Berberine inhibits glucose oxidation and insulin secretion in rat islets

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Abstract. Glucose promotes insulin secretion primarily via its metabolism and the production of metabolic coupling factors in beta-cells. The activation of AMP-activated protein kinase (AMPK), an energy sensor, results in a decrease in insulin secretion from beta-cells, but its mechanism remains largely unknown. Berberine, an oral anti-diabetic drug, has been shown to activate AMPK in multiple peripheral tissues. Here, we examined the effects of berberine and AMPK activation on insulin secretion and glucose oxidation in rat islets. Our results showed that berberine inhibited glucose-stimulated insulin secretion from rat islets with AMPK activation. When glucose concentration was elevated to 25 mmol/L, the inhibitory action of berberine on insulin secretion disappeared. Furthermore, berberine significantly decreased oxygen consumption rate (OCR) and ATP production induced by high glucose in rat islets. Although adenovirus-mediated overexpression of constituent-activated AMPK markedly decreased GSIS and OCR in rat islets, the inhibition of AMPK by compound C did not reverse berberine-suppressed OCR. In addition, berberine attenuated glucose-stimulated expression of fatty acid synthase. These results indicate that berberine-mediated deceleration of glucose oxidation is tightly link to the decreased insulin secretion in islets independent of AMPK activation and inhibition of fatty acid synthesis may also contribute to the effect of berberine on insulin secretion.

Key words: Berberine, Glucose oxidation, Islets, Insulin secretion, AMP-activated protein kinase
molecular mechanism by which berberine regulates insulin secretion remains largely unknown.

Pancreatic beta-cells secrete insulin in response to blood nutrient for maintaining metabolic homeostasis [11]. Glucose is the most important driver for insulin secretion, and its action in beta-cells is tightly linked to glycolysis and mitochondrial metabolism for the quantitative flux of glucose carbons into mitochondria [12]. Increased glucose metabolism results in an elevation of ATP/ADP ratio, followed by closure of ATP-sensitive K\(^{+}\) (K\(_{ATP}\)) channels and depolarization of the plasma membrane. The activation of L-type voltage-dependent Ca\(^{2+}\) channels leads to a rise in intracellular Ca\(^{2+}\) and triggers insulin exocytosis [13]. In addition to the classical ATP/K\(_{ATP}\)/Ca\(^{2+}\) signaling pathway, compelling evidence supports the existence of additional metabolic molecules that provide amplification signals (e.g., fatty acyl-CoA, glutamate, adenine nucleotides) for GSIS [11, 14]. Recently, it has been reported that glucose increases oxygen consumption, ATP production, free fatty acid (FFA) content and release from rat islets and INS-1 cell [12]. Metformin inhibited these metabolic events at glucose concentrations over 3 mmol/L, with marked shifting of glucose oxidation, ATP production, and GSIS response to the right [15]. These data indicate that metformin regulates insulin secretion via decreasing glucose metabolism in beta-cells. However, whether berberine-inhibited insulin secretion is related to its effect on glucose oxidation in islets remains unclear.

In the present study, we synchronously detected the effects of berberine on insulin secretion and oxygen consumption rate (OCR) at different concentrations of glucose in isolated rat islets. The results showed that berberine-inhibited insulin secretion was closely related to OCR in response to glucose.

Materials and methods

Reagents

Berberine was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Bovine serum albumin (BSA), 5-aminimidazole-4-carboxamide riboside (AICAR), and collagenase type XI were obtained from Sigma (St Louis, MO, USA). Fetal bovine serum (FBS), RPMI 1640 medium, and other culture reagents were gained from Gibco Life Technologies (Grand Island, NY). Anti-\(\alpha\)-tubulin, anti-AMPK\(\alpha\), anti-phospho-AMPK\(\alpha\) (Thr172), anti-acetyl-CoA carboxylase (ACC), anti-phospho-ACC (Ser79), and anti-rabbit IgG conjugated with horseradish peroxidase were purchased from Cell Signaling Technology (Beverly, MA, USA). Rat insulin ELISA kit was obtained from Mercodia (Uppsala, Sweden).

Islet isolation and treatment

Male Sprague–Dawley rats weighing 180–220 g were obtained from Shanghai Slack Experimental Center. Islets were isolated by in situ pancreas collagenase infusion and separated by density gradient as described previously [16]. Freshly isolated islets were transferred to 6-well plates and cultured overnight in RPMI 1640 medium containing 10 mmol/L HEPES, 0.25% BSA, 100 U/mL penicillin G sodium and 100 \(\mu\)g/mL streptomycin sulfate at 37°C and 5% CO\(_2\). All animal protocols were reviewed and approved by the Animal Care Committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine.

Insulin secretion

Islets were washed once in Krebs-Ringer bicarbonate (KRB) buffer [128.8 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L KH\(_2\)PO\(_4\), 5 mmol/L NaHCO\(_3\), 10 mmol/L HEPES (pH = 7.4), 1.2 mmol/L MgSO\(_4\), 2.5 mmol/L CaCl\(_2\) with 0.25% BSA] containing 3.3 mmol/L glucose and then were pre-incubated in the same medium at 37°C. Then this buffer was replaced with 1 mL of pre-warmed KRB containing other additions as indicated for a further 60 min at 37°C. An aliquot was then removed for analysis of insulin secretion. Islets were extracted with 75% acid-ethanol for the determination of insulin content.

Adenovirus infection

The adenovirus encoding constituent-activated AMPK (AMPK-CA) and control adenovirus from Gene Chem Co., Ltd. (Shanghai, China) were transfected into rat islets for 48 h according to the manufacturer’s instructions.

Quantitative real-time PCR

Total RNA was extracted from rat islets with Trizol (Invitrogen) and reverse transcribed from Random Primers (Promega). Quantitative real-time PCR (qRT-PCR) was performed using a SYBR Premix Ex Taq\(^{TM}\) (Takara, Japan). The primer sequences used for qRT-PCR were designed as follows: 18S, 5'-ACCGCAGCTAGGAATGGA-3' (forward), 5'-GCCTCAGTTCCGAAAA CCA-3' (reverse); FASN, 5'-CTGGCCACCTCAGT
CCTGTTATCAC-3' (forward), 5'-AGGAGTAGTAGGC/TTAGAC-3' (reverse). Equal amounts of
cDNA were analyzed in triplicate for each run. The com-
parative Ct method was used to evaluate differences in
gene expressions and the results of relative expression
were normalized by 18S mRNA levels.

**Western blotting**

Islets were washed twice with ice-cold PBS and
immediately placed in lysis buffer containing phenyl-
methylsulfonyl fluoride (PMSF), protease inhibitor cock-
tail I and phosphatase inhibitor cocktail V (Merck,
Darmstadt, Germany). Lysates were gently mixed for 10
min at 4°C and then centrifuged at 12,000 g for 15 min at
4°C. The protein concentration of the extracts was deter-
mined according to the method of Bradford, using BSA
as the standard. Samples were separated by SDS-PAGE
on 10% polyacrylamide gels and transferred to PVDF
membranes (Bio-Rad). Membranes were blocked with
5% BSA. Primary antibodies were detected with donkey
anti-rabbit IgG at 1:2,000 for 2 h at room temperature.
Visualization was detected with a LAS-4000 Super CCD
Remote Control Science Imaging System (Fuji, JAP).

**Oxygen consumption measurement**

Rat islets were seeded in Seahorse XF 24-well islet
capture microplate (Seahorse biosciences, North
Billerica, MA) at 60 islets/well in 500 μL of Assay
Media (Supplemented with 2 mmol/L glucose and 0.1%
BSA) in the presence or absence of berberine. Islets were
incubated at 37°C without CO2 for 30 min to allow cells
to pre-equilibrate with the medium before the first mea-
surement. After the equilibration period, islets were sub-
jected to basal measurements, followed by injection of
different concentration of glucose or 5 μmol/L oligomy-
cin, an inhibitor of ATP synthesis for distinguishing oxy-
gen consumption devoted to ATP synthesis; 1 μmol/L
Carboxyl cyanide 4-trifluoromethoxy phenylhydrazone
(FCCP), an uncoupling agent added to measure uncou-
pled respiration; 5 μmol/L rotenone plus antimycin A,
electron transport chain inhibitors used to inhibit the
whole oxygen consumption.

**Statistical analysis**

Data were expressed as mean ± SEM. Comparisons
were performed by using ANOVA for multiple groups or
the Student’s test for two groups. Significance was estab-
lished at $p < 0.05$.

**Results**

Berberine acutely inhibits GSIS

To select an appropriate concentration of berberine,
freshly isolated islets were treated with 0.5, 2.5, 5, and
10 μmol/L berberine for 1 h. As shown in Fig. 1A, ber-
berine inhibited insulin secretion in a dose-dependent
manner, showing a significant action at the concentration
of 0.5 μmol/L and reaching the maximal effect at 5
μmol/L. We applied 2.5 μmol/L berberine in the next
experiments. We detected the dose-dependent effect of
glucose on acute insulin release from isolated rat islets.
As expected, insulin secretion started to increase at the
concentration of 5.6 mmol/L glucose and reached a pla-
teau at 16.7 mmol/L (Fig. 1B). Berberine significantly
lowered insulin secretion at 5.6, 8.3, 11.1, and 16.7
mmol/L glucose, but not at 3.3 and 25 mmol/L glucose
(Fig. 1B). Since berberine had no effect on 35 mmol/L
KCl-induced insulin secretion (Fig. 1C), the inhibitory
effect of berberine on GSIS was unlikely to be due to
non-specific toxicity. It has been shown that glucose
acutely inhibits AMPK activity and stimulates insulin
release from beta-cells [17]. Thus, we observed the effect
of berberine on AMPK phosphorylation in freshly isola-
ted rat islets incubated with various concentrations of
glucose. The phosphorylation levels of AMPK and ACC
were gradually decreased with the elevation of glucose
concentration from 3.3 to 16.7 mmol/L (Fig. 1D). Ber-
berine markedly stimulated the phosphorylations of
AMPK and ACC at 8.3 and 16.7 mmol/L glucose (Fig.
1D).

Berberine suppresses mitochondrial respiration and
ATP production in rat islets

It is widely recognized that glucose oxidation is well
correlated with GSIS. We measured OCR in response to
glucose as an indicator of glucose oxidation with Sea-
horse XF24 analyzers. Rat islets were pretreated with
berberine for 30 min before the detection began. OCR
significantly increased when glucose concentration was
elevated to 8.3 mmol/L and reached a plateau at 16.7
mmol/L (Fig. 2A). These results reveal the tight correla-
tion between GSIS and glucose oxidation in beta-cells.
Berberine markedly decreased glucose-stimulated OCR
at the concentrations above basal (Fig. 2B). To further
investigate the effect of berberine on mitochondria respi-
ration, we performed mitochondria stress assay in rat
islets cultured with 11.1 mmol/L glucose in the presence
or absence of 2.5 μmol/L berberine. Oligomycin (an ATP
synthase inhibitor), FCCP (uncouples oxygen consumption from ATP production), and rotenone (an inhibitor of complex I)/antimycin A (an inhibitor of complex III) were added at the indicated time (Fig. 2C). Berberine significantly decreased the maximal OCR induced by 11.1 mmol/L glucose (Fig. 2D), accompanied by approximately 40% reduction in ATP synthesis calculated by subtracting the lowest OCR after oligomycin injection from the maximum OCR induced by 11.1 mmol/L glucose (Fig. 2E). These results suggest that berberine may exert a decelerating effect on glucose metabolism.

**Berberine inhibits glucose-induced fatty acid synthase expression**

Some studies showed that glucose utilization was quantitatively higher than its oxidation [18, 19]. An important fate of glucose carbons in rat islets is to synthesis fatty acid *de novo*. To further explore the effects of berberine on this aspect of glucose metabolism in islets, we incubated rat islets with 3.3 and 16.7 mmol/L glucose in the presence or absence of berberine for 24 h. As shown in Fig. 3A, berberine markedly diminished insulin secretion from islets cultured in 16.7 mmol/L glucose. qRT-PCR result demonstrated that glucose increased the mRNA level of FASN (Fig. 3B), which catalyzes the *de novo* synthesis of fatty acid. Berberine repressed high glucose-induced FASN mRNA expression, but without effect at 3.3 mmol/L glucose (Fig. 3B). Western blotting showed a similar result of fatty acid synthase protein expression inhibited by berberine at 16.7 mmol/L glucose (Fig. 3C, D). Thus, it is possible that berberine suppresses high glucose-promoted fatty acid biosynthesis and lessens lipid signal molecules via decreasing fatty acid synthase expression in rat islets, which contributes to its inhibitory effect on insulin secretion.
AMPK activation inhibits insulin secretion and glucose-induced OCR

Like berberine, AICAR, an AMPK agonist, also inhibited both acute and chronic insulin secretion (Fig. 4A, B). To exclude the non-specific action of the agent, we further investigated the effect of AMPK on insulin secretion using adenovirus-mediated overexpression of AMPK-CA (Fig. 4C). The islets transfected with AMPK-CA adenovirus showed significantly reduced GSIS (Fig. 4D). To determine whether AMPK exerts an

Fig. 2 Effect of berberine on mitochondrial respiration in rat islets. Oxygen consumption rate (OCR) was recorded in rat islets treated with 2.5 μM berberine (BBR) or vehicle (CON). Berberine was added 30 min before the experiment began. (A) Glucose (G) was added from 2 to 8.3, 16.7, and 25 mM. (B) ΔOCR was calculated by subtracting basal OCR from the maximum OCR induced by 8.3, 16.7 and 25 mM glucose in the presence or absence of berberine. (C) Mitochondria stress assay was performed in rat islets treated with 11.1 mM glucose in the presence or absence of berberine. 5 mM oligomycin (O), 1 μM FCCP (F), and 5 μM rotenone plus antimycin (R + A) were added at the indicated time. (D) The maximal OCR induced by glucose in control and berberine groups. (E) OCR for ATP synthesis was calculated by subtracting the lowest OCR after oligomycin injection from the maximum OCR induced by glucose. Data were given as mean ± SEM for three separate experiments. *p < 0.05, **p < 0.01 vs. control (CON) group, #p < 0.05 vs. 8.3G group.
impact on glucose oxidation, we measured OCR in islets transfected with AMPK-CA adenovirus at 3.3 and 16.7 mmol/L glucose, AMPK-CA markedly decreased high glucose-stimulated OCR, but had no effect at 3.3 mmol/L glucose (Fig. 4E). To determine the role of AMPK in berberine-suppressed OCR, rat islets were preincubated with 10 μmol/L compound C (an AMPK inhibitor) for 30 min in the presence of berberine. Unexpectedly, compound C could not reverse the inhibitory effect of berberine on OCR (Fig. 4F). These results suggest that berberine may regulate the OCR of islets independent of AMPK signal pathway.

Discussion

Type 2 diabetes is characterized by insulin resistance and impaired islet function. There exists evidence supporting that in some circumstances insulin hypersecretion can induce insulin resistance and is regarded as an important pathogenetic factor in type 2 diabetes [1]. Thus pharmacological intervention that can reduce insulin hypersecretion and simultaneously alleviate insulin resistance is an attractive strategy to prevent beta-cell dysfunction due to their exhaustion. Berberine exerts hypoglycemic and hypolipidemic action via stimulating glucose uptake, reducing lipogenesis, and increasing energy expenditure in peripheral tissues [8, 9, 20]. Berberine can also decrease insulin levels in high-fat-fed rats [10]. In this current study, we showed for the first time that berberine significantly inhibited glucose-induced OCR in rat islets with decreased GSIS. These data suggests that berberine can regulate insulin secretion through affecting glucose metabolism in beta-cells.

Pancreatic beta-cell has the ability to rapidly and reversibly adapt to changes in metabolic demand, thus glucose-induced insulin secretion is tightly coupled to its metabolism in these cells. Although the stimulus-secretion coupling is complex and still incompletely understood, multiple metabolic signaling factors including ATP, fatty acyl-CoA, glutamate and adenine nucleotides, are believed to take part in this process [11, 21]. Although berberine has been reported to block the activ-
ity of respiratory chain complex I [22, 23], little is known about the metabolic effects of berberine on islets. Applying Seahorse XF24 Extracellular Flux Analyzer to assess the mitochondrial OCR, we revealed the inhibitory action of berberine on oxygen consumption in islets induced by glucose, consisted with the inhibition of complex I previously reported [22]. It was demonstrated that GSIS increased from basal to 16 mmol/L glucose and reached a plateau between 16 and 25 mmol/L. Glucose oxidation, oxygen consumption, and ATP production were well correlated with GSIS, indicating that ATP production from glucose oxidation is tightly related to insulin secretion [12]. In our study, berberine significantly suppressed OCR and ATP production induced by glucose. The inhibition of ATP synthesis in turn alters the ATP/AMP ratio, which blocks K\textsubscript{ATP} channel closure and thereby inhibits insulin secretion [11]. Interestingly, berberine markedly inhibited OCR at 25 mmol/L glucose in rat islets, but without effect on GSIS, which is similar to the effects of metformin on GSIS and OCR in beta-cells.

Fig. 4  AMPK activation inhibits glucose-stimulated insulin secretion and oxygen consumption rate. Rat islets were incubated with 0.5 mM AICAR at 16.7 mM glucose for 1 h (A) and 24 h (B) for insulin secretion assay. (C) AMPK protein expression in rat islets transfected with control adenovirus (CON) or adenovirus encoding constituent-activated AMPK adenovirus (AMPK-CA). (D and E) Insulin secretion and oxygen consumption rate (OCR) were assayed in rat islets transfected with control adenovirus and AMPK-CA adenovirus at 3.3 and 16.7 mM glucose. (F) Rat islets were preincubated with 10 μM compound C (CC) for 30 min, and then treated with 2.5 μM berberine (BBR) at 16.7 mM glucose for the measurement of OCR. Data were given as mean ± SEM for three separate experiments. *p < 0.05, **p < 0.01, ***p < 0.001 vs. 3.3G group, †p < 0.05, ‡p < 0.01 vs. 16.7G alone group.
It has been reported that short-term exposure to physiological concentration of FFA potentiates insulin secretion [21]. Fatty acid plays an important role in the regulation of GSIS through glycolipid (GL)/FFA cycle. The GL/FFA cycle is thought to be central for the generation of lipid-signaling molecules as metabolic coupling factors [19]. Moreover, glucose-derived acetyl-CoA via citrate/pyruvate cycle provides building blocks for the de novo synthesis of fatty acid [24, 25]. In our study, high glucose increased the expression of fatty acid synthase, which is involved in the lipogenesis process. Berberine inhibited the glucose-induced increase in fatty acid synthase mRNA and protein levels. Our previous study revealed that berberine inhibited lipolysis in 3T3-L1 adipocytes [26]. Thus, it is likely that berberine suppresses GSIS partially via interrupting glucose-promoted GL/FFA cycle and reducing lipid-signaling molecules.

AMPK is an intracellular fuel sensor and plays an important role in regulating energy balance at cellular and whole-body levels [27]. The increase in AMP/ATP ratio can activate AMPK, which in turn redirects metabolism towards increased catabolism and decreased anabolism through the phosphorylation of key proteins in multiple pathways to increase ATP generation and decrease ATP consumption [28]. Elevations in glucose concentration from 3.3 to 16.7 mmol/L markedly increased insulin secretion, with reduced phosphorylation of AMPK at Thr-172 [29, 30]. These data suggested that AMPK activity was negatively related to insulin secretion. Our results also support this conclusion. AICAR- and AMPK-CA-treated islets exhibited decreased insulin secretion. Berberine treatment led to the activation of AMPK in rat islets, along with an inhibition of glucose-induced ATP production, suggesting that berberine activates AMPK through changing AMP/ATP ratio. However, little is known about how AMPK activation affects insulin secretion. In the present study, the increased OCR induced by high glucose was reversed by adenovirus-mediated overexpression of AMPK-CA in rat islets, suggesting that AMPK-inhibited OCR is involved in its inhibitory effect on GSIS. Although berberine exhibited a similar action for OCR with AMPK activation, the inhibitor of AMPK compound C did not reverse berberine-suppressed OCR. These results indicate that berberine-mediated inhibition of GSIS is attributed to the decreased OCR and ATP production independent of AMPK activation as the latter is the downstream event of the former. However, the exact mechanism remains to be further explored.

In conclusion, berberine inhibits glucose oxidation and ATP synthesis in rat islets, which likely contributes to its inhibitory action on GSIS through K<sub>ATP</sub>-dependent triggering pathway. In addition, berberine-suppressed fatty acid synthase expression may decrease the lipid-signaling molecules of insulin secretion via preventing GL/FFA cycle. AMPK activation by berberine-decreased ATP production is not necessary for berberine-inhibited GSIS.

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**Disclosure**

None of the authors have any potential conflicts of interest associated with this research.

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