UCF-101 mitigates streptozotocin-induced cardiomyocyte dysfunction: role of AMPK

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Li Q, Li J, Ren J. UCF-101 mitigates streptozotocin-induced cardiomyocyte dysfunction: role of AMPK. Am J Physiol Endocrinol Metab 297: E965–E973, 2009. First published August 18, 2009; doi:10.1152/ajpendo.00323.2009.— Diabetic heart disease contributes to the high mortality in diabetics, although effective clinical management is lacking. The protease inhibitor 5-[5-(2-nitrophenyl) furfuryliodine]-1,3-diphenyl-2-thiobarbituric acid (UCF-101) was reported to protect the hearts against ischemic injury. This study examined the role of UCF-101 on streptozotocin (STZ)-induced diabetic heart defect. Vehicle or UCF-101 was administrated to STZ diabetic mice, and cardiomyocyte mechanical properties were analyzed. UCF-101 reduced STZ-induced hyperglycemia and alleviated STZ-induced aberration in cardiomyocyte contractile mechanics. Diabetes dramatically decreased AMPK phosphorylation at Thr\textsuperscript{172} of catalytic α-subunit, which was restored by UCF-101. Neither diabetes nor UCF-101 affected the expression of HtrA2/Omi and XIAP or caspase-3 activity. The AMPK activator resveratrol mimicked the UCF-101-induced beneficial effect against diabetic cardiac dysfunction. Mechanical properties in cardiomyocytes from the AMPK-kinase-dead (KD) mice displayed markedly impaired contractile function reminiscent of diabetes. STZ injection in AMPK-KD mice failed to elicit any additional cardiomyocyte contractile defect. UCF-101 significantly downregulated the AMPK-degrading enzymes PP2A and PP2C, the effect of which was mimicked by resveratrol. Taken together, these results indicate that UCF-101 protects against STZ-induced cardiac dysfunction, possibly through AMPK signaling.

contractile function; diabetes; adenosine 5'-monophosphate-activated protein kinase

MOUNTING EVIDENCE HAS INDICATED that cardiovascular complications are the leading cause of morbidity and mortality in diabetic population. With the rapidly increasing burden of diabetes mellitus worldwide coupled with cardiovascular complications, diabetic heart diseases have been the subject of intensive research over the last three decades. Although a cadre of hypoglycemic or antihyperglycemic drugs is used clinically for the management of diabetes, new classes of drugs with specific benefit on diabetic cardiomyopathy are still lacking (12, 25). Recent evidence has revealed a rather unique cardioprotective role of the protease inhibitor 5-[5-(2-nitrophenyl) furfuryliodine]-1,3-diphenyl-2-thiobarbituric acid (UCF-101) (2, 17). UCF-101 is a specific inhibitor of high-temperature requirement A2 (HtrA2)/Omi, a mitochondrial serine protease released into cytosol from mitochondria to promote caspase activation by proteolyzing the antiapoptotic X chromosome-linked inhibitor of apoptosis protein (XIAP) (3). It is believed that UCF-101 elicits its protective role through antiapoptotic mechanism in myocardial ischemia-reperfusion injury and cerebral ischemia (1, 2, 17). Although recent observations from our laboratory have demonstrated that UCF-101 may be cardioprotective against experimental diabetes in an in vitro setting (14), the impact of UCF-101 on diabetes-associated cardiac complications has not been examined in vivo.

AMP-activated protein kinase (AMPK) is a widely conserved serine/threonine-specific protein kinase that emerges as a metabolic master switch. Changes in AMPK activity have been shown to regulate glucose production and transport. Under acute metabolic stresses, cardiac AMPK activation seems to be involved primarily in promoting energy generation to maintain or restore intracellular ATP levels (10). AMPK plays a key role in the regulation of insulin release (10, 13, 30) and preserved cardiac integrity (10, 28). Not surprisingly, dysregulation in AMPK signaling has been demonstrated in many disorders, such as myocardial ischemia, heart failure, diabetes, and lipotoxic heart disease, to be responsible for altered cardiac energy metabolism (10). The aim of this study was to examine the impact of UCF-101 on cardiomyocyte contractile dysfunction in streptozotocin (STZ)-induced experimental diabetes and participating subcellular signaling mechanisms with a focus on AMPK. In addition, the level of AMPK phosphorylation appears to be reciprocally correlated with the expression of protein phosphatases 2A and 2C (PP2A and PP2C), enzymes that were shown to inactivate AMPK by dephosphorylation (31, 32). Protein expression of PP2A and PP2C was also monitored in response to UCF-101 treatment.

MATERIALS AND METHODS

Experimental animals. The experimental procedure described in this study was approved by our Institutional Animal Use and Care Committee investigation and was in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85-23, revised 1996). In brief, 4- to 6-mo-old adult male C57BL/6 mice were made diabetic using STZ (50 mg·kg\textsuperscript{-1}·day\textsuperscript{-1} for 5 consecutive days) dissolved in a sterile citrate buffer (0.05 M sodium citrate, pH 4.5). Vehicle or UCF-101 (7.15 mg/kg ip) was administrated 30 min prior to STZ injection on the 1st day and once/day for 6 days. The AMPK-kinase-dead (KD) mice (obtained from Dr. Morris Birnbaum, University of Pennsylvania, Philadelphia, PA) were made diabetic using STZ (50 mg·kg\textsuperscript{-1}·day\textsuperscript{-1} for 5 consecutive days). AMPK-KD mice express a dominant negative AMPK-kinase-dead (KD) mice failed to elicit any additional cardiomyocyte contractile defect. UCF-101 significantly downregulated the AMPK-degrading enzymes PP2A and PP2C, the effect of which was mimicked by resveratrol. Taken together, these results indicate that UCF-101 protects against STZ-induced cardiac dysfunction, possibly through AMPK signaling.
using a glucose monitor (Accu-Chek II, model 792; Boehringer Mannheim Diagnostics, Indianapolis, IN).

**Cardiomyocyte isolation and resveratrol treatment.** Mouse hearts were rapidly removed under anesthesia (ketamine-xylazine at 3:1, 1.32 mg/kg) and were perfused with a Ca\(^{2+}\)-free Krebs-Henseleit bicarbonate buffer containing (in mM) 118 NaCl, 4.7 KCl, 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 25 NaHCO\(_3\), 10 HEPES, 11.1 glucose, and 10 butanedione with 5% CO\(_2\)-95% O\(_2\). Hearts were subsequently digested with 0.1 mg/ml Liberase Blendzymes (Roche Diagnostics, Indianapolis, IN) for \(\sim 10\) min at 37°C. After digestion, left ventricles were removed and minced. Extracellular Ca\(^{2+}\) was added back slowly to 1.25 mM. Mechanical assessment was performed within 8 h of isolation. Myocytes with obvious sarcolemmal blebs or spontaneous contractions were not used for study (16). A cohort of isolated cardiomyocytes from C57 nondiabetic control mice were incubated with resveratrol (50 \(\mu\)M, dissolved in DMSO) at 37°C for 1 h prior to functional assessment and protein extraction. The final concentration of DMSO was \(<0.5\%\), which did not affect myocyte mechanical function.

**Cell shortening/relengthening.** Mechanical properties of murine cardiomyocytes were assessed using a SoftEdge MyoCam system (IonOptix, Milton, MA) (16). In brief, cardiomyocytes were placed in a chamber mounted on the stage of an inverted microscope (Model IX-70; Olympus, Tokyo, Japan) and superfused at 25°C with a buffer containing (in mM) 131 NaCl, 4 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), 10 glucose, and 10 HEPES, pH 7.4. The cells were field stimulated with suprathreshold voltage at a frequency of 0.5 Hz (unless stated otherwise), 3 ms duration, using a pair of platinum wires placed on opposite sides of the chamber connected to an FHC (Brunswick, NE) stimulator. The myocyte being studied was displayed on a computer monitor using an IonOptix MyoCam camera. IonOptix SoftEdge software was used to capture changes in cell length during shortening and relengthening.

**Caspase-3 assay.** Caspase-3 is an enzyme activated during the induction of apoptosis. Caspase-3 activity was determined using the Caspase-3 assay kit according to the manufacturer's instructions (Sigma Chemical, St. Louis, MO) (15). Briefly, 1 ml of phosphate-buffered saline was added to heart tissues. Tissues were homogenized and centrifuged at 10,000 g for 10 min at 4°C. The supernatant was discarded, and pellets were lysed in 100 \(\mu\)l of ice-cold lysis buffer containing HEPES (50 mM), 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (CHAPS; 0.1%), dithiothreitol (DTT; 1 mM), EDTA (0.1 mM), and NP-40 (0.1%), pH 7.4. The assay for caspase-3 activity was performed in a 96-well plate. Each well contained 5 \(\mu\)l of lysate, 85–90 \(\mu\)l of assay buffer (50 mM HEPES, pH 7.4, 0.1% CHAPS, 100 mM NaCl, 10 mM DTT, and 1 mM EDTA), and 20 \(\mu\)l of caspase-3 colorometric substrate Ac-DEVD-pNA. The 96-well plate was incubated at 37°C overnight, during which time caspase-3 in the sample was allowed to cleave the chromophore p-NA from the

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**Fig. 1.** Contractile properties of cardiomyocytes isolated from hearts of C57 control and streptozotocin (STZ)-diabetic groups treated with or without 5-[5-(2-nitrophenyl)furfuryliodine]-1,3-diphenyl-2-thiobarbituric acid (UCF-101) (7.15 mg/kg ip). A: resting cell length. B: peak shortening (normalized to cell length). C: maximal velocity of shortening (+dL/dt). D: maximal velocity of relengthening (–dL/dt). E: time-to-PS relengthening (TPS). F: time-to-90% relengthening (TR\(_{90}\)). Values are means ± SE; \(n = 66–160\) cells/group. *\(P < 0.05\) vs. C57 group; #\(P < 0.05\) vs. STZ group.
substrate molecule. Absorbance readings were obtained at 405 nm. Protein content was determined using the Bradford method (5).

Western blot analysis of XIAP, HtrA2/Omi, AMPK, phosphorylated AMPK, PP2A, and PP2C. Myocardial tissues or isolated murine cardiomyocytes following treatment with resveratrol or UCF-101 were sonicated in a lysis buffer containing (in mM) 20 Tris (pH 7.4), 150 NaCl, 1 EDTA, 1 EGTA, 1% Triton, 0.1% SDS, and protease inhibitor cocktail for protein extraction. Protein levels of XIAP, HtrA2/Omi, AMPK, phosphorylated (p)-AMPK, PP2A, and PP2C were determined using a standard immunoblotting technique (15). Membranes were probed with mouse anti-human IAP-like protein (XIAP) (1:1,000; BD Biosciences, San Jose, CA), rabbit anti-HtrA2/Omi monoclonal (1:1,000; Abcam, Cambridge, MA), rabbit anti-p-AMPK (Thr172, 1:1,000; Cell Signaling Technology, Beverly, MA), rabbit anti-AMPK (1:1,000; Cell Signaling Technology), rabbit anti-PP2A (1:1,000; Cell Signaling Technology), rabbit anti-PP2C (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA), and rabbit anti-α-tubulin (1:1,000 as the internal loading control; Cell Signaling Technology) antibodies. Blots were incubated with horseradish peroxidase-coupled anti-rabbit or anti-mouse secondary antibodies (1:5,000; Cell Signaling Technology). After immunoblotting the film was scanned and detected with a Bio-Rad calibrated densitometer, and the intensity of immunoblot bands was normalized to α-tubulin.

Statistical analysis. Data were presented as means ± SE. Statistical significance (P < 0.05) was determined by analysis of variance followed by Tukey’s post hoc analysis.

RESULTS

Fasting plasma glucose test. Two weeks after STZ injection, the plasma glucose levels were measured in C57 control and STZ-induced diabetic mice with or without UCF-101 treatment. STZ injection significantly elevated the fasting blood levels (390.8 ± 20.5 mg/dl) compared with the C57 controls (80.6 ± 5.9 mg/dl, P < 0.05 vs. STZ group). Although UCF-101 treatment failed to impose any effect in the C57 control group (66.8 ± 6.7 mg/dl), it significantly reduced the fasting plasma glucose levels in diabetic mice (217.8 ± 34.8 mg/dl, P < 0.05 vs. all other groups). These data indicate that UCF-101 treatment is capable of attenuating STZ-induced elevation in the fasting glucose levels.

Baseline cardiomyocyte mechanical properties in STZ and UCF-101-treated mice. Data shown in Fig. 1 indicate that diabetes significantly reduced resting cell length, peak shortening (PS), maximal velocity of shortening/relengthening,
(±dL/dt), and prolonged time-to-PS (TPS) and time-to-90% relengthening (TR90). Although UCF-101 treatment elicited little effect on the mechanical properties in the control group, it ablated STZ-induced changes in cardiomyocyte contractile properties, with the exception of PS. These observations favor a beneficial effect of UCF-101 against cardiomyocyte contractile dysfunction in diabetes.

Expression of AMPK signaling, HtrA2/Omi, and XIAP as well as caspase-3 activity. To determine the potential mechanism(s) involved in UCF-101-elicited cardioprotection in STZ-induced diabetes, expression of the cellular fuel AMPK and its activation (p-AMPK) were examined in control and diabetic mice treated with or without UCF-101. Our data revealed that STZ treatment significantly suppressed AMPK activation without affecting AMPK expression, the effect of which was nullified by UCF-101 treatment. Given the nature of protease inhibition for UCF-101, the apoptosis-related signals such as HtrA2/Omi and XIAP, as well as caspase-3 activity, were also examined. Our data revealed that neither STZ nor UCF-101 significantly altered expression of HtrA2/Omi and XIAP as well as caspase-3 activity. These results suggest that UCF-101 may improve cardiomyocyte contractile function in experimental diabetes through a mechanism(s) associated with AMPK but not HtrA2/Omi or XIAP-related apoptotic pathways (Fig. 2).

Effect of resveratrol on STZ-induced cardiomyocyte contractile dysfunction. To examine the possible role of AMPK signaling in UCF-101- and STZ-induced cardiac contractile response, cardiomyocyte contractile properties were examined in myocytes isolated from control and STZ-diabetic mice incubated with or without the AMPK activator resveratrol (50 μM, 1 h) (6). Data shown in Fig. 3 reveal that resveratrol abolished STZ-induced cardiomyocyte contractile dysfunction in a manner reminiscent of UCF-101. The depressed PS and ±dL/dt as well as the prolonged TPS and TR90 in STZ-treated murine cardiomyocytes were negated by resveratrol. In addition, resveratrol itself did not elicit any overt effect on cardiomyocyte mechanics. Further Western blot analysis confirmed that resveratrol restored STZ-induced decrease in AMPK activation. Neither resveratrol nor STZ treatment affected the expression of AMPK (Fig. 4). This result consolidates the beneficial role of resveratrol.

![Fig. 3](http://ajpendo.physiology.org/)

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Resting Cell Length (μm)</th>
<th>Peak Shortening (% Cell Length)</th>
<th>+dL/dt (μm/s)</th>
<th>-dL/dt (μm/s)</th>
<th>TPS (msec)</th>
<th>TR90 (msec)</th>
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<td>7</td>
<td>150</td>
<td>-120</td>
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<td>150</td>
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<tr>
<td>STZ</td>
<td>100</td>
<td>5</td>
<td>130</td>
<td>-100</td>
<td>100</td>
<td>130</td>
</tr>
<tr>
<td>CON + RSV</td>
<td>115</td>
<td>6</td>
<td>140</td>
<td>-110</td>
<td>115</td>
<td>140</td>
</tr>
<tr>
<td>STZ + RSV</td>
<td>105</td>
<td>5</td>
<td>135</td>
<td>-105</td>
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Values are means ± SE; n = 87–111 cells/group; *P < 0.05 vs. control (CON) group; #P < 0.05 vs. STZ group.
and PP2C negatively regulate AMPK activity through dephosphorylation of AMPK (31, 32), protein expression of PP2A and PP2C was explored. Our result indicated that UCF-101 (4–100 μM) significantly downregulated the levels of both PP2A and PP2C, indicating a likely role of the lessened PP2A and PP2C protein expression in the UCF-101-induced increase in AMPK activity (Fig. 6).

Effect of resveratrol on protein phosphatase expression in control or diabetic cardiomyocytes. To further elucidate the potential mechanism of AMPK signaling in UCF-101-induced protection against diabetic cardiac contractile dysfunction, expression of PP2A and PP2C was examined in cardiomyocytes from control and STZ-diabetic mice incubated with or without the AMPK activator resveratrol (50 μM, 1 h) (6). Data shown in Fig. 7 reveal that expression of both PP2A and PP2C was upregulated in STZ-diabetic cardiomyocytes, the effect of which was abolished (PP2C) or significantly attenuated (PP2A) by resveratrol. Furthermore, resveratrol significantly reduced the protein abundance of both PP2A and PP2C in control cardiomyocytes in a manner reminiscent of UCF-101. This result supports the notion that UCF-101 may exert its cardioprotective benefit in diabetes through the reversal of protein phosphatase-suppressed AMPK signaling in a manner reminiscent of resveratrol.

**DISCUSSION**

The cardiovascular risk associated with diabetes is becoming increasingly evident, accounting for ~80% of mortality in diabetic patients (18). The salient findings of our present study indicate a cardiac protective potential of the candidate drug UCF-101 against experimental diabetes-elicited cardiac contractile dysfunction, with a possible involvement of AMPK. UCF-101 mitigates cardiomyocyte contractile dysfunction and lowered fasting plasma glucose level in diabetes. These effects were associated with UCF-101-induced recovery of diabetes-induced decrease in AMPK phosphorylation. Our data did not favor a role of UCF-101-related apoptotic proteins such as HtrA2/Omi and XIAP or apoptosis in UCF-101-induced cardioprotection. The role of AMPK in UCF-101-elicited protection against diabetic cardiomyocyte dysfunction received further support that resveratrol mimicked UCF-101-induced mechanical response and downregulation of protein phosphatase expression, whereas AMPK knockdown masked STZ-induced changes in cardiomyocyte contractile function.

Our data revealed that cardiomyocytes from diabetic mice exhibited reduced cell length, peak shortening, and depressed maximal velocity of shortening/relengthening as well as prolonged duration of shortening and relengthening. These data are consistent with our previous findings from both chemically induced and genetically predisposed rodent diabetic models (11, 21, 22, 24, 26, 33). UCF-101 itself failed to alter these mechanical properties in the absence of diabetes, whereas it restored almost all STZ-induced mechanical defects with the exception of peak shortening. This finding indicates the therapeutic potential of UCF-101 against cardiac dysfunction in diabetes. The apparent discrepancy in the UCF-101-induced responsiveness in peak shortening and other mechanical indexes may be related to the disparate drug response in various cardiac contractile and intracellular Ca2+-regulating proteins, although further study is warranted. As a specific inhibitor of

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Fig. 4. Protein expression of AMPK (A) and p-AMPK (B) in cardiomyocyte from control and STZ-diabetic groups treated with or without RSV (50 μM for 1 h). A and B, top: representative gel blots of AMPK and p-AMPK using specific antibodies. Values are means ± SE; n = 4–7/group. *P < 0.05 vs. CON group; #P < 0.05 vs. STZ group.
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Fig. 5. Contractile properties of cardiomyocytes from C57 control and AMPK-kinase-dead (AMPK-KD) mice treated with or without STZ (50 mg·kg\(^{-1}\)·day\(^{-1}\) for 5 consecutive days) to elicit experimental diabetes. A: resting cell length. B: peak shortening (normalized to resting cell length). C: +dL/dt. D: −dL/dt. E: TPS. F: TR\(_{90}\). Values are means ± SE; n = 92–135 cells/group. *P < 0.05 vs. C57 group.

HtrA2/Omi, UCF-101 was reported to possess potent cardio-protective properties. HtrA2, a death-promoting mitochondrial serine protease, is released into cytosol from mitochondria in response to pathological stimuli (7). HtrA2 has been shown to serve as an essential element in apoptosis in pathological conditions such as myocardial ischemia-reperfusion injury (2, 17). Following its translocation from mitochondria to the cytosol under pathological conditions, HtrA2 promotes apoptosis via a protease activity-dependent/caspase-mediated pathway. Cytosolic HtrA2 is capable of degrading XIAP, thus favoring caspase activation and apoptosis (3). As an inhibitor of HtrA2, UCF-101 suppresses HtrA2 protease activity to offer an attractive therapeutic action. To our surprise, data from our present study revealed the beneficial effect of UCF-101 in diabetic cardiomyocyte defects independently of any antiapoptotic capacity. UCF-101 treatment in vivo failed to affect the expression of HtrA2/Omi and XIAP or the caspase-3 activity. Our data revealed that neither UCF-101 nor diabetes affects the XIAP protein expression, which seems to favor the absence of XIAP degradation and subsequent apoptosis in our current experimental setting.

AMPK is an essential regulator of energy balance, the activation of which plays a pivotal role in the protection against ischemia-reperfusion injury, diabetes, and metabolic syndrome (20, 28). Our data showed depressed AMPK phosphorylation in diabetes, the effect of which was nullified by UCF-101. To further scrutinize the role of AMPK in the UCF-101- and/or STZ-induced cardiac contractile response, cardiomyocyte contractile function was assessed using both the AMPK activator resveratrol and an AMPK-KD mouse model. Our data showing that resveratrol mimicked UCF-101-induced mechanical response while activating AMPK strongly support the involvement of AMPK in UCF-101-elicited beneficial effect in diabetes. On the other side of the coin, the AMPK-KD mice exhibited a “masking effect” in response to STZ-induced experimental diabetes. AMPK KD-mice carry a dominant negative AMPK\(^{\alpha 2}\) replacing the functional \(\alpha 1\), \(\alpha 2\), and \(\alpha 3\) subunits, resulting in low AMPK activity in muscles and hearts (19, 28). Our results revealed that AMPK knockdown significantly reduced peak shortening and maximal velocity of shortening/relengthening, as well as prolonged TPS and TR\(_{90}\), in a manner reminiscent of
STZ-induced diabetes. Interestingly, STZ treatment failed to impose any further change on the mechanical indexes in AMPK-KD mice. These findings favor the presence of a commonality in cardiac contractile defect between STZ-induced diabetes and AMPK knockdown. AMPK is a heterotrimeric serine/threonine kinase widely recognized as a key regulator of fatty acid and glucose homeostasis (27, 34). AMPK activation suppresses expression of two key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, which in turn inhibits gluconeogenesis (8). AMPK activation is important to the heart function through the activation of energy-generating pathways and inhibition of energy-consuming pathways. AMPK facilitates the energy production via several mechanisms, such as accelerated glucose uptake, increased fatty acid uptake and oxidation, and enhanced glycolysis (10). In our hand, UCF-101 antagonized STZ-induced hyperglycemia, in line with a preserved AMPK activity and possibly improved glucose uptake. Our further study revealed that UCF-101 did not directly stimulate AMPK phosphorylation but rather reduced the protein phosphatases PP2A and PP2C in a manner similar to the AMPK activator resveratrol. Both phosphatases are known to directly dephosphorylate and thus inactivate AMPK phosphorylation in mammals (31, 32). It is thus possible that the UCF-101-elicited effects on AMPK phosphorylation and cardiomyocyte mechanics represent a secondary action to the protein phosphatase of the drug. This is supported by our present observation of the resveratrol-induced antagonism of diabetes-triggered upregulation in PP2A and PP2C. In our study, it is somewhat surprising that resveratrol failed to stimulate AMPK phosphorylation in the control group, whereas the polyphenol promoted AMPK phosphorylation in diabetic cardiomyocytes. Although no precise explanation may be offered at this point, a possible time-dependent response may contribute to the apparent absence of direct AMPK activation for resveratrol in our current experimental setting. Resveratrol is well known to increase phosphorylation of AMPK and its downstream target acetyl-CoA carboxylase (35). On the other hand, resveratrol may protect against cell death through its antioxidant property (23), although regulation of oxidative stress may eventually lead to regulation of the AMPK kinase circuit (9). In an earlier study, it was shown that resveratrol at concentrations used in the current study requires 8 h (1 h for our current study) to facilitate glucose uptake, a downstream effector for AMPK (23). Those authors found that resveratrol promoted AMPK phosphorylation following a 2-wk in vivo treatment (23). Thus, it is possible that resveratrol promotes AMPK phosphorylation indirectly through inhibition of protein phosphatase acutely, whereas it facilitates AMPK activation in a more chronic mode. Occurrence of diabetes may have shifted the lipid/glucose metabolism to allow a quick response for resveratrol. Further experiments are warranted to examine whether such a scenario holds for resveratrol and UCF-101 in both normal and diabetic conditions.

In conclusion, our present study revealed that UCF-101 mitigates STZ-induced diabetic cardiomyocyte contractile dysfunction and hyperglycemia, possibly associated with an AMPK-dependent mechanism rather than antiapoptosis. Through its central role in the regulation of glucose and lipid homeostasis, AMPK may be a critical therapeutic target for diabetes treatment.

Fig. 6. Protein expression of AMPK (A), p-AMPK (B), protein phosphatase 2A (PP2A; C), and protein phosphatase 2C (PP2C; D) in cardiomyocytes from C57 control mice exposed to different concentrations of UCF-101 for 1 h. A–D, top: representative gel blots depicting AMPK, p-AMPK, PP2A, and PP2C using specific antibodies. Values are means ± SE; n = 4–8/group, *P < 0.05 vs. control (0 concentration).
metabolism, AMPK may serve as a novel molecular target for the treatment of diabetes and diabetic complications. Further work should focus on the molecular mechanism of the protease inhibitor UCF-101 in the therapeutics of diabetes and its strategic application in the management of diabetes mellitus and, more broadly, cardiovascular diseases.

GRANTS

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