA-to-free CoA ratios, and succinyl CoA content in vehicle-treated and ANG II-treated hearts

<table>
<thead>
<tr>
<th></th>
<th>WT + Vehicle</th>
<th>WT + ANG II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl CoA, nmol/g wet wt</td>
<td>11.7 ± 1.5</td>
<td>12.0 ± 1.7</td>
</tr>
<tr>
<td>Free CoA, nmol/g wet wt</td>
<td>105.8 ± 10.8</td>
<td>105.9 ± 6.3</td>
</tr>
<tr>
<td>Acetyl CoA/CoA</td>
<td>0.10 ± 0.002</td>
<td>0.11 ± 0.012</td>
</tr>
<tr>
<td>Succinyl CoA, nmol/g wet wt</td>
<td>32.8 ± 2.4</td>
<td>22.1 ± 2.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. WT, wild-type. *P < 0.05 compared with ANG II-treated group.

Acetylation of PDH is critical for PDH activity. Deletion of PDK4 only partially rescues ANG II-induced perturbation in cardiac energy metabolism, suggesting potential alternative pathway(s) for ANG II-induced reduction in glucose oxidation. We therefore examined the potential role for the SIRTs family in this process, since SIRTs have recently been reported to regulate important pathways in energy metabolism (32, 33). We hypothesized that a decrease in SIRTs deacetylates activity could lead to an increased acetylation of PDH and/or Akt, thus leading to reduction in glucose oxidation and insulin resistance, respectively. Acetylation of PDH was significantly increased in ANG II-treated WT hearts (Fig. 6A), whereas acetylation of Akt was unaffected (Fig. 6B). Next, we checked the expression of members of SIRTs family (SIRT1, SIRT3, and SIRT6) and Nmnat2 (an important enzyme involved in biosynthesis of NAD+), a necessary cofactor for SIRT activity. The expression of SIRT1, which is ubiquitously distributed, was not significantly changed (Fig. 6C). The expression of mitochondrial SIRT3 (Fig. 6D) was significantly decreased in ANG II-treated WT hearts, as well as nuclear SIRT6 (Fig. 6E), in the absence of changes in the expression of Nmnat2 (Fig. 6F). These results support the possibility of reduced SIRT activity, especially SIRT3 and SIRT6, leading to increased acetylation of PDH.

ANG II-induced perturbation in cardiac energy metabolism precedes diastolic dysfunction. To assess the time course of ANG II-induced pathological effects on the heart, we treated...
WT mice with ANG II for a 1-wk period, which resulted in cardiac hypertrophy (Fig. 7, A and B) without inducing either diastolic dysfunction (Fig. 7, C and D) or systolic dysfunction (Fig. 7, E and F). The rates of glucose oxidation were significantly blunted in response to insulin in the ANG II-treated WT hearts, whereas the rates of palmitate oxidation in the presence or absence of insulin were similar between vehicle-treated and ANG II-treated hearts (Fig. 7, G and H). The impairment in

Table 2. Echocardiographic assessment of cardiac function in WT and PDK4 knockout mice infused with ANG II

<table>
<thead>
<tr>
<th></th>
<th>WT + Vehicle</th>
<th>WT + ANG II</th>
<th>PDK4 + Vehicle</th>
<th>PDK4 + ANG II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>493 ± 11</td>
<td>506 ± 14</td>
<td>486 ± 15</td>
<td>492 ± 14</td>
</tr>
<tr>
<td>E-wave, mm/s</td>
<td>716 ± 24</td>
<td>705 ± 31</td>
<td>689 ± 27</td>
<td>668 ± 29</td>
</tr>
<tr>
<td>A-wave, mm/s</td>
<td>431 ± 17</td>
<td>548 ± 19*</td>
<td>445 ± 23</td>
<td>475 ± 27</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.66 ± 0.12</td>
<td>1.29 ± 0.13*</td>
<td>1.55 ± 0.13</td>
<td>1.41 ± 0.15</td>
</tr>
<tr>
<td>Isovolumetric relaxation time, ms</td>
<td>13.6 ± 1</td>
<td>14.3 ± 1.2</td>
<td>13.9 ± 1.1</td>
<td>13.7 ± 1.2</td>
</tr>
<tr>
<td>Deceleration time, ms</td>
<td>26.2 ± 1.6</td>
<td>25.1 ± 1.5</td>
<td>25.4 ± 1.7</td>
<td>25.7 ± 1.8</td>
</tr>
<tr>
<td>E', mm/s</td>
<td>24.4 ± 2.1</td>
<td>16.3 ± 2.4*</td>
<td>26.1 ± 2.7</td>
<td>25.8 ± 2.4</td>
</tr>
<tr>
<td>E/E' ratio</td>
<td>29.3 ± 2.9</td>
<td>43.3 ± 3.2*</td>
<td>26.4 ± 2.5</td>
<td>25.9 ± 3.6</td>
</tr>
<tr>
<td>A', mm/s</td>
<td>17.9 ± 1.3</td>
<td>18.8 ± 1.9*</td>
<td>18.4 ± 1.5</td>
<td>20.3 ± 1.6</td>
</tr>
<tr>
<td>E/A'</td>
<td>1.36 ± 0.12</td>
<td>0.87 ± 0.09*</td>
<td>1.42 ± 0.14</td>
<td>1.27 ± 0.13</td>
</tr>
<tr>
<td>Left ventricular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-diastolic diameter, mm</td>
<td>3.72 ± 0.08</td>
<td>3.43 ± 0.11</td>
<td>3.75 ± 0.09</td>
<td>3.42 ± 0.11</td>
</tr>
<tr>
<td>End-systolic diameter, mm</td>
<td>2.46 ± 0.06</td>
<td>2.21 ± 0.07</td>
<td>2.48 ± 0.08</td>
<td>2.19 ± 0.08</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>33.9 ± 2</td>
<td>35.6 ± 2.8</td>
<td>33.9 ± 2.4</td>
<td>36 ± 2.2</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>63.1 ± 2.5</td>
<td>66.9 ± 3.2</td>
<td>62.6 ± 2.6</td>
<td>65.3 ± 2.9</td>
</tr>
<tr>
<td>Velocity of circumferential shortening corrected for heart rate, circ/s</td>
<td>6.47 ± 0.22</td>
<td>6.61 ± 0.25</td>
<td>6.56 ± 0.23</td>
<td>6.69 ± 0.31</td>
</tr>
<tr>
<td>Left ventricular posterior wall thickness, mm</td>
<td>0.67 ± 0.05</td>
<td>1.02 ± 0.09*</td>
<td>0.68 ± 0.06</td>
<td>1.03 ± 0.10*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. PDK4, pyruvate dehydrogenase kinase 4; E-wave, peak early transmitral inflow mitral E velocity; A-wave, transmitral inflow velocity due to atrial contraction; E', early diastolic tissue Doppler velocity. *P < 0.05 compared with vehicle-treated group.
insulin-stimulated glucose oxidation was accompanied by increased expression of PDK4 levels, increased phosphorylation of PDH and AMPK (Fig. 7, I–K), reduced SIRT3 levels (Fig. 7L), and unchanged SIRT6 levels (data not shown). These results suggest that ANG II-induced perturbations in glucose oxidation precede diastolic dysfunction and correlate with increased phosphorylation of PDH linked with increased PDK4 levels and/or acetylation of PDH due to reduced SIRT3 levels.

**DISCUSSION**

This study provides the novel finding that: 1) ANG-II induced hypertrophy lowers the cardiac response to insulin (insulin resistance), leading to a reduction in glucose oxidation contribution to overall cardiac energy metabolism; 2) Ang II-induced cardiac insulin resistance evokes a cardiac metabolic switch from glucose oxidation to fatty acid β-oxidation, accompanied by the development of a cardiac inefficiency; 3) ANG II-induced cardiac metabolic switch precedes diastolic dysfunction; 4) PDK4 deletion prevents the ANG II-induced reduction in glucose oxidation and prevents diastolic dysfunction; and 5) ANG II promotes acetylation of the PDH complex, due to a decreased deacetylation of PDH by mitochondrial SIRT3. In this study, we show that in ex vivo working heart perfusions, ANG II-treated WT mice have cardiac insulin resistance and are significantly lower in the rate of glucose oxidation compared with vehicle-treated WT mice. Also, less response to insulin in phosphorylation of Akt is suggestive of insulin resistance in response to ANG II treatment. ANG II-induced whole body insulin resistance, impairment in phosphorylation of myocardial Akt, and ANG II ability to inhibit insulin-stimulated GLUT4 translocation (9) may have contributed to myocardial insulin resistance. ANG II-induced myocardial hypertrophy, insulin resistance, and diastolic dysfunction predispose the heart to the development of heart failure (15, 26, 39) and mimic the LV diastolic dysfunction seen in diabetic cardiomyopathy (3, 5). ANG II-induced insulin resistance has been reported in adipocyte, vascular smooth muscle cells, and cardiac myocytes (7, 16, 22) and is likely due to dephosphorylation and inactivation of Akt (9, 16), leading to impairment in insulin signaling. ANG II-mediated reduction in glucose oxidation coupled with ANG II-induced reactive oxygen species can potentially damage mitochondria (10, 41), thereby reducing fatty acid oxidation. However, our study showed that both AMPK and ACC were significantly phosphorylated in ANG II-treated hearts, leading to decrease in malonyl CoA, resulting
in a decreased inhibition of fatty acid β-oxidation. Importantly, while total ATP production remains unaltered, ATP analysis showed that ANG II-treated hearts produced a relatively greater amount of ATP from fatty acid β-oxidation, which is likely a compensatory mechanism to counter the impaired glucose oxidation. Complete oxidation of 1 mol palmitate produces 105 ATP. On the contrary, complete oxidation of 1 mol glucose produces 31 ATP. However, fatty acid β-oxidation is less efficiency, because P/O of glucose oxidation and palmitate oxidation is 105/46 and 31/12, respectively (20, 31). As a result, the decreased cardiac efficiency we observed in the ANG II-treated hearts may have occurred, in part, as a result of the greater contribution of fatty acid oxidation to energy production in these hearts. We likely underestimated the contribution of lipid to the TCA cycle and ATP production since we did not assess endogenous triglyceride oxidation.

Insulin has a profound ability to suppress PDK4 expression in skeletal muscle and hepatocyte (8, 36), which raises the possibility that Ang II-mediated insulin resistance and inhibition of insulin signaling may have mediated the increased expression of PDK4, leading to the reduction in glucose oxidation. In addition, ANG II-mediated hypertrophy can uncouple glycolysis from glucose oxidation (21) resulting in intracellular acidosis. This can lead to a secondary elevation of intracellular Ca²⁺ due to the reverse mode Na⁺-Ca²⁺ exchanger, further exacerbating insulin resistance, as well as diastolic dysfunction (23, 38). Impaired energetics and a rise in the ADP-to-ATP ratio have been linked to diastolic dysfunction in hypertrophied hearts (19, 35). Our results support a key role of ANG II-mediated diastolic dysfunction mediated by PDK4-induced reduction in PDH activity and impaired glucose oxidation. In fact, ANG II reduces glucose oxidation in PDK4KO mice to the basal levels of that in vehicle-treated WT mice, suggesting that PDK4 is, in part, responsible for ANG II-induced reduction in glucose oxidation. Since cardiac glucose oxidation was similarly decreased by ANG II-treatment in both WT and PDK4KO mice, it is likely that mechanisms other than PDK4 are also involved in the decrease in glucose oxidation seen in the ANG II-treated mice. To address alter-
native mechanisms by which ANG II may decrease glucose oxidation (other than PDK4 stimulation), we also examined what effect ANG II has on PDH acetylation. PDH acetylation was markedly increased following ANG II treatment. This may have contributed to the decrease in glucose oxidation following ANG II treatment, independent of alterations in PDK4. Importantly, however, the rates of cardiac glucose oxidation in PDK4KO mice after ANG II treatment were increased to levels seen in the WT mice treated with vehicle. This was also associated with the prevention of diabetic dysfunction. Combined, this data do suggest that low glucose oxidation in ANG II-treated mice contributes to diastolic dysfunction and that inhibition of PDK4 can prevent this from occurring. Furthermore, PDK4 is also expressed in fibroblast (14) and as such we cannot exclude the possible modulation of myocardial fibrosis and ECM remodeling in the PDK4 knockout model as a mechanism of preservation of diastolic function. As a result, inhibition of PDK4 might be a potential therapy for conditions associated with diastolic dysfunction (21, 37).

The SIRTs have received considerable attention, and recently it has been shown that the SIRTs regulate the important pathways of energy metabolism (33). Deacetylation of Akt by SIRTs before phosphorylation of Akt is involved in insulin signaling (32). Deacetylation of Akt is also necessary for activation of Akt; however, acetylation of Akt was not increased in ANG II-treated hearts compared with vehicle-treated hearts. In contrast, acetylation of PDH was significantly increased in ANG II-treated hearts, which is likely induced by SIRT3 downregulation. PDH activity is decreased in ANG II-induced diabetic dysfunction model. Consequently, ANG II-induced downregulation of SIRT3 acetylates and inactivates PDH as well as upregulates the phosphorylation of PDH via PDK4. Since ANG II induces the degradation of Nmnat2 in cultured cardiomyocytes, we also determined whether Nmnat2 could be involved in the observed acetylation of PDH (6). However, ANG II did not alter Nmnat2 in the present study, although NAD+ is expected to decrease, as sirtuins are NAD+ dependent. There is still a concern whether the increased acetylation of PDH is cause or consequence of the metabolic change, and further experimental will be needed.

In conclusion, ANG II-induced insulin resistance causes cardiac metabolic switch from glucose to fatty acid oxidation and produces both a diastolic dysfunction and cardiac inefficiency. Inhibition of PDK4 (and possibly activation of SIRT3) can prevent these changes and may provide the new therapeutic strategy against diastolic dysfunction and heart disease.

ACKNOWLEDGMENTS

J. Mori is a fellow of the Mazankowski Alberta Heart Institute. G. Y. Oudit is an Alberta Innovates Health Solution (AIHS) Clinician Investigator and a
H1112

ANG II, PDK4, and INSULIN RESISTANCE

REFERENCES


