Metabolic Switch and Hypertrophy of Cardiomyocytes following Treatment with Angiotensin II Are Prevented by AMP-activated Protein Kinase*

Bettina Johanna Stuck1,2, Matthias Lenski1, Michael Böhm, and Ulrich Laufs

From the Klinik für Innere Medizin III, Kardiologie, Angiologie und Internistische Intensivmedizin, Universitätsklinikum des Saarlandes, Homburg/Saar, D-66421 Homburg/Saar, Germany

Angiotensin II induces cardiomyocyte hypertrophy, but its consequences on cardiomyocyte metabolism and energy supply are not completely understood. Here we investigate the effect of angiotensin II on glucose and fatty acid utilization and the modifying role of AMP-activated protein kinase (AMPK), a key regulator of metabolism and proliferation. Treatment of H9C2 cardiomyocytes with angiotensin II (Ang II, 1 μM, 4 h) increased [3H]leucine incorporation, up-regulated the mRNA expression of the hypertrophy marker genes MLC, ANF, BNP, and β-MHC, and decreased the phosphorylation of the negative mTOR-regulator tuberin (TSC-2). Rat neonatal cardiomyocytes showed similar results. Western blot analysis revealed a time- and concentration-dependent down-regulation of AMPK-phosphorylation in the presence of angiotensin II, whereas the protein expression of the catalytic α-subunit remained unchanged. This was paralleled by membrane translocation of glucose-transporter type 4 (GLUT4), increased uptake of [3H]glucose and transient down-regulation of phosphorylation of acetyl-CoA carboxylase (ACC), whereas fatty acid uptake remained unchanged. Similarly, short-term transaortic constriction in mice resulted in down-regulation of P-AMPK and P-ACC but up-regulation of GLUT4 membrane translocation in the heart. Preincubation of cardiomyocytes with the AMPK stimulator 5-aminoimidazole-4-carboxamide (AICAR; 1 mM, 4 h) completely prevented the angiotensin II-induced cardiomyocyte hypertrophy. In addition, AICAR reversed the metabolic effects of angiotensin II: GLUT4 translocation was reduced, but ACC phosphorylation and TSC phosphorylation were elevated. In summary, angiotensin II-induced hypertrophy of cardiomyocytes is accompanied by decreased activation of AMPK, increased glucose uptake, and decreased mTOR inhibition. Stimulation with the AMPK activator AICAR reverses these metabolic changes, increases fatty acid utilization, and inhibits cardiomyocyte hypertrophy.

Left ventricular (LV)3 hypertrophy is a major independent risk factor for premature death (1). Myocardial hypertrophy is a response to hemodynamic overload such as hypertension or aortic valve stenosis. A fundamental mechanism leading to both hypertrophy and subsequent heart failure is activation of the renin-angiotensin-aldosterone system. All components of the system are expressed at the mRNA and protein levels in the heart (2), circulating plasma concentrations of angiotensin II (Ang II) correlate to the extend of LV hypertrophy (3) and the progression to heart failure (4), and the increase in left ventricular mass can be prevented with an angiotensin converting enzyme inhibitor or angiotensin receptor blocker (5).

Molecular mechanisms by which the renin-angiotensin-aldosterone system evokes cardiac hypertrophy are activation of protein kinase cascades, initiation of a fetal-like gene program, impaired calcium handling, and increased production of reactive oxygen species (6). Changes in substrate utilization occurring during cardiomyocyte hypertrophy are not well known, and their consequences on cardiomyocyte metabolism and energy supply are not completely understood. A shift in the substrate pattern from fatty acid oxidation to glucose utilization appears to occur in different forms of LV hypertrophy (7), but whether metabolic changes precede hypertrophy or result from it and which pathways are involved is unknown.

A key metabolic regulator is AMP-activated protein kinase (AMPK), a heterotrimeric protein that is centrally involved in controlling cellular energy homeostasis. Its activation through metabolic stress stimulates energy-generating processes and has been shown to regulate hypertrophy-regulating genes in several models (8). When AMPK binds AMP the α-subunit gets sensitized to phosphorylation by upstream kinases and increases kinase activity. Upon activation AMPK increases fatty acid oxidation by phosphorylating ACC, in turn decreasing malonyl-CoA content and activating carnitine palmitoyltransferase-I. In several tissues AMPK has been shown to also regulate glucose metabolism (9) and translocation of glucose transporter 4 (GLUT4) to the plasma membrane (10). Activated AMPK mediates changes in protein expression through regul-

---

1 Both authors contributed equally to this work.
2 To whom correspondence should be addressed: Kirberger Strasse, Universitätsklinikum des Saarlandes, 66421 Homburg/Saar, Germany. Tel.: 49-6841-1623000; E-mail: bettina.stuck@web.de.
3 The abbreviations used are: LV, left ventricular; ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AMPK, AMP-activated protein kinase; Ang II, angiotensin II; GLUT4, glucose transporter 4; PBS, phosphate-buffered saline; TAC, transaortic constriction; TSC-2, tuberous sclerosis complex 2; MLC, myosin light chain; ANF, atrial natriuretic factor; BNP, brain natriuretic peptide; β-MHC, β-myosin heavy chain.
AMPK Reverses Ang II Effects on Cardiomyocyte Metabolism and Hypertrophy

Cell Culture—Rat neonatal cardiomyocytes were isolated as described (12). Briefly, hearts of neonatal rats 2–4 days of age were digested with pankreatin and collagenase type 2, the cell suspension was centrifuged and filtered to extract fibroblasts, and cardiomyocytes were counted and seeded at 2–4 x 10^6 per well. After overnight incubation viability and contractile function was evaluated microscopically, cardiomyocytes were washed and serum starved before using for experiments. H9C2 rat cardiomyocytes (LGC Promochem, Wesel, Germany) are an immortalized cell line derived from embryonic rat hearts using selective serial passage. Cells were grown on tissue culture dishes in Dulbecco’s modified Eagle’s medium complete cell culture medium (Invitrogen), supplemented with 10% fetal calf serum, 1% penicillin and streptomycin, maintained in 95% air and 5% CO2 at 37 °C. Cells were not allowed to become confluent in the development and progression of LV hypertrophy (11). But the relevance of AMPK activation in cardiomyocyte hypertrophy and metabolism is still in question. Therefore, the aim of the study was to examine the effects of angiotensin II on glucose and fatty acid utilization in cardiomyocytes, and whether metabolic alterations through modulation of AMP-activated protein kinase would prevent angiotensin II-induced hypertrophy.

EXPERIMENTAL PROCEDURES

Treatment Conditions—For angiotensin II treatment cells were incubated with 1 μM angiotensin II (purchased from Sigma) for the indicated time periods (5 min to 24 h). AICAR (5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside) treatment was done in a concentration of 1 mM for 2 h (from Sigma). In the case of combined incubation, AICAR pre-treatment was done for 2 h with 1 mM, followed by angiotensin treatment for 4 h with 1 μM. Finally cells were washed with 2 ml of PBS, lysed with 200 μl of ice-cold lysis buffer, scraped, and homogenized with 5 min ultrasound. After homogenization debris was removed by centrifugation at 14,000 x g for 15 min at 4 °C and supernatant was transferred to Eppendorf cups for storage at −20 °C.

Western Analysis—For Western blot analysis protein concentration in cell lysates was measured using a Bio-Rad DC Protein Assay Kit. Protein (50 μg) was loaded in each slot of a 10% polyacrylamide gel, separated at 120 V for 2 h and transferred with 350 mA for 1.5 h. Membrane were stained with Ponceau solution, washed with TBS-Tween, and blocked with 5% milk for 30 min at 4 °C.

Incubations with primary antibodies were done at 4 °C for 12 h. Primary antibodies were purchased from New England Biolabs (P-AMPK, P-ACC) and Santa Cruz Biotechnology (GLUT4, α2-AMPK, glyceraldehyde-3-phosphate dehydrogenase, P-tubulin). Secondary antibodies were purchased from Santa Cruz and incubated at room temperature for 30 min (goat) and 90 min (rabbit), respectively. After washing with PBS-Tween (20 min, twice) and PBS (10 min, twice) membranes were developed with ECL scintillation fluid obtained from GE Healthcare and exposed to hyperfilm.

The amount of GLUT4 in the membrane fraction was determined after membrane preparation. Cells were harvested as described above, mixed at equal volumes with 1 mM KCl, and centrifuged for 1 h at 100,000 x g at 4 °C. The resultant supernatant was collected as cytosolic fraction. The pellet was resuspended, centrifuged at 100,000 x g for 1 h, and the resulting supernatant regarded as the membrane fraction. Separation of cytosolic and membrane proteins was controlled by Western blotting using glyceraldehyde-3-phosphate dehydrogenase. Bands were scanned using XnView normalized for glyceraldehyde-3-phosphate dehydrogenase and the density values were expressed as % of control.

Real-time Reverse Transcriptase-PCR—For reverse transcriptase-PCR RNA was extracted by the chloroform/isopropyl alcohol method. After photometric quantification of the isolated RNA, reverse transcriptase-PCR was performed with the TaqMan System (13). mRNA expression was normalized to expression of the housekeeping gene 18S.

The primers were as follows: for ANF, 5-TGG GCT CCT TCT CCA TCA CC and 5-GCC AAA AGG CCA GGA AGG; for BNP, 5-CAG AAC AAT CCA CGA TGC AG and 5-GCT GTC TCT GAG CCA TTT CC; for β-MHC, 5-GCC TAC CTC ATG GGA CTG AA and 5-ACA TTC TGC CCT TTG GTG AC; for MLC, 5-AGG CCT TCA CAA TCA TGG AC and 5-TCG TTT TTC ACG TCT ACT CG; and for 18S, 5-CTG ATT AAA GTC CTA CTG CCC TTT GT and 5-GCA TCC TAG GGC GTA ACT A.

Leucine Incorporation Assay—Incorporation of radioactively labeled amino acid [3H]leucine was quantified as a parameter of protein synthesis in cardiac myocytes. Assay protocol was performed as previously described (6). Cells were serum-starved overnight, incubated with the indicated treatment (Ang II for 6 h; AICAR for 2 h; AICAR pretreatment for 4 h), and 1 μCi/ml L-4,5-3H-leucine was added for another 30 min at 37 °C, cells were washed with PBS, and harvested in ice-cold lysis buffer. Aliquots were collected for liquid scintillation counting.

Glucose Uptake Assay—To quantify glucose uptake cardiomyocytes were either untreated (basal) or treated with Ang II (1 μM), AICAR (1 mM), or AICAR + Ang II for 10 min, followed by the addition of 2-deoxy-d,2-6-[3H]glucose (0.1 μCi/well) for an additional 30 min at 37 °C. Reaction was stopped by aspirating the medium, washing the cells with PBS, and solubilization in 1 ml of lysis buffer. Samples were assayed for 2-deoxy-d,2-6-[3H]glucose uptake as disintegrations per min as assessed by liquid scintillation counting. Glucose uptake was expressed as a percentage of basal uptake.

Palmitic Acid Uptake Assay—To quantify uptake of palmitic acid, cardiomyocytes were either untreated (basal) or treated with Ang II (1 μM), AICAR (1 mM), or AICAR + Ang II for 10 min. Reaction was initiated by adding 1 μCi of [14C]palmitic acid, incubation at 37 °C for another 30 min, and terminated by...
AMPK Reverses Ang II Effects on Cardiomyocyte Metabolism and Hypertrophy

rapid washing with 1 ml of ice-cold PBS. Cells were disrupted with 1 ml of lysis buffer and scraping, and cell-associated radioactivity was determined by scintillation counting.

**Ferroni Test.**—To assess cellular fatty acid content, cardiomyocytes were seeded on cell culture coverslips (Nalge Nunc, Rochester, NY) in 24-well plates and treated with 1 μM angiotensin II for 4 h, and/or 1 mM AICAR for 2 h. Cells were washed twice with 500 μl of ice-cold PBS and fixed with formalin. The slides were immersed in 7% aqueous solution of nile blue stain for 10 min at 60 °C, then washed with water to remove the excess stain, followed by a 10-min 1% acetic acid wash, rinse in water, and air-dried. Quantification was done by a blinded observer using a Nicon eclipse TS 100 microscope, 40 magnification, and digital image analysis with Lucia G, version 4.60.

**Statistical Analysis.**—Band intensities were analyzed by densitometry. All values are expressed as mean ± S.E. Unpaired Student’s t tests and analysis of variance for multiple comparisons were applied. Post hoc comparisons were performed with the Bonferroni test. Prism software, version 4.0, was used. Differences were considered significant at p < 0.05.

**RESULTS**

**Angiotensin II-induced Changes in Leucine Incorporation, TSC Phosphorylation, and mRNA Expression of Hypertrophy Markers in H9C2 Cardiomyocytes.**—Treatment of H9C2 cardiomyocytes with angiotensin II (Ang II, 1 μM, 4 h) up-regulates (A) [3H]leucine incorporation (n = 4), (B) mRNA expression of MLC, ANF, BNP, and β-MHC standardized to expression of 18S and (n = 5 for MLC, ANP; n = 4 for BNP and β-MHC) and (C) phosphorylation of tuberin (TSC-2) (n = 8). **, p < 0.01, ***p < 0.001. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

**Figure 1.** Effect of angiotensin II on leucine incorporation, TSC phosphorylation, and mRNA expression of hypertrophy markers.

**Transaortic Constriction.**—Animal experiments were approved by the animal ethics committee of the Universita¨t des Saarlandes and conform with National Institutes of Health publication number 85-23, revised 1996, and conducted in accordance with institutional guidelines. 10-Week-old male C57/Bl6 mice (Charles River Laboratories, Sulzfeld, Germany) were housed under standard conditions. Animals were anesthetized with ketamine/xylazine for transaortic constriction (TAC) (14). After orotracheal intubation mice were ventilated by a volume cycled rodent ventilator (Harvard Apparatus, Holliston, MA) on supplemental oxygen. The chest cavity was entered in the second intercostal space and aortic constriction was performed by tying a 7-0 nylon suture ligature against a 27-gauge needle to yield a narrowing 360 mm in diameter and a transverse aortic constriction of 65–70%. A 1.4 French pressure-transducing catheter (Mikro Tip Catheter, Millar instruments) was used for LV pressure measurements. Effective ligation was defined as an increase in left ventricular systolic pressure to at least 200%. All effectively constricted mice developed an increase in left ventricular end-diastolic pressure of 200–400%. Control mice underwent a sham operation and showed no significant increase in systolic or diastolic pressures. After 30 min of LV pressure measurements post-constriction hearts were rapidly excised, weighed, and frozen in liquid nitrogen.

**Statistical Analysis.**—Band intensities were analyzed by densitometry. All values are expressed as mean ± S.E. Unpaired Student’s t tests and analysis of variance for multiple comparisons were applied. Post hoc comparisons were performed with the Bonferroni test. Prism software, version 4.0, was used. Differences were considered significant at p < 0.05.

**RESULTS**

**Angiotensin II-induced Changes in Leucine Incorporation, TSC Phosphorylation, and mRNA Expression of Hypertrophy Markers in H9C2 Cardiomyocytes.**—Treatment of H9C2 cells with angiotensin II (Ang II, 1 μM, 4 h) increased incorporation of [3H]leucine (Fig. 1A). Treatment with Ang II (1 μM, 4 h) increased the membranous fraction of GLUT4 in rat cardiomyocytes with angiotensin II (1 μM, 4 h) increased the mRNA expression of hypertrophy markers (Fig. 1B). Treatment with Ang II (1 μM, 4 h) increased the mRNA expression of hypertrophy markers (Fig. 1C). Treatment with Ang II (1 μM, 4 h) increased the membrane fraction of GLUT4 in rat neonatal cardiomyocytes to 185 ± 21%, p < 0.01 (n = 4), accompanied by an increase in cellular uptake of 2-deoxy-D-glucose to 180 ± 12%, p < 0.01 (n = 12) (Fig. 2).

**Angiotensin II Transiently Decreases ACC Phosphorylation in Cardiomyocytes.—**Exposure of H9C2 cardiomyocytes to angiotensin II (1 μM) for 2 h decreased the phosphorylation of acetyl-
CoA carboxylase to 63.7 ± 8.9%, p < 0.05 (n = 3 per group). This effect was no longer significant after 4 h. Angiotensin (1 μM for 4 h) did not alter the uptake of 14C-labeled palmitic acid (Fig. 3).

**Angiotensin II Decreases Phosphorylation of AMP-activated Protein Kinase**—To evaluate a possible involvement of AMPK we assessed the phosphorylation status of this key metabolic regulator. Treatment with angiotensin II led to a significant reduction of AMPK phosphorylation in a time- and concentration-dependent manner. The maximum depressive effect of 58 ± 4% was reached with 1 μM Ang II at an incubation period of 4 h, p < 0.01. Expression of AMPK represented by the α2-subunit remained unchanged (n = 4 control versus 8 for Ang II) (Fig. 4). Preincubation with the AMPK-activator AICAR (1 mM, 1 h) completely reversed the angiotensin II-induced inhibition of AMPK, p < 0.001 (n = 8 control versus 4 Ang II/AICAR) (Fig. 5).

**Effect of AMPK Stimulation on Angiotensin-induced Metabolic Changes**—To test whether these changes in AMPK phosphorylation mediate the observed shift in glucose utilization, we performed the metabolic measurements upon treatment with the AMPK activator alone (1 mM, 4 h) and after preincubation followed by angiotensin II treatment (1 μM, 4 h) (n = 6 control versus 4 Ang II/AICAR/Ang II + AICAR). We found a significant decrease in GLUT4 translocation to 19 ± 2% with AICAR treatment alone and 43 ± 3% after AICAR preincubation with Ang II treatment, p < 0.001. Glucose-uptake remained unchanged after AICAR pre-treatment and AICAR treatment alone as well. In contrast, ACC phosphorylation and early fatty acid uptake were markedly increased by AMPK activation to 284 ± 23%, p < 0.001, and 160 ± 17%, p < 0.05, respectively, an effect that was not reversed by consecutive angiotensin II treatment. Angiotensin II significantly, but only partially reversed the AMPK-induced activation of palmitic acid uptake to 132 ± 7% of control, p < 0.001 versus Ang II (Fig. 6).

**Effect of Angiotensin II and AICAR Treatment on Fatty Acid Content**—To clarify the consequences of a differential regulation of fatty acid uptake and fatty acid oxidation upon Ang II and AICAR treatments, Nile blue staining was performed. Treatment with Ang II (1 μM, 4 h) induced a significant increase of fatty acids to 221 ± 19% versus control, p < 0.001 (Fig. 7), which was prevented by pre-treatment with the AMPK activator AICAR (130 ± 11%, p < 0.01 versus control) (n = 6 control versus 4 Ang II/AICAR/Ang II + AICAR).

**FIGURE 2. Effect of angiotensin II treatment on glucose metabolism.** Angiotensin II (1 μM, 4 h) increases translocation of the glucose transporter GLUT4 to the plasma membrane (n = 4) (A) and incorporation of [3H]glucose in H9C2 cardiomyocytes (n = 12). **, p < 0.01.

**FIGURE 3. Effect of angiotensin II treatment on fatty acid metabolism.** A, representative Western blots and quantification show that angiotensin II (Ang II, 1 μM) exerted a significant down-regulation of the phosphorylation of acetyl-CoA-carboxylase (P-ACC) after 2 h that was no longer significant after 4 h treatment (n = 3 per group); *, p < 0.05. Ang II (1 μM, 4 h) did not change the incorporation of [14C]palmitic acid in H9C2 cells (n = 6 per group). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
Short-term Increase of Cardiac Afterload Decreases AMPK Phosphorylation and Increases GLUT4 Translocation—To test the cardiac regulation of AMPK, P-ACC, and GLUT4 in vivo, TAC was applied to increase cardiac afterload in mice (n = 4 – 8 per group). **, p < 0.01. Sham versus 4 TAC (14). Compared with sham-operated controls, 30 min after TAC the hearts showed a profound downregulation of P-AMPK to 8.5 ± 3.3%, p < 0.01. This was associated with a decrease of P-ACC to 12.3 ± 2.2%, p < 0.01, and up-regulation of GLUT4 membrane translocation to 238 ± 56%, p < 0.05 (Fig. 8).

Inhibition of Angiotensin-induced Hypertrophy by Stimulation of AMPK and Inhibition of Glucose Uptake—Consequently, the impact of the AMPK-mediated substrate changes on cardiomyocyte protein synthesis and regulation of hypertrophy markers was assessed. Activation of AMPK by AICAR (1 mM, 1 h) decreased GLUT4 translocation, pre-treatment with the AMPK-activator AICAR (1 mM, 1 h) inhibits glucose utilization and increases phosphorylation of acetyl-CoA carboxylase (n = 4 – 8 per group). **, p < 0.01.

FIGURE 4. Effect of angiotensin II treatment on AMPK. Angiotensin II (1 μM, 4 h) induces a significant reduction of AMPK phosphorylation in a (A) time- and (B) concentration-dependent manner with maximum effect at a concentration of 1 μM at 2–6 h. C, treatment with Ang II (1 μM, 4 h) reduced AMPK phosphorylation (P-AMPK), expression of total AMPK represented by the α2-subunit remained unchanged (n = 4 control versus 8 Ang II). **, p < 0.01. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

FIGURE 5. AICAR reverses angiotensin II-induced AMPK inhibition. Preincubation with the AMPK activator AICAR (1 mM, 1 h) reverses the angiotensin II-induced inhibition of AMPK phosphorylation, expression of the catalytic subunit stays unchanged (n = 8 control versus 4 Ang II/Ang II + AICAR). *, p < 0.05; **, p < 0.001. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

FIGURE 6. Effect of AMPK activation on angiotensin II-induced changes in glucose and fatty acid metabolism. Preincubation with the AMPK activator AICAR (1 mM, 1 h) before Ang II treatment (1 μM, 4 h) decreases GLUT4 translocation (A) and prevents Ang II-induced increase of glucose incorporation (B). Effects on fatty acid metabolism: treatment with the AMPK activator AICAR (1 mM, 4 h) increases ACC phosphorylation (C) and induces incorporation of [14C]palmitic acid (D), the latter was only partially reversed by Ang II (n = 6 control versus 4 Ang II/AICAR/Ang II). *, p < 0.05; **, p < 0.01; ***, p < 0.001. E, effects of Ang II and AMPK stimulation on metabolic parameters in rat neonatal cardiomyocytes: Ang II (1 μM, 4 h) decreases AMPK phosphorylation and induces GLUT4 translocation, pre-treatment with the AMPK-activator AICAR (1 mM, 1 h) inhibits glucose utilization and increases phosphorylation of acetyl-CoA carboxylase (n = 4 – 8 per group). **, p < 0.01.
AMPK Reverses Ang II Effects on Cardiomyocyte Metabolism and Hypertrophy

DISCUSSION

These experiments show that angiotensin II-induced hypertrophy in cultured cardiomyocytes is accompanied by profound changes in substrate utilization. The data identify phosphorylation of AMP-activated protein kinase as a metabolic switch reconstituting fatty acid consumption that is able to prevent cardiomyocyte hypertrophy.

There is mounting evidence that substrate utilization plays a crucial role in cardiac contractile performance and in limiting damage to the heart (15). The metabolic changes in left ventricular hypertrophy differ between adaptive and maladaptive forms of remodeling and may depend on the course of the disease (16). The particular consequences of angiotensin-induced hypertrophy are not known. It is therefore of interest to identify the influence of the key players in left ventricular remodeling on

FIGURE 7. Fatty acid content in H9C2 cells is increased by angiotensin II treatment. A. representative sections (×40 magnification), and B, quantification of H9C2 cardiomyocytes stained with Nile blue upon treatment with Ang II (1 μM, 4 h), AICAR (1 mM, 1 h) and co-treatment with Ang II and AICAR (n = 6 control versus 4 Ang II/AICAR/Ang II + AICAR). **, p < 0.01; ***, p < 0.001.

FIGURE 8. Short-term increase of cardiac afterload reduces AMPK and ACC phosphorylation and induces GLUT4 translocation. TAC for 30 min increased the maximal left ventricular systolic pressure (LV-Pmax) to at least 200% and left ventricular end-diastolic pressure to 200–400% compared with sham operated mice; A shows a representative recording. B, representative Western blots and quantification of P-AMPK, P-ACC, and GLUT4 membrane translocation (n = 3 Sham versus 4 TAC). *, p < 0.05; **, p < 0.01.

FIGURE 9. Angiotensin II-induced protein synthesis and expression of hypertrophy markers are reversed by AMPK activation. Pre-treatment of H9C2 cardiomyocytes with AICAR (1 mM, 1 h) before incubation with 1 μM Ang II for 4 h prevents the effects on TSC phosphorylation (A) (n = 8), expression of markers of hypertrophy (B): ANF, BNP, and β-MHC (n = 6); and C on Ang II-induced leucine incorporation (n = 12 control versus 6 Ang II/AICAR/Ang II + AICAR). *, p < 0.05; **, p < 0.01; ***, p < 0.001.
AMPK Reverses Ang II Effects on Cardiomyocyte Metabolism and Hypertrophy

cardiomyocyte metabolism and energy supply, such as the members of the renin-angiotensin-aldosterone system cascade.

Angiotensin II mediates cardiomyocyte hypertrophy directly and indirectly through autocrine and paracrine effects (17, 18). Overexpression of its type I receptor induces cardiac hypertrophy and remodeling, and treatment with angiotensin converting enzyme inhibitors or angiotensin receptor blockers prevents these effects (19). But only a little is known about its influence on cardiac energy metabolism. Here we show in cardiomyocytes that angiotensin II-induced hypertrophy is associated with increased translocation of GLUT4 to the plasma membrane and increased utilization of glucose. This observation is in agreement with up-regulation of glucose utilization observed during increases of afterload, e.g. arterial hypertension (7).

Increased glucose utilization may be an adaptive response with potentially protective effects because ATP production from glucose consumes less oxygen than that from fatty acids (20). On the other hand, oxidation of 1 mol of carbon yields about 29% more ATP for free fatty acid than for glucose, so that in the oxygen abundant state, oxidation of free fatty acid is more high-energy phosphate efficient than glucose. Therefore it is not known whether an increase in glucose utilization would be indeed favorable in cardiomyocyte hypertrophy.

In cultured cardiac myocytes, we observed a transient decrease of ACC phosphorylation. Furthermore, the cells show an increased content of fatty acids upon Ang II treatment. Importantly, our in vivo experiments using short-term transaortic constriction show a profound down-regulation of P-ACC by cardiac afterload. These findings are in agreement with data describing reduced fatty acid oxidation in failing human hearts (21) and in murine hearts with specific angiotensinogen overexpression (22). The resulting energetic consequences are largely unknown. On the one hand the suppression of fatty acid oxidation decreases cardiac oxygen consumption per mol of ATP produced and could therefore be protective. On the other hand decreased fatty acid oxidation may impair cardiac energetics through lipid accumulation causing lipotoxicity (23). Several models of myocardial lipid accumulation, Zucker diabetic fatty (ZDF) rats (24), overexpression of lipoprotein lipase at cardiomyocytes (25), and cardiac restricted overexpression of peroxisome proliferator activated receptor-α (26), show ventricular hypertrophy and systolic ventricular dysfunction associated with an increased rate of apoptosis. In idiopathic-dilated cardiomyopathy fatty acid oxidation is decreased and associated with an intracellular triglyceride accumulation (27). In our experiments, angiotensin II-treated, hypertrophied cardiomyocytes show a metabolic phenotype of increased glucose utilization and intracellular fatty acid accumulation. Similarly, cardiac afterload induced down-regulation of P-ACC and up-regulation of GLUT4 membrane expression in mice heart.

Therefore, induction of fatty acid oxidation, e.g. by AMPK, may be metabolically favorable not only to increase ATP yield but also to reduce toxic effects of intracellular free fatty acids. AMPK is a major stimulator of β-oxidation in many tissues (28, 29). An unexpected finding of our experiments was that angiotensin II-induced hypertrophy and glucose utilization were accompanied by down-regulation of AMPK, despite stimulation of energy demanding processes such as cell growth and protein synthesis.

The existing literature on the role of AMPK in cardiac pathology is not entirely clear. Russell et al. (30) found an AMPK-dependent increase in GLUT4-membrane translocation in the working heart ischemia model. Similarly, ischemia induced GLUT4 translocation in isolated cardiac myocytes (31). On the other hand, three decades ago Finck et al. (32) had shown an ischemia-induced reduction of glucose uptake, a finding that was supported by a recent study that found no increase in glucose uptake despite AMPK activation in ischemic isolated working hearts (33). Similarly, the data regarding ACC are not entirely consistent. Frederich et al. (34) show a negative correlation between the activities of AMPK and ACC in the heart. The same has been shown for AMPK-induced phosphorylation in exercised skeletal muscle (35). However, newer data suggest a differential regulation of AMPK and ACC that depends on the time course and the intensity of exercise. Sripiupparat and colleagues (36) have shown that after 40 min of low intensity exercise the phosphorylation of ACC was increased even though AMPK phosphorylation was unchanged, whereas in the post-exercise period ACC phosphorylation was no longer elevated, whereas AMPK phosphorylation was now slightly increased. Therefore AMPK-dependent metabolic regulation appears to be differentially regulated in different cell types, tissues, and different experimental models. It will be important for future research to confirm cell culture observations in animal models and eventually clinical studies. Here we confirm the cell culture studies in vivo observing a similar regulation upon a short-term increase of afterload in mice heart. However, eventually these data have to be confirmed in human disease.

To test the implications of the observed role of AMPK, we treated cardiomyocytes with the AMPK activator AICAR alone or before the induction of hypertrophy with angiotensin II. AMPK activation caused a metabolic switch, favoring utilization of fatty acids over glucose metabolism: AMPK activation induced a robust increase of the phosphorylation of ACC that was not antagonized by angiotensin II treatment. This was accompanied by an increase in palmitic acid uptake, indicating both an increased uptake and intracellular cleavage of lipids. This effect was partially reversed by Ang II co-treatment, suggesting a second modulator that has yet to be elucidated. Regarding glucose metabolism we found a massive decrease in GLUT4 translocation upon AICAR treatment, whereas glucose uptake remained unchanged compared with control cells. The constancy in glucose uptake is most likely due to the expression of other glucose transporters (e.g. GLUT1) in the cardiomyocyte plasma membrane, maintaining the basal cellular need (37).

Importantly, the metabolic effects of AMPK activation in cardiomyocytes were paralleled by anti-hypertrophic effects on protein synthesis and reduced expression of maladaptive hypertrophic markers. These data identify AMPK-dependent substrate utilization as a key hypertrophy regulator in cardiac myocytes and give new insights into the link between metabolism and hypertrophy.
AMPK Reverses Ang II Effects on Cardiomyocyte Metabolism and Hypertrophy

We conclude that angiotensin II-induced hypertrophy of cardiomyocytes depends on the inhibition of AMPK and changes in substrate utilization to the favor of glucose metabolism. Stimulation of fatty acid oxidation by AMPK activation prevents cardiomyocyte hypertrophy and therefore identifies a novel potential therapeutic target. Whether these observations are of functional significance in vivo has to be tested in further studies.

Acknowledgment—We thank Ellen Becker for excellent technical assistance.

REFERENCES

1. Bayes-Genis, A., Guindo, J., Vinolas, X., Tomas, L., Elosua, R., Duran, I., and Bayes, D. L. (1995) Am. J. Cardiol. 76, 54D–59D
2. Suzuki, J., Matsubara, H., Urakami, M., and Inada, M. (1993) Circ. Res. 73, 439–447
3. Weber, K. T., Brilla, C. G., Campbell, S. E., Guarda, E., Zhou, G., and Sriman, K. (1993) Basic Res. Cardiol. 88, Suppl. 1, 107–124
4. Neri Serneri, G. G., Boddi, M., Modesti, P. A., Cecioni, I., Coppo, M., Padeletti, L., Michelucci, A., Colella, A., and Galanti, G. (2001) Circ. Res. 89, 977–982
5. Baker, K. M., Chernin, M. I., Wixson, S. K., and Aceto, J. F. (1990) Am. J. Physiol. 259, H324–H332
6. Custodis, F., Eberl, M., Kilter, H., Bohm, M., and Laufs, U. (2006) Cardiovasc. Res. 71, 342–351
7. Sambandam, N., Lopaschuk, G. D., Browney, R. W., and Allard, M. F. (2002) Heart Fail. Rev. 7, 161–173
8. Motoshima, H., Goldstein, B. J., Igata, M., and Araki, E. (2006) J. Physiol. 574, 63–71
9. Mimokoshi, Y., Alquier, T., Furukawa, N., Kim, Y. B., Lee, A., Xue, B., Mu, J., Foufelle, F., Ferre, P., Birnbaum, M. J., Stuck, B. J., and Kahn, R. B. (2004) Nature 428, 569–574
10. Yamaguchi, S., Katabira, H., Ozawa, S., Nakamichi, Y., Tanaka, T., Shimoyama, T., Takahashi, K., Yoshimoto, K., Imaiizu, M. O., Nagamatsu, S., and Ishida, H. (2005) Am. J. Physiol. 289, E643–E649
11. McMullen, J. R., Sherwood, M. C., Tarnavski, O., Zhang, L., Dorfman, A. L., Shioi, T., and Izumo, S. (2004) Circulation 109, 3050–3055
12. Laufs, U., Kilter, H., Konkol, C., Wassmann, M., Bohm, M., and Nickenig, G. (2002) Cardiovasc. Res. 53, 911–920
13. Laufs, U., Werner, N., Link, A., Endres, M., Wassmann, S., Jurgens, K., Miche, E., Bohm, M., and Nickenig, G. (2004) Circulation 109, 220–226
14. Adam, O., Frost, G., Custodis, F., Sussman, M. A., Schafers, H. J., Bohm, M., and Laufs, U. (2007) J. Am. Coll. Cardiol. 50, 359–367
15. Frey, N., and Olson, E. N. (2003) Annu. Rev. Physiol. 65, 45–79
16. Ritchie, R. H., and Delbridge, L. M. (2006) Clin. Exp. Pharmacol. Physiol. 33, 159–166
17. Gray, M. O., Long, C. S., Kalinyak, J. E., Li, H. T., and Karliner, J. S. (1998) Circ. Res. 80, 352–363
18. Malhotra, R., Sadoshima, J., Brosius, F. C., III, and Izumo, S. (1999) Circ. Res. 85, 137–146
19. Paradis, P., Dali-Youcef, N., Paradis, F. W., Thibault, G., and Nemer, M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 931–936
20. Grynpberg, A., and Dena, L. (1996) J. Cardiovasc. Pharmacol. 28, Suppl. 1, S11–S17
21. Razeghi, P., Young, M. E., Alcorn, J. L., Moravec, C. S., Frazier, O. H., and Taegtmeyer, H. (2001) Circulation 104, 2923–2931
22. Pelleux, C., Aasum, E., Larsen, T. S., Montessuit, C., Papageorgiou, I., Pedrazzini, T., and Lerch, R. (2006) J. Mol. Cell Cardiol. 41, 459–466
23. Ingwall, J. S., and Weiss, R. G. (2004) Circ. Res. 95, 135–145
24. Zhou, Y. T., Grayburn, P., Karim, A., Shimabukuro, M., Higa, M., Baetens, D., Orsi, L., and Unger, R. H. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1784–1789
25. Yagyu, H., Chen, G., Yokoyama, M., Hirata, K., Augustus, A., Kako, Y., Seo, T., Hu, Y., Lutz, E. P., Merkel, M., Bensadoun, A., Homma, S., and Goldberg, I. J. (2003) J. Clin. Investig. 111, 419–426
26. Finck, B. N., Lehman, J. I., Leone, T. C., Welch, M. J., Bennett, M. J., Kovacs, A., Han, X., Gross, R. W., Kozak, R., Lopaschuk, G. D., and Kelly, D. P. (2002) J. Clin. Investig. 109, 121–130
27. Tomita, T., Wilson, L., and Chiga, M. (1990) Am. J. Cardiovasc. Pathol. 3, 81–85
28. Winders, W. W., Wilson, H. A., Hardie, D. G., Rasmussen, B. B., Hutber, C. A., Call, G. B., Clayton, R. D., Conley, L. M., Yoon, S., and Zhou, B. (1997) J. Appl. Physiol 82, 219–225
29. Dadd, M., Gamble, J., Witters, L. A., and Lopaschuk, G. D. (1993) J. Biol. Chem. 268, 25836–25845
30. Russell, R. R., III, Li, J., Coven, D. L., Pypoaret, M., Zechnner, C., Palmeri, M., Giordano, F. J., Mu, J., Birnbaum, M. J., and Young, L. H. (2004) J. Clin. Investig. 114, 495–503
31. Sun, D., Nguyen, N., DeGrado, T. R., Schweiger, M., and Brosius, F. C., III (1994) Circulation 89, 793–798
32. Neely, J. R., Rovetto, M. J., Whitmer, J. T., and Morgan, H. E. (1973) Am. J. Physiol. 225, 651–668
33. Omar, M. A., Fraser, H., and Clanachan, A. S. (2008) Am. J. Physiol. 294, H1266–H1273
34. Frederich, M., and Balschi, J. A. (2002) J. Biol. Chem. 277, 1928–1932
35. Park, S. H., Gammon, S. R., Knipppers, J. D., Paulsen, S. R., Rubins, D. S., and Winder, W. W. (2002) J. Appl. Physiol. 92, 2475–2482
36. Sveerikamol, A., Coletta, D. K., Wajcberg, E., Balbontin, G. B., Reyna, S. M., Barrientes, J., Eagan, P. A., Jenkinson, C. P., Cersosimo, E., Defronzo, R. A., Sacamato, K., and Musi, N. (2007) Diabetes 56, 836–848
37. Kraegen, E. W., Sowden, J. A., Halstead, M. B., Clark, P. W., Rodnick, K. J., Chisholm, D. J., and James, D. E. (1993) Biochem. J. 295, 287–293