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DOI : 10.1074/jbc.M114.632547
PMID : 25548283
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Received for publication, December 15, 2014. Published, JBC Papers in Press, December 30, 2014,DOI 10.1074/jbc.M114.632547

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Background: Mitochondrial Ca\(^{2+}\) uptake affects energy metabolism and insulin secretion. Knockdown of mitochondrial Ca\(^{2+}\) unipporter (MCU) mediates Ca\(^{2+}\) uptake into the organelle, where energy metabolism is further stimulated for sustained second phase insulin secretion. Here, we have studied the contribution of the MCU to the regulation of oxidative phosphorylation and metabolism-secretion coupling in intact and permeabilized clonal β-cells as well as rat pancreatic islets. Knockdown of MCU with siRNA transfection blunted matrix Ca\(^{2+}\) rises, decreased nutrient-stimulated ATP production as well as insulin secretion. Furthermore, MCU knockdown lowered the expression of respiratory chain complexes, mitochondrial metabolic activity, and oxygen consumption. The pH gradient formed across the inner mitochondrial membrane following nutrient stimulation was markedly lowered in MCU-silenced cells. In contrast, nutrient-induced hyperpolarization of the electrical gradient was not altered. In permeabilized cells, knockdown of MCU ablated matrix acidification in response to extramitochondrial Ca\(^{2+}\). Suppression of the putative Ca\(^{2+}/H^+\) antiporter leucine zipper-EF hand-containing transmembrane protein 1 (LETM1) also abolished Ca\(^{2+}\)-induced matrix acidification. These results demonstrate that MCU-mediated Ca\(^{2+}\) uptake is essential to establish a nutrient-induced mitochondrial pH gradient which is critical for sustained ATP synthesis and metabolism-secretion coupling in insulin-releasing cells.

Results: Knockdown of mitochondrial Ca\(^{2+}\) unipporter decreases respiratory chain activity and mitochondrial pH gradient generation.

Conclusion: Mitochondrial Ca\(^{2+}\) uptake via unipporter is essential for oxidative phosphorylation and metabolism-secretion coupling.

Significance: The present study identifies mechanisms of action and bioenergetic consequences of mitochondrial Ca\(^{2+}\) transporters in insulin-releasing cells.

In pancreatic β-cells, ATP acts as a signaling molecule initiating plasma membrane electrical activity linked to Ca\(^{2+}\) influx, which triggers insulin exocytosis. The mitochondrial Ca\(^{2+}\) unipporter (MCU) mediates Ca\(^{2+}\) uptake into the organelle, where energy metabolism is further stimulated for sustained second phase insulin secretion. Here, we have studied the contribution of the MCU to the regulation of oxidative phosphorylation and metabolism-secretion coupling in intact and permeabilized clonal β-cells as well as rat pancreatic islets. Knockdown of MCU with siRNA transfection blunted matrix Ca\(^{2+}\) rises, decreased nutrient-stimulated ATP production as well as insulin secretion. Furthermore, MCU knockdown lowered the expression of respiratory chain complexes, mitochondrial metabolic activity, and oxygen consumption. The pH gradient formed across the inner mitochondrial membrane following nutrient stimulation was markedly lowered in MCU-silenced cells. In contrast, nutrient-induced hyperpolarization of the electrical gradient was not altered. In permeabilized cells, knockdown of MCU ablated matrix acidification in response to extramitochondrial Ca\(^{2+}\). Suppression of the putative Ca\(^{2+}/H^+\) antiporter leucine zipper-EF hand-containing transmembrane protein 1 (LETM1) also abolished Ca\(^{2+}\)-induced matrix acidification. These results demonstrate that MCU-mediated Ca\(^{2+}\) uptake is essential to establish a nutrient-induced mitochondrial pH gradient which is critical for sustained ATP synthesis and metabolism-secretion coupling in insulin-releasing cells.

Pancreatic β-cells maintain blood glucose homeostasis by adapting insulin secretion to the changes in circulating nutrients. A major signaling molecule in this metabolism-secretion coupling linking nutrient metabolism to insulin secretion is cytosolic ATP most of which is synthesized from oxidative phosphorylation. Mitochondrial ATP synthesis is driven by the electrical (\(\Delta\Psi_{\text{mito}}\), membrane potential) and chemical (\(\Delta p_{\text{H}_{\text{mito}}}\)) gradients across the mitochondrial inner membrane. These gradients are established as a result of electron transport and the associated export of protons mediated by the respiratory chain. Reducing equivalents in mitochondrial matrix are mainly produced by the tricarboxylic acid (TCA) cycle and mitochondrial metabolite shuttles. Thus, the metabolic status of the β-cell mitochondria critically controls ATP synthesis and insulin secretory activity (1). Accumulating evidence suggests that defective mitochondrial function results in impaired glucose-stimulated insulin secretion (GSIS) and may contribute to the development of type 2 diabetes (2–5).

The matrix Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_{\text{mito}}\)]) is a key activator of mitochondrial metabolic function (1, 6, 7). The [Ca\(^{2+}\)\(_{\text{mito}}\)] activates several matrix enzymes including α-ketoglutarate dehydrogenase in the TCA cycle (8). The ATP synthase is also directly activated by a rise in [Ca\(^{2+}\)\(_{\text{mito}}\)] (9). In pancreatic β-cells [Ca\(^{2+}\)\(_{\text{mito}}\)] is strictly required for ATP synthase-dependent respiration stimulated by glucose (10). Given its importance, mitochondrial Ca\(^{2+}\) uptake has been a research focus for...
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decades, starting with the functional characterization in isolated mitochondria. Nevertheless, it took 50 years to elucidate the molecular identity of the mitochondrial Ca\(^{2+}\)-uniporter (MCU) (11, 12). Mitochondrial Ca\(^{2+}\) uptake through MCU is regulated by a number of recently discovered proteins, including mitochondrial Ca\(^{2+}\) uptake 1 and 2 (MICU1/2) (13–15), mitochondrial Ca\(^{2+}\) uniporter regulator 1 (MCUR1) (16), and essential MCU regulator (EMRE) (17). Especially MICU1/2 negatively regulate MCU activity under resting cytosolic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{cyt}\) ) levels decreasing MCU activity, implying that the regulatory subunits of the MCU complex modulate mitochondrial Ca\(^{2+}\) loads of \(\Delta \Psi_{mito}\)-driven Ca\(^{2+}\) uptake without perturbing the important signal propagation from ER to mitochondria (13, 18, 19).

Mitochondrial Ca\(^{2+}\) homeostasis is maintained by balanced Ca\(^{2+}\) influx and efflux. Mitochondrial Ca\(^{2+}\) export is mediated by antiporters exchanging Ca\(^{2+}\) for Na\(^{+}\) or K\(^{+}\) (20). Two mitochondrial antiporters promoting Ca\(^{2+}\) efflux have been identified: The leucine zipper-EF hand-containing transmembrane protein 1 (LETM1) and the mitochondrial sodium calcium exchanger (NCLX). LETM1, which is defective in Wolfram syndrome, works as a K\(^{+}\)/H\(^{+}\) exchanger in yeast mitochondria (21) or mammalian ER (22). LETM1 was also shown to mediate Ca\(^{2+}\)/H\(^{+}\) exchange in mitochondria with a [Ca\(^{2+}\)]\(_{mito}\)-dependent biphasic mode (23). NCLX was confirmed as an electrogenic Na\(^{+}\)/Ca\(^{2+}\) antiporter (exchanging 3 or 4 Na\(^{+}\) per Ca\(^{2+}\) ) (24). Inhibition of NCLX in pancreatic \(\beta\)-cells increases [Ca\(^{2+}\)]\(_{mito}\) accelerates mitochondrial oxidative metabolism and GSIS (25–28).

In addition to [Ca\(^{2+}\)]\(_{mito}\) the matrix pH has been identified as a regulator of mitochondrial energy metabolism in \(\beta\)-cells. In contrast to other cell types, pancreatic \(\beta\)-cells have acidic pH\(_{mito}\) under resting conditions. Nutrient stimulation causes matrix alkalization without any marked cytosolic pH change (29). Preventing the resulting nutrient-induced increase of the \(\Delta \Psi_{mito}\)-changes using ionophores abrogated proton-coupled mitochondrial ion/metabolite transport, ATP synthesis, and GSIS regardless of elevated \(\Delta \Psi_{mito}\) (29–31). Therefore, pathogenic conditions causing a reduction of \(\Delta \Psi_{mito}\) may seriously deteriorate ATP generation and insulin secretion in pancreatic \(\beta\)-cells.

Several recent reports demonstrate the functional role of MCU in pancreatic \(\beta\)-cells (26, 32). MCU mediates glucose-stimulated [Ca\(^{2+}\)]\(_{mito}\) rise and second phase ATP/ADP increase (26). Knockdown of either MCU or MICU1 diminishes insulin secretion associated with defects in mitochondrial Ca\(^{2+}\) uptake (32). Mice lacking MCU show a significant reduction of [Ca\(^{2+}\)]\(_{mito}\) and Ca\(^{2+}\)-stimulated oxygen consumption in muscle mitochondria, without changes in the basal respiration in embryonic fibroblasts (33). It remains unclear, however, how reduced MCU activity attenuates mitochondrial signal generation in pancreatic \(\beta\)-cell metabolism-secretion coupling. In this study, we observed that reduced mitochondrial Ca\(^{2+}\) uptake following silencing of MCU significantly attenuated respiratory chain activity and \(\Delta \Psi_{mito}\) increase in permeabilized as well as in intact insulin-secreting cells. These defects lead to impaired ATP synthesis and insulin secretion, demonstrating the crucial role of mitochondrial Ca\(^{2+}\) uptake for the establishment of the \(\Delta \Psi_{mito}\) in metabolism-secretion coupling. We also provide evidence for a novel role of the putative Ca\(^{2+}\)/H\(^{+}\) antiporter leucine zipper-EF-hand-containing transmembrane protein 1 (LETM1) as a Ca\(^{2+}\) efflux route in insulin secreting cells, the role of which is altered in the absence of MCU.

EXPERIMENTAL PROCEDURES

Cell Culture and Drugs—Rat insulinoma INS-1E cells were cultured in a humidified atmosphere (37 °C) containing 5% CO\(_{2}\) in a complete medium composed of RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% FBS (Invitrogen), 1 mm sodium pyruvate, 50 \(\mu\)M 2-mercaptoethanol, 2 mm glutamine, 10 mm HEPES, 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin (HyClone, Thermo Fisher Scientific Inc., Lafayette, CO). Experiments were performed with cells of passage number 80–120. Most chemicals were purchased from Sigma except JC-1 from Molecular Probes (Eugene, OR).

Pancreatic islets were isolated from 200–300-g male Sprague-Dawley rats ( Orient Bio, Seongnam, Korea) by collagenase (Sigma) digestion (29) and dispersed by a brief incubation with trypsin (Invitrogen). Islet cells were seeded on multiwell-plates coated with 804G matrix and cultured in RPMI 1640 medium supplemented with 10% FBS, 10 mm HEPES, 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin (7).

Permeabilization with \(\alpha\)-Hemolysin Toxin—INS-1E cells were seeded and cultured onto well-plates or coverslips coated with 804G matrix. Cells were washed with Ca\(^{2+}\)-free Krebs-Ringer bicarbonate (KRB) solution (mm; 140 NaCl, 3.6 KCl, 0.5 NaH\(_{2}\)PO\(_{4}\), 0.5 MgSO\(_{4}\), 1.5 CaCl\(_{2}\), 10 HEPES, 2 NaHCO\(_{3}\), 5.5 glucose, pH 7.4 titrated with NaOH) and then incubated for 10 min at 37 °C with 1 \(\mu\)g/ml of Staphylococcus aureus \(\alpha\)-hemolysin toxin (Sigma) in an intracellular buffer (mm; 140 KCl, 5 NaCl, 7 MgSO\(_{4}\), 1 KH\(_{2}\)PO\(_{4}\), 20 HEPES, 10.2 EGTA, 1.65 CaCl\(_{2}\), 0.1 ATP, pH 7.0 with KOH), which has about 120 nm of free Ca\(^{2+}\) concentration. After \(\alpha\)-toxin permeabilization, cells were washed once with 0.5% bovine serum albumin (BSA) containing intracellular buffer and used for experiments (29).

siRNA Transfection—Cells were transfected with non-targeting or target-specific small interfering RNA (siRNA) using DharmaFECT1 (Dharmacon, Thermo Fisher Scientific Inc.). The target-specific siRNAs for rat MCU and LETM1 were purchased from Dharmacon, which is composed of siRNAs for four different targets of each gene (SMART pool, Dharmacon).

Quantitative Real-time PCR—Total RNA was isolated from cells 72 h after siRNA transfection using the RNasy kit (Cat. 74134, Qiagen GmbH, Hilden, Germany). First strand cDNA was synthesized from 1 \(\mu\)g of total RNA with a reverse transcription kit (Applied Bioscience, Foster City, CA) using oligo-dT. Quantitative PCR was performed using sequence-specific primers for rat MCU (forward: 5'-GAAGTGGATTGGCAGGTTCCA-3', reverse: 5'-AGGAAAGCGGAGAAGGAC-3'), and LETM1 (forward: 5'-GGCTGACCTTGCACCTGATT-3', reverse: 5'-CAGGTTGACCTTCAGTGGT-3'). Rat \(\beta\)-actin was used as the reference control. For the analysis of each gene expression, experiments were conducted in a triplicate in the real-time PCR system (7900HT, Applied Bioscience) using SYBR Green PCR Master Mix (Cat. 204143, Qiagen GmbH). Data were analyzed following \(\Delta \Delta C_{T}\) method (34).

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Western Blot—The protein level of MCU or LETM1 was determined in a cell extract using Western blotting as described previously (31). Primary antibodies for MCU (1:1000, Cat. HPA016480, Sigma), LETM1 (1:500, Cat. sc134672, Santa Cruz Biotechnology, Dallas, TX), complex I NDUFA9 (1:2000 dilution, Cat. 459100, Invitrogen, Carlsbad, CA), complex III UQCR2 (1:2500, Cat. MS304, Mitosciences, Abcam), complex IV subunit I (1:1000, Cat. 459600, Invitrogen), complex V (1:5000, Cat. MS604-300, Mitosciences, Abcam), TOM20 (1:1000, Cat. sc11415, Santa Cruz Biotechnology), and β-actin (1:5000, Cat. ab6276, Abcam, Cambridge, UK) were used. Horseradish peroxydase (HRP)-conjugated secondary antibody against either mouse or rabbit IgG (Cat. 31450 and 31460, Thermo Scientific) was incubated for 1 h at room temperature. The bands were visualized with a UVP Biospectrum-600 imaging system using enhanced chemiluminescence (ECL) solution (Luminata Forte, Millipore).

Measurement of Mitochondrial Matrix Ca$^{2+}$—To measure mitochondrial matrix Ca$^{2+}$ level, we used a mitochondria-targeted ratio-pericam (RPmit) plasmid, generously provided by Prof. Roger Tsien (UC San Diego). Cells were transfected with siRNA, and 24 h after, transfected with RPmit using X-tremeGENE (Roche Diagnostics GmbH, Mannheim, Germany). Fluorescence imaging of Ca$^{2+}$ was performed by using an inverted microscope (IX-81, Olympus, Tokyo, Japan) with an array laser confocal spinning disk (CSU10, Yokogawa Electric Corporation, Tokyo, Japan) and a cooled charge-coupled device (CCD) camera (Cascade 512B, Photometrics, Tucson, AZ). Intact or permeabilized cells on the confocal microscope were perfused with KRB solution or intracellular buffer, respectively, and fluorescence images (435 nm excitation and 535 nm emission) were acquired every 10 s and analyzed using Metafluor 6.3 software (Universal Imaging, Molecular Devices).

Measurement of Cytosolic ATP and Insulin—Cells plated onto 48 well-plates (2 × 10$^5$ cells/well) were permeabilized with α-toxin and incubated for 5 or 15 min in an intracellular buffer containing ADP (10 μM) with or without succinate (3 mM). To determine mitochondrial ATP release, the supernatant was harvested after incubation, and ATP level was measured by using the microplate reader (Synergy$^\text{TM}$2, BioTek Instruments Inc., Winooski, VT) with a bioluminescence assay kit (HS II, Roche Diagnostics, Mannheim, Germany).

For static insulin secretion measurement, cells in a 804G-coated 24 well-plate (1.5 × 10$^5$ cells/well) were transfected with siRNA and grown for 72 h. After deprivation of glucose for 1 h, cells were preincubated for 30 min with a KRB solution containing 2.8 mM glucose and 0.1% BSA. Then, cells were washed and incubated for 30 min with 2.8 mM or 16.7 mM glucose-containing KRB solution. Supernatant was collected for estimation of insulin release. Cellular insulin content was determined in acid-ethanol extracts. Insulin levels were measured by using an insulin ELISA kit (Shibayagi Co., Gunma, Japan).

Mitochondrial Enzyme Activity and MTT Assay—For cytochrome c oxidase (COX) activity measurement, INS-1E cells were permeabilized by freeze-thaw cycle three times and mixed with isotonic solution (10 mM KH$_2$PO$_4$, 250 mM sucrose, 0.1% BSA, pH 6.5) with detergent (laurylmalatoside, 2.5 mM). Traces were started with the addition of reduced cytochrome c (25 mM with a tiny amount of sodium hydrosulfite) and the enzymatic activity of COX was estimated by measuring absorbance at 550 nm continuously with a spectrophotometer (Amersham Biosciences, GE Healthcare Biosciences, Pittsburgh, PA). COX activity was expressed as moles of oxidized cytochrome c per min. Citrate synthase activity was measured with citrate synthase assay kit (Sigma) based on the manufacturer’s instructions.

For the MTT assay, siRNA-transfected cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (50 μg/well) for 2 h and then treated with dimethyl sulfoxide (100 μl/well). The absorbance (A$_{570}$ − A$_{630}$) of each well was measured by using a microplate reader ( Molecular Devices, Sunnyvale, CA).

Measurement of Oxygen Consumption—Cellular oxygen consumption rate (OCR) was determined by Extracellular Flux Analyzer (XF-24, Seahorse Bioscience, North Billerica, MA). Cells (2 × 10$^4$ cells/well) seeded on 24-well plates (Seahorse Bioscience) were transfected with siRNA and cultured for 72 h. On the experiment day, cells were incubated for 1 h at 37°C with KRB solution containing 2.8 mM glucose prior to 20 min of basal OCR measurement. Then, glucose (16.7 mM), oligomycin (3 μg/ml), FCCP (3 μM), and antimycin A (3 μM) were added consecutively and the changes in OCR were recorded.

Measurement of Mitochondrial Matrix pH—Mitochondrial matrix pH (pH$_{mito}$) was measured using adenovirus expressing mtAlpHi (Ad-tetON-mAlpHi) as described previously (29). Intact or permeabilized cells on the confocal microscope were perfused with KRB solution (pH 7.4) or intracellular buffer (pH 7.0), respectively, and the fluorescence signals (488 nm excitation and 535 nm emission) were recorded. Titration of the mitochondrial pH was performed by clamping the matrix pH with high K$^+$ buffer (mM) (125 KCl, 5 NaCl, 1 NaH$_2$PO$_4$, 1 MgSO$_4$, 10 HEPES) of defined pH containing the ionophores nigericin (5 μM) and monensin (5 μM) (31).

Measurement of Mitochondrial Membrane Potential—To measure the mitochondrial membrane potential (ΔΨ$_{mito}$), cells seeded onto black-walled 96-well plates (5 × 10$^4$ cells/well) were loaded with JC-1 (500 nM, Invitrogen) for 30 min and then permeabilized with α-toxin. The ratio of red (540 nm excitation and 590 nm emission) over green (490 nm excitation and 540 nm emission) fluorescence intensity was monitored from permeabilized cells in the presence of intracellular buffer containing JC-1 (500 nM) using a multi-well fluorescence reader (FlexStation, Molecular Devices) (35).

As an alternative method to measure the mitochondrial membrane potential, cells seeded on coverslips were loaded with 5 nM TMRM for 20 min, and perfused with KRB solution containing TMRM (5 nM) on the inverted microscope. Fluorescence imaging with 514 nm excitation and 560 nm emission were recorded with the array laser confocal spinning disk microscopic system and analyzed by using Metamorph 6.1 software.

Statistical Analysis—Values are presented as mean ± S.E. and N is the number of independent experiments. p values were obtained by Student’s t test or one-way ANOVA and < 0.05 was considered to be significant.
RESULTS

Effects of MCU Knockdown on Mitochondrial Ca\(^{2+}\) Uptake—
To understand the role of mitochondrial Ca\(^{2+}\) transport in metabolism-secretion coupling, we transfected non-targeting siRNA (siControl) or siRNA selectively targeted to MCU (siMCU) in INS-1E cells, and assessed the effect of silencing after 72 h using quantitative real-time PCR and Western blotting. Application of siMCU efficiently reduced the transcript levels of MCU (73.8 ± 5.3% reduction, Fig. 1A) compared with siControl-treated cells. Western blot analysis also revealed a strong siRNA-induced reduction of the MCU protein by 82.3 ± 2.3% (Fig. 1, B and C).

To examine the impact of MCU knockdown on mitochondrial Ca\(^{2+}\) uptake, we determined the effect of extramitochondrial Ca\(^{2+}\) ([Ca\(^{2+}\)\(_{\text{o}}\)]\(_{\text{mito}}\)) on [Ca\(^{2+}\)\(_{\text{mito}}\]) in \(\alpha\)-toxin-permeabilized INS-1E cells expressing mitochondria-targeted ratiopericam (RPmit). When the cells were perfused with intracellular buffer containing ~10 nM Ca\(^{2+}\) and 3 mM succinate, switching [Ca\(^{2+}\)\(_{\text{mito}}\)] to 120 nm gradually increased [Ca\(^{2+}\)\(_{\text{mito}}\)]\(_{\text{mito}}\) (Fig. 1D). The [Ca\(^{2+}\)\(_{\text{mito}}\)]\(_{\text{mito}}\) declined slowly after returning to 10 nM [Ca\(^{2+}\)\(_{\text{mito}}\)]\(_{\text{mito}}\).

Effect of MCU Knockdown on ATP Synthesis and Insulin Secretion—It is well known that [Ca\(^{2+}\)\(_{\text{mito}}\)]\(_{\text{mito}}\) amplifies metabolism-secretion coupling in \(\beta\)-cells and reduction of [Ca\(^{2+}\)\(_{\text{mito}}\)]\(_{\text{mito}}\) inhibits GSIS (6, 7). To further investigate the role of [Ca\(^{2+}\)\(_{\text{mito}}\)]\(_{\text{mito}}\) in energy metabolism we studied succinate-dependent ATP synthesis in permeabilized cells. In the absence of substrate, silencing of MCU did not affect basal ATP formation (Fig. 1F). However, time-dependent ATP synthesis stimulated by succinate was markedly lowered in siMCU-treated cells. Consistent with the role of ATP as a signaling molecule for insulin exocytosis, GSIS in intact INS-1E cells was also dramatically decreased in MCU knockdown cells (76.3% inhibition, Fig. 1G). The role of MCU in metabolism-secretion coupling in pancreatic \(\beta\)-cells, was assessed following siMCU transfection of isolated rat pancreatic islets. Successful silencing of MCU in dispersed islet cells (Fig. 1H), resulted in significantly attenuated GSIS (Fig. 1I). Our data emphasize the importance of MCU-dependent mitochondrial Ca\(^{2+}\) uptake in metabolism-secretion coupling of pancreatic \(\beta\)-cells.

Effects of MCU Knockdown on Mitochondrial Respiratory Function—Mitochondrial ATP synthesis by the F\(\text{F}_0\)-ATPase (complex V) is driven by the proton electrochemical gradient across the inner mitochondrial membrane, which is generated by the proton pumping activity of the respiratory complexes I, III, and IV. Protein expression of selected subunits of complex I, III, IV, and V was examined using Western blot analysis. MCU knockdown markedly reduced complex I (NDUFA9, nuclear DNA-en-
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Knockdown of MCU decreased respiratory chain protein levels and mitochondrial enzyme activities. A and B, total cellular protein was extracted 72 h after transfection with non-targeting siRNA (siControl) or siRNA against MCU (siMCU). A, Western blots and B, densitometric analyses for respiratory chain complex I, III, IV, V, and TOM20 (n = 4–8). C–G, MTT intensities (n = 7) (C), COX activity (n = 6) (D and E), and citrate synthase activity (n = 8) (F and G) were measured 72 h after siRNA transfection and compared between siControl- and siMCU-treated cells. *, **, and *** denote p < 0.05, < 0.01, and < 0.001, respectively.

FIGURE 2. Knockdown of MCU decreased respiratory chain protein levels and mitochondrial enzyme activities. A and B, total cellular protein was extracted 72 h after transfection with non-targeting siRNA (siControl) or siRNA against MCU (siMCU). A, Western blots and B, densitometric analyses for respiratory chain complex I, III, IV, V, and TOM20 (n = 4–8). C–G, MTT intensities (n = 7) (C), COX activity (n = 6) (D and E), and citrate synthase activity (n = 8) (F and G) were measured 72 h after siRNA transfection and compared between siControl- and siMCU-treated cells. *, **, and *** denote p < 0.05, < 0.01, and < 0.001, respectively.

coded, −47.9%), complex III (UQCRC2, nuclear DNA-encoded, −47.5%), complex IV (subunit I, mitochondrial DNA-encoded, −67.1%), and complex V (ATP5A, nuclear DNA-encoded, −29%) (Fig. 2, A and B). Consistently, the enzyme activity of Complex IV was reduced by 16.6% after MCU knockdown (Fig. 2, D and E). Other mitochondrial functions such as citrate synthase activity (TCA cycle) or the TOM20 protein expression (subunit of mitochondrial protein import) were not altered when lowering MCU (Fig. 2, F and G) (Fig. 2, A and B). These results demonstrate that lowering mitochondrial calcium uptake selectively affect the expression of respiratory chain complexes.

Knockdown of MCU causes neither cell loss nor alteration in total soluble proteins (data not shown). Given these findings, we used the MTT assay as a read-out of mitochondrial reductive activity (36). Using an identical number of cells, silencing of MCU lowered the ability of cells to reduce MTT by 15% (Fig. 2C).

Finally, we investigated the effect of MCU knockdown on OCR. Under basal conditions (2.8 mM glucose), the OCR was modestly reduced in MCU knockdown cells (19.3% inhibition; Fig. 3, A and B). The OCR induced by high glucose (16.7 mM) was strongly impaired following MCU knockdown (35.0% inhibition; Fig. 3, A and C), suggesting that the activation of mitochondrial respiration is highly dependent on MCU activity. Taken together, in insulin-secreting cells, mitochondrial Ca2+ uptake via MCU is necessary for mitochondrial functions at multiple levels from nutrient oxidation to ATP synthesis.

Nigericin-induced Mitochondrial Hyperpolarization Was Lowered in MCU-silenced Cells—The mitochondrial electrical gradient (\( \Delta \Psi_{\text{mito}} \)) is the main driving force for ATP synthesis as well as Ca2+ transport through the MCU. MCU knockdown in turn may alter the \( \Delta \Psi_{\text{mito}} \). We therefore measured the ratio of fluorescence intensities (red/green) after loading with JC-1, which reflects the \( \Delta \Psi_{\text{mito}} \) (35). The hyperpolarizing response to succinate in permeabilized control and MCU knockdown cells, was not significantly different (Fig. 4A). Glucose-induced hyperpolarization in intact cells was also not different between the two groups, which was measured by using the fluorescence probe TMRM in a non-quenching redistribution mode to measure \( \Delta \Psi_{\text{mito}} \) (on the figure it says JC-1 not TMRM). We also confirmed these findings by using JC-1 dye (data not shown), showing that silencing of MCU does not significantly alter \( \Delta \Psi_{\text{mito}} \) in insulin-secreting cells.

The K+/H+ electroneutral ionophore nigericin, dissipates the \( \Delta p \text{H}_{\text{mito}} \) across the inner mitochondrial membrane. This results in a compensatory elevation of \( \Delta \Psi_{\text{mito}} \) in order to maintain the total proton motive force (30). Therefore, hyperpolarization of \( \Delta \Psi_{\text{mito}} \) by nigericin is proportional to the \( p \text{H} \) gradient prior to the addition of the ionophore. Interestingly, hyperpolarization by nigericin was markedly decreased (53.0% inhibition) following knockdown of MCU in permeabilized INS-1E cells (Fig. 4, C and D). This result suggests that MCU-silenced cells have defects in the establishment of a \( \Delta \text{pH}_{\text{mito}} \) gradient but not \( \Delta \Psi_{\text{mito}} \) in response to nutrients.
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Knockdown of MCU Reduced Basal and Glucose-Stimulated Oxygen Consumption. INS-1E cells (2 × 10⁴ cells/well) were seeded onto 24-well plates and transfected with non-targeting siRNA (siControl) or siRNA against MCU (siMCU). After 72 h, culture medium was replaced with 2.8 mM glucose KR8 solution and incubated for 1 h at 37 °C prior to measurement of basal OCR for 20 min. Subsequently, glucose (16.7 mM) was applied to the incubation solution, followed by the addition of mitochondrial inhibitors; oligomycin (3 μg/ml), FCCP (3 μM), and antimycin A (3 μM). The levels of basal (B) and glucose-stimulated OCR (C) were compared between control and MCU-silenced cells. Values of OCR are presented as pmol of consumed oxygen per min per μg protein, n = 14, * and ** denote p < 0.05 and < 0.01, respectively.

Knockdown of MCU Did Not Affect Mitochondrial Membrane Potential but Decreased the Hyperpolarizing Response Induced by Dissipating the pH Gradient with Ionophore. A, mitochondrial membrane potential (Ψmito) in α-toxin-permeabilized INS-1E cells was measured in the presence of JC-1, a potential-sensitive fluorescence dye (500 nM). Increased JC-1 ratio (red/green) reflects hyperpolarization of the Ψmito. B, intact cells were loaded and per fused with another potential-sensitive fluorescence dye, TMRM (5 nM), and the Ψmito was measured in a nonquenching redistribution mode. Hyperpolarization of the Ψmito by succinate (3 mM) in permeabilized cells (n = 15) (A) and by high glucose (16.7 mM) in intact cells (n = 6) (B) were compared between control and MCU-silenced cells. In α-toxin-permeabilized cells, nigericin (500 μM)-induced hyperpolarization of the Ψmito as compensation for the collapsed mitochondrial pH gradient (ΔpHmito) was compared between control and MCU-silenced cells (n = 6) (C and D). * denotes p < 0.05.

Knockdown of MCU Impaired Nutrient-generated pH Gradient. The ability of β-cells to elevate their ΔpHmito following glucose stimulation is important for mitochondrial energy metabolism and thereby metabolism-secretion coupling (1). To directly assess the effect of MCU knockdown on pHmito, we expressed the mitochondria-targeted pH-sensitive protein mtAlpHi in siMCU-treated INS-1E cells. As shown in Fig. 5, A and C, succinate-induced alkalization of pHmito in MCU-silenced cells was blunted compared with control cells (44.7% reduction). In intact MCU-silenced cells, glucose-induced matrix alkalinization was also strongly decreased (52.4% inhibition; Fig. 5, B and E). These results show that suppression of MCU-dependent mitochondrial Ca²⁺ uptake also prevents the establishment of the nutrient-generated ΔpHmito.

Addition of extramitochondrial Ca²⁺ to succinate-stimulated mitochondria resulted in mitochondrial matrix acidification in permeabilized control cells. Interestingly, Ca²⁺-induced matrix acidification was not observed in MCU knockdown cells, indicating that this acidification is a secondary consequence of MCU-mediated Ca²⁺ uptake (Fig. 5, A and D). Ca²⁺ loading via MCU may be followed by Ca²⁺ efflux in exchange for H⁺ in energized mitochondria causing the observed net acidification.

Acute Blocking of MCU Did Not Affect Metabolism-secretion Coupling—Gene silencing with siMCU transfection reduces the protein expression of MCU slowly over a time-course of several days. In order to understand the acute effects of blocking mitochondrial calcium import, we performed insulin measurement with the specific MCU blocker, Ru360. Pretreatment with Ru360 did not affect glucose-stimulated insulin secretion (Fig. 6A). MCU is a selective Ca²⁺ channel mediating inward current and this Ca²⁺ influx through MCU depolarizes the ΔΨmito. We measured the effect of Ru360 on ΔΨmito in intact cell during high glucose stimulation. As shown in Fig. 6, B and C, Ru360 further hyperpolarized the ΔΨmito by blocking MCU-mediated inward depolarizing currents. These results also confirm the effectiveness of Ru360 in intact cells. Taken together, long term reduction of mitochondrial Ca²⁺ uptake leads to down-regulation of mitochondrial bioenergetics and metabolism-secretion coupling, which is not reproduced by acute pharmacological blocking of MCU.

LETM1 Participated as a Mitochondrial Ca²⁺-H⁺ Antiporter in INS-1E Cells—We hypothesized that the Ca²⁺/H⁺ antiporter LETM1 may be required for the observed matrix acidification triggered by Ca²⁺ (23). To elucidate the role of LETM1 on Ca²⁺-coupled pH regulation, the change in pHmito upon extramitochondrial addition of Ca²⁺ was measured in control or LETM1-silenced INS-1E cells. After 72 h of siRNA treatment, the knockdown effect was evaluated by using quantitative real-time PCR and Western blotting. Application of siLETM1 efficiently reduced the transcript (78.0 ± 5.7% reduc-
tion, Fig. 7A) and LETM1 protein (78.9 ± 0.9% reduction, Fig. 7, B and C) compared with siControl-treated cells. In α-toxin-permeabilized LETM1 knockdown cells, succinate-induced matrix alkalinization was not significantly altered (Fig. 7, D and E). Moreover, the increase in $[\text{Ca}^{2+}]_{\text{mito}}$ by extramitochondrial $\text{Ca}^{2+}$ (500 nm)-induced acidification (D) was compared between control (clear bar) and MCU knockdown cells (gray bar) ($n = 6–28$). Alkalinization of the $\text{pH}_{\text{mito}}$ by high glucose (16.7 mM) in intact cells was also compared between control (clear bar) and MCU knockdown cells (gray bar) ($n = 8$) (E). ** and *** denote $p < 0.01$ and $< 0.001$, respectively.

**FIGURE 5.** Knockdown of MCU markedly impaired nutrient-stimulated mitochondrial matrix alkalinization. INS-1E cells were transfected with non-targeting siRNA (siControl) or siRNA against MCU (siMCU) followed by infection with an adenovirus carrying mtAlpHi 24 h after siRNA transfection. After 48 h of further incubation, mtAlpHi fluorescence was recorded by using the confocal microscope system in α-toxin permeabilized cells (A) or intact cells (B) and expressed as mitochondrial matrix pH ($\text{pH}_{\text{mito}}$) based on the subsequent pH titration. For titrations of mtAlpHi fluorescence, mitochondrial pH was clamped to the defined pH with ionophores. Succinate-induced alkalinization (C) and extramitochondrial $\text{Ca}^{2+}$ (500 nm)-induced acidification (D) were compared between control (clear bar) and MCU knockdown cells (gray bar) ($n = 6–28$). Alkalinization of the $\text{pH}_{\text{mito}}$ by high glucose (16.7 mM) in intact cells was also compared between control (clear bar) and MCU knockdown cells (gray bar) ($n = 8$) (E). ** and *** denote $p < 0.01$ and $< 0.001$, respectively.

**FIGURE 6.** Acute blocking of MCU-mediated $\text{Ca}^{2+}$ uptake did not affect metabolism-secretion coupling. A, effect of a selective MCU blocker, Ru360, on glucose-stimulated insulin secretion were analyzed in INS-1E cells ($n = 4$). B and C, changes in mitochondrial membrane potential ($\Psi_{\text{mito}}$) by Ru360 were measured with JC-1 fluorescence dye. * denotes $p < 0.05$.

We further investigated the functional consequences of LETM1 silencing on mitochondrial bioenergetics and metabolism-secretion coupling. Even though the stimulus-induced mitochondrial $\text{Ca}^{2+}$ response was augmented in LETM1 knockdown cells, glucose-induced insulin secretion and hyperpolarization of the $\Psi_{\text{mito}}$ were attenuated (Fig. 8, A and B). Reduction of LETM1 expression also lowered protein levels of subunits of respiratory chain complexes.

These results strongly suggest that LETM1 mediates at least one important component of $\text{Ca}^{2+}$ efflux in insulin secreting cells. In MCU knockdown cells, extramitochondrial $\text{Ca}^{2+}$ is taken up inefficiently, and therefore LETM1-mediated $\text{Ca}^{2+}$ efflux is strongly reduced. As a consequence the acidifying response of $\text{pH}_{\text{mito}}$ to extramitochondrial $\text{Ca}^{2+}$ is abrogated.
analyzed in intact INS-1E cells (n = 5). β-Actin was used as the reference control. INS-1E cells were transfected with non-targeting siRNA (siControl) or siRNA against LETM1 (siLETM1) and infected with an adenovirus carrying mtAlpHi or transfected with a ratiometric-pericam plasmid 24 h after transfection. D–F, changes in mitochondrial matrix pH (pH$_{\text{mito}}$) in response to succinate (3 mM) or extramitochondrial Ca$^{2+}$ (500 nM) were measured after 48 h of further incubation and compared between control (clear bar) and LETM1 knockdown cells (gray bar) (n = 14–21). G and H, increases in mitochondrial matrix Ca$^{2+}$ ([Ca$^{2+}$]$_{\text{mito}}$) by the addition of extramitochondrial Ca$^{2+}$ (120 mM and 500 mM) in α-toxin-permeabilized cells were compared between two groups (n = 5–12). The changes in [Ca$^{2+}$]$_{\text{mito}}$ by the application of high K$^+$ (30 mM) in intact cells were measured using ratiopericam probe (n = 5) (I and J) or Rhod-2 dye (K and L). ** and *** denote p < 0.01 and < 0.001, respectively.

level of the electron transport chain likely explain defective insulin secretion in LETM1-silenced cells (Fig. 8, C and D).

**DISCUSSION**

Increases in cytosolic Ca$^{2+}$ stimulate numerous energy consuming processes, including muscle contraction and neurotransmitter release. In particular, elevated mitochondrial matrix Ca$^{2+}$ ([Ca$^{2+}$]$_{\text{mito}}$) is a key stimulator of energy provision. The rise in [Ca$^{2+}$]$_{\text{mito}}$ activates TCA cycle dehydrogenases and ATP synthase leading to accelerated mitochondrial ATP production (6). For the proper coupling between energy demand and supply, propagation of Ca$^{2+}$ waves from the cytosol to the mitochondrial matrix through Ca$^{2+}$ transporters is necessary. MCU has been suggested to be the main channel linking cytosolic and mitochondrial Ca$^{2+}$ signaling driven by the mitochondrial electrical gradient.

In this study, we aimed to better understand the role of MCU as a regulator of mitochondrial metabolism and bioenergetics in pancreatic islet cells and clonal β-cells. Our findings demonstrate that silencing of MCU in insulin-releasing cells 1) decreases mitochondrial Ca$^{2+}$ uptake, 2) down-regulates electron transport chain proteins and enzyme activities, 3) reduces glucose-stimulated oxygen consumption, 4) impairs the generation of nutrient-stimulated ΔΨ$_{\text{mito}}$ (5) does not affect ΔΨ$_{\text{mito}}$, 6) reduces nutrient-stimulated ATP generation, and 7) impairs glucose-stimulated insulin secretion. We provide strong evidence that mitochondrial Ca$^{2+}$ uptake through MCU is a prerequisite for the establishment of the ΔΨ$_{\text{mito}}$ and activation of...
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mitochondrial energy metabolism. The critical role of the $\Delta p_{\text{H}_\text{mito}}$ in mitochondrial ATP synthesis and insulin secretion has been demonstrated previously (29, 30). It is inferred, therefore, that the reduced $[\text{Ca}^{2+}]_{\text{mito}}$ together with impaired $\Delta p_{\text{H}_\text{mito}}$ generation are the main reasons for defective metabolism-secretion coupling in MCU-silenced cells.

MCU is an inwardly rectifying, highly $\text{Ca}^{2+}$-selective ion channel driven by the negative $\Psi_{\text{mito}}$ generated by the respiratory chain (39). Because of high $\Delta \Psi_{\text{mito}}$ (assumed as 180mV), energized mitochondria have the ability to capture cytosolic $\text{Ca}^{2+}$ over a wide range of concentrations (40, 41). We observed that knockdown of MCU significantly diminished mitochondrial $\text{Ca}^{2+}$ influx at relatively low concentrations of extramitochondrial $\text{Ca}^{2+}$ (< 500 nM). These data imply that the MCU is the main mediator of mitochondrial $\text{Ca}^{2+}$ uptake from the cytosol under physiological conditions despite the negative regulation by other subunits of this complex such as MICU1/2 (18). Independent of the $\text{Ca}^{2+}$ source, either release from the ER or influx from the extracellular space, MCU works as the main $\text{Ca}^{2+}$ transport route into mitochondria (42). In MCU-silenced insulinoma cells, $[\text{Ca}^{2+}]_{\text{mito}}$ rises following either ER $\text{Ca}^{2+}$ release or high K+-induced $\text{Ca}^{2+}$ influx are reduced (32).

We found a close functional connection between mitochondrial $\text{Ca}^{2+}$ uptake and $\Delta p_{\text{H}_\text{mito}}$ regulation, not previously observed. Mitochondria in pancreatic $\beta$-cells are of relatively high volume density, facilitating nutrient metabolism and signal generation (35). Indeed, detection of plasma glucose levels is strictly dependent on mitochondrial oxidative phosphorylation in the $\beta$-cells (2). In this context, matrix alkalinization by glucose is a distinctive characteristic of $\beta$-cell mitochondria (29). In contrast, glycolytic cells such as HeLa cells or HepG2 cells have high resting $p_{\text{H}_\text{mito}}$ and do not respond to nutrient stimulation (29, 43). Our previous studies in insulin-secreting cells showed that short term attenuation of $[\text{Ca}^{2+}]_{\text{mito}}$ rises in an extracellular $\text{Ca}^{2+}$ free condition did not affect nutrient-stimulated alkalinization of $p_{\text{H}_\text{mito}}$. Conversely, a $[\text{Ca}^{2+}]_c$, transient caused by tolbutamide, a $K_{\text{ATP}}$, channel blocker, also did not affect $\Delta p_{\text{H}_\text{mito}}$ (29). In the present study, however, continuous suppression of calcium uptake after knockdown of MCU had profound effects on matrix pH and oxidative phosphorylation. As strong evidence for impaired mitochondrial metabolism following MCU knockdown, we observed that protein levels and function of electron transport chain complexes, mitochondrial enzyme activities, and oxygen consumption rate were all reduced. Our findings are consistent with an earlier study which showed that effective buffering of matrix $\text{Ca}^{2+}$ lowered NAD(P)H levels, oxygen consumption, and ATP synthesis in hormone secreting cells (7). We propose that persistent inhibition of $[\text{Ca}^{2+}]_{\text{mito}}$ rises perturbs Krebs cycle and electron transport chain activities, which in turn causes defective $\Delta p_{\text{H}_\text{mito}}$ generation and ATP synthesis, leading to impaired nutrient-stimulated insulin secretion.

Nigericin-induced hyperpolarization of $\Psi_{\text{mito}}$ reflects the preexisting $\Delta p_{\text{H}_\text{mito}}$. Therefore, the reduced hyperpolarizing response in MCU-silenced cells suggests reduction of the preexisting $\Delta p_{\text{H}_\text{mito}}$ (Fig. 4, C and D). This effect on $\Delta p_{\text{H}_\text{mito}}$ was confirmed using the mitochondrial pH-sensitive probe mtAlpHi (Fig. 5). On the other hand, the mitochondrial electrical gradient, the main component of the proton motive force, was not affected by MCU silencing (Fig. 4, A and B). This finding is similar to those observed in other cell types (11, 12). It is not clear why there is a selective defect in $\Delta p_{\text{H}_\text{mito}}$ generation without alterations in $\Delta \Psi_{\text{mito}}$ $\text{Ca}^{2+}$ influx through MCU uses the electrical gradient as a driving force therefore rapid $\text{Ca}^{2+}$ influx elicits depolarization of $\Delta \Psi_{\text{mito}}$ (40, 41). In MCU-silenced cells, mitochondrial $\text{Ca}^{2+}$ inward currents are reduced contributing to the preservation of $\Delta \Psi_{\text{mito}}$. Another mechanism contributing to the maintenance of $\Delta \Psi_{\text{mito}}$ could be lower activity of the mitochondrial $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCLX) because the amplitude of $[\text{Ca}^{2+}]_{\text{mito}}$ is decreased in MCU-silenced cells. $\text{Ca}^{2+}$ influx through NCLX leads to depolarization of $\Delta \Psi_{\text{mito}}$ because of its electrogenic property (24). Taken together, we can infer that reduced $\text{Ca}^{2+}$ influx and efflux in MCU-silenced cells prevent depolarization of $\Psi_{\text{mito}}$ which may counteract the effect of attenuated respiratory chain activity.

An interesting finding in this study is the acidification of $p_{\text{H}_\text{mito}}$ by extramitochondrial $\text{Ca}^{2+}$ addition to energized mitochondria of permeabilized INS-1E cells. This $p_{\text{H}_\text{mito}}$ acidification was dependent on mitochondrial $\text{Ca}^{2+}$ uptake via MCU. Mitochondrial $\text{Ca}^{2+}$ transport is coupled with $H^+$ through a $\text{Ca}^{2+}/H^+$ exchanger, the molecular identity of which is not clear. In a genome-wide RNA interference screen Jiang et al. identified LETM1 to mediate this exchange in mitochondria (23). To investigate the role of LETM1 on $\text{Ca}^{2+}/H^+$ -coupled transport in insulin-secreting cells, we tested whether there is an alteration in $p_{\text{H}_\text{mito}}$ acidification after knockdown of LETM1. Similar to the response in MCU knockdown cells, the $p_{\text{H}_\text{mito}}$ in LETM1 knockdown cells was not acidified, instead slight alkalinization by extramitochondrial $\text{Ca}^{2+}$ was observed (Fig. 6C). Furthermore, the $[\text{Ca}^{2+}]_{\text{mito}}$ rises by extramitochondrial $\text{Ca}^{2+}$ addition were increased in LETM1-silenced cells (Fig. 6F). Our findings demonstrate that LETM1 mediates $\text{Ca}^{2+}$ efflux from mitochondria of insulin-secreting cells working in parallel with NCLX. A recent publication shows that purified human LETM1 mediates electroneutral $2\text{H}^+/1\text{Ca}^{2+}$ antiport when reconstituted in artificial liposomes (44). Thus, in intact cells, $\text{Ca}^{2+}$ efflux via LETM1 is preferred, which is driven by the $H^+$ gradient across the inner mitochondrial membrane (45). We suggest that $\text{Ca}^{2+}$ influx through MCU is coupled to LETM1-mediated $\text{Ca}^{2+}$ efflux with proton uptake favored by high $[\text{Ca}^{2+}]_{\text{mito}}$ and alkaline $p_{\text{H}_\text{mito}}$. In MCU-silenced cells, $\text{Ca}^{2+}$ efflux via LETM1 was prevented or even reversed to $\text{Ca}^{2+}$ influx due to reduced $\text{Ca}^{2+}$ and pH gradients, explaining the disappearance of $p_{\text{H}_\text{mito}}$ acidification.

Why LETM1 knockdown has negative effects on the expression of subunits of the respiratory chain, glucose-stimulated $\Psi_{\text{mito}}$ hyperpolarization and insulin secretion is not clear. A recent publication presented evidence that LETM1 haploinsufficiency (+/-) increases mitochondrial superoxide levels which is responsible for mitochondrial dysfunction (46). We propose that oxidative stress in LETM1-silenced insulin-releasing cells may negatively affect mitochondrial bioenergetics, respiratory activity, and metabolism-secretion coupling.

Our findings herein demonstrate that MCU-mediated $\text{Ca}^{2+}$ uptake is essential for the respiratory chain activity and the generation of $\Delta p_{\text{H}_\text{mito}}$ in insulin-releasing cells. This chemical
gradient ($\Delta p_{H_{\text{mito}}}$) is critically required for the substrate transport into the mitochondrial matrix, including pyruvate and inorganic phosphate (31) and ATP synthesis (29). Therefore, evidence in the present study suggests the bioenergetic mechanism to explain the definitive metabolism–secretion coupling by MCU knockdown. In addition, mitochondrial Ca$^{2+}$ uptake regulates cytosolic Ca$^{2+}$ signaling and contributes to prevent local Ca$^{2+}$ overload in the cytosol (37). On the contrary, accumulation of Ca$^{2+}$ in the mitochondrial matrix induces permeability transition (PT) pore opening and apoptosis. To maintain Ca$^{2+}$ homeostasis, there is an interactive operation of mitochondrial transporters involved in Ca$^{2+}$ influx and efflux pathways. Further research focusing on the comprehensive understanding of mitochondrial Ca$^{2+}$ transporters may lead to the identification of novel therapeutic targets to improve mitochondrial energy metabolism and to prevent cytotoxicity. This may be especially relevant in insulin-releasing cells, where mitochondrial Ca$^{2+}$ transport plays a key role in metabolism–secretion coupling, dysfunction of which leads to the development of type 2 diabetes.

Acknowledgments—We thank Dr. Young-Kyoung Lee and Prof. Gye-soon Yoon (Ajou Univ., Suwon, Korea) for sincere support for the measurements of oxygen consumption.

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Role of MCU for Mitochondrial pH Gradient


Bioenergetics: Essential Role of Mitochondrial Ca\textsuperscript{2+} Uniporter in the Generation of Mitochondrial pH Gradient and Metabolism-Secretion Coupling in Insulin-releasing Cells

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doi: 10.1074/jbc.M114.632547 originally published online December 29, 2014

Access the most updated version of this article at doi: 10.1074/jbc.M114.632547

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