Ginsenoside Rb₁ stimulates glucose uptake through insulin-like signaling pathway in 3T3-L1 adipocytes

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Abstract

A series of clinical trials and animal experiments have demonstrated that ginseng and its major active constituent, ginsenosides, possess glucose-lowering action. In our previous study, ginsenoside Rb₁ has been shown to regulate peroxisome proliferator-activated receptor γ activity to facilitate adipogenesis of 3T3-L1 cells. However, the effect of Rb₁ on glucose transport in insulin-sensitive cells and its molecular mechanism need further elucidation. In this study, Rb₁ significantly stimulated basal and insulin-mediated glucose uptake in a time- and dose-dependent manner in 3T3-L1 adipocytes and C2C12 myotubes; the maximal effect was achieved at a concentration of 1 μM and a time of 3 h. In adipocytes, Rb₁ promoted GLUT1 and GLUT4 translocations to the cell surface, which was examined by analyzing their distribution in subcellular membrane fractions, and enhanced translocation of GLUT4 was confirmed using the transfection of GLUT4-green fluorescence protein in Chinese Hamster Ovary cells. Meanwhile, Rb₁ increased the phosphorylation of insulin receptor substrate-1 and protein kinase B (PKB), and stimulated phosphatidylinositol 3-kinase (PI3K) activity in the absence of the activation of the insulin receptor. Rb₁-induced glucose uptake as well as GLUT1 and GLUT4 translocations was inhibited by the PI3K inhibitor. These results suggest that ginsenoside Rb₁ stimulates glucose transport in insulin-sensitive cells by promoting translocations of GLUT1 and GLUT4 by partially activating the insulin signaling pathway. These findings are useful in understanding the hypoglycemic and anti-diabetic properties of ginseng and ginsenosides.


Introduction

Ginseng has been used as a tonic in oriental medicine to promote health for several millennia, and its constituents possess multiple pharmacological effects on the central nervous, cardiovascular, endocrine, and immune systems (Attele et al. 1999). Historical records in traditional Chinese medicine have shown that ginseng has been used to treat type 2 diabetes for a long time (Xie et al. 2005a). More recently, a series of clinical trials and animal experiments have demonstrated that ginseng and its major active components, ginsenosides, exert hypoglycemic and insulin-sensitizing action (Vuksan et al. 2000a,b, 2008, Attele et al. 2002, Yun et al. 2004, Liu et al. 2005, Xie et al. 2005b,c, R ey et al. 2006, Sievenpiper et al. 2006). These studies suggest that ginseng may be a useful therapy in the management of type 2 diabetes. However, cellular and molecular mechanisms of hypoglycemic action produced by ginseng and ginsenosides remain unclear.

Ginsenosides are the major active constituents responsible for pharmacological properties of ginseng. All ginsenosides have the same structure of four trans-ring rigid steroid skeleton with the difference in their number and site of attachment of hydroxyl groups (Gillis 1997, Attele et al. 1999). Ginsenoside Rb₁ is the most abundant one among more than 20 ginsenosides in ginseng root despite different sources and species (Attele et al. 2002, Washida & Kitanaka 2003, Lim et al. 2005). Our recent study showed that ginsenoside Rb₁, (Washida & Kitanaka 2003), facilitated adipogenesis of 3T3-L1 preadipocytes by enhancing peroxisome proliferator-activated receptor γ 2 (PPARγ2) and CCAAT/enhancer-binding protein α gene expression. In addition, Rb₁ binds to PPARγ-ligand-binding domain as measured by surface plasmon resonance, which suggests that Rb₁ has the ability to activate PPARγ (Shang et al. 2007b). Although treatment of differentiating adipocytes with Rb₁ lead to augmented basal and insulin-mediated glucose uptake, which reflects the degree of adipogenesis (Shang et al. 2007a), the direct effect of Rb₁ on glucose uptake in mature adipocytes remains unknown. We also found that Rb₁ could inhibit lipolysis in adipocytes (Shang et al. 2007b), which suggests that Rb₁ may possess insulin-like effect. Recent studies have reported that ginseng extract and ginsenoside Re stimulates glucose transport and inhibits lipolysis in adipocytes through activation of insulin signaling (Edens et al. 2001, Wang et al. 2006, Zhang et al. 2008).
It has been indicated that the structural diversity of ginsenosides may lead to the different effects (Attele et al. 1999). Therefore, the effect of Rb1 on glucose transport in insulin-sensitive cells and insulin signaling pathway in mature adipocytes merits further elucidation.

Insulin-mediated glucose transport in appropriate tissue is essential for maintenance of whole-body glucose homeostasis (Herman & Kahn 2006). Adipocytes and muscle cells are highly insulin-sensitive cells. In adipocytes, insulin stimulates glucose transport by enhancing the translocation of GLUT4 and, to a lesser extent, GLUT1 from the intracellular pool to the plasma membrane (PM) (Weiland et al. 1990, Watson et al. 2004). The classic pathway of insulin signaling involved in glucose transporter translocation to the cell surface is triggered by autophosphorylation of the insulin receptor on multiple tyrosine residues following insulin binding. This results in the tyrosine phosphorylation of a family of insulin receptor substrate (IRS) proteins and activation of a complex network of downstream molecules, including phosphatidylinositol 3-kinase (PI3K) and the serine/threonine kinase AKT (Khan & Pessin 2002, Watson et al. 2004). Muscle cells share the same signal cascade of regulating glucose transport (Zorzano et al. 2005).

The present study sought to explore the effects of Rb1 on glucose transport in the differentiated 3T3-L1 adipocytes and myotubes, as well as elucidation of its mechanism in adipocytes. Our results presented here show that Rb1 stimulates glucose transport in adipocytes by inducing the translocations of GLUT1 and GLUT4 through activating insulin-like signaling pathways. These findings may help to understand the hypoglycemic and anti-diabetic effects of ginseng and ginsenosides.

**Materials and Methods**

**Reagents**

Dulbecco modified Eagle medium (DMEM) and horse serum were purchased from Gibco. Fetal calf serum (FCS) was obtained from PAA Laboratories (Pasing, Austria). Ginsenoside Rb1, dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX), and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich. Recombinant human insulin (Ins) was purchased from Sigma–Aldrich. Rabbit anti-insulin receptor (IR) antibody was purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-p85 subunit of PI3K antibody was purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-phospho-p85 antibody was purchased from Upstate (Temecula, CA, USA). Anti-phospho-PI3K antibody was obtained from R&D (Minneapolis, MN, USA). Polyclonal IRS-1 (phosphor Y612) and mouse monoclonal GLUT1 rabbit polyclonal antibody was purchased from R&D (Minneapolis, MN, USA). GLUT4 monoclonal antibody was obtained from Upstate (Temecula, CA, USA). Phospho-insulin receptor antibody (Tyr1135/1136), phospho-AKT antibody (Ser473 and Thr308), AKT antibody, horseradish peroxidase-linked anti-rabbit immunoglobulin G (IgG) and anti-mouse IgG were purchased from Cell Signaling Technology (Danvers, MA, USA). Wortmannin was obtained from Biomol International LP (Plymouth Meeting, PA, USA). LY294002 and hydroxy-2-naphthalenylmethylphosphonic acid trisacetoxy-methyl ester (HNMPA-(AM)3) were purchased from Calbiochem (La Jolla, CA, USA).

**Cell culture**

Mouse 3T3-L1 fibroblast cells and C2C12 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). 3T3-L1 fibroblast cells were cultured and differentiated into adipocytes as described previously with some modifications (Perrini et al. 2004). Briefly, the fibroblasts were grown in DMEM containing 10% FCS, 25 mM glucose, 2 mM glutamine, 3.4 mg/ml NaHCO3, 2-47 mM CaCl2, 1.24 mM MgSO4, 0.5 mM IBMX, and 1% DMSO at 37 °C in 5% CO2 atmosphere. After confluence, the fibroblasts were maintained for another 2 days. Differentiation was induced by treating the cells with standard differentiation inducers (DMEM containing 0.5 mM IBMX, 1 μM DEX, 1-7 μM Ins, and 10% FCS) for 48 h. The cells were re-fed with DMEM supplemented with 1-7 μM Ins and 10% FCS for the following 48 h, then the medium was replaced with growth medium, changed every 2 days. More than 90% of the cells expressed the adipocyte phenotype between 8 and 10 days after the initiation of differentiation and were used for the experiments. C2C12 cells were maintained in growth medium. When the cells achieved 70% confluence, they were differentiated in DMEM containing 2% horse serum for 4 days. The differentiated C2C12 cells were fused into myotubes and were used for the experiments. The cells used for the experiments were serum starved overnight in DMEM containing 0.2% BSA (Roche Applied Science). Ginsenosides and all inhibitors were dissolved in DMSO as a 1000-fold stock, and added to 0.2% BSA–DMEM at various concentrations as shown in each figure. DMSO was present in the control culture at a same concentration less than 0.2% (v/v).

**Glucose uptake measurement**

The assay was performed essentially as described previously (Perrini et al. 2004). The cells were washed thrice with PBS, then incubated in Krebs–Ringer phosphate buffer (KRP, 1-32 mM NaCl, 4-71 mM KCl, 47 mM CaCl2, 1-24 mM MgSO4, 2-48 mM Na3PO4, 10 mM HEPES (pH 7-4)) for 10 min at 37 °C, then 0-5 μCi/ml 2-DOG as the final concentration was added to the cells. After 10-min incubation, the medium was aspirated and the cells were washed thrice with

**Clathrin heavy chain antibody** was purchased from Abcam (Cambridge, UK). Anti-phosphorylated PI3K antibody was obtained from Cell Signaling Technology (Danvers, MA, USA). Phospho-insulin receptor antibody (Tyr1135/1136), phospho-AKT antibody (Ser473 and Thr308), AKT antibody, horseradish peroxidase-linked anti-rabbit immunoglobulin G (IgG) and anti-mouse IgG were purchased from Cell Signaling Technology (Danvers, MA, USA). Wortmannin was obtained from Biomol International LP (Plymouth Meeting, PA, USA). LY294002 and hydroxy-2-naphthalenylmethylphosphonic acid trisacetoxy-methyl ester (HNMPA-(AM)3) were purchased from Calbiochem (La Jolla, CA, USA).
ice-cold KRP containing 10 mM glucose to terminate the reaction. The cells were lysed with 0.1 M NaOH and the radioactivity taken up by the cells was determined using a scintillation counter (Beckman Instruments, Fullerton, CA, USA). The d.p.m. value was corrected by protein content in each well which was measured using a BCA protein assay kit.

**Extract of total cell protein and subcellular membrane fractions**

For extracting total protein from adipocytes, the cells were washed twice with ice-cold PBS and harvested in a lysis buffer (radioimmunoprecipitation assay (RIPA), 50 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM phenylmethylsulphonyl fluoride (PMSF), 10 μg/ml aprotinin, 10 μg/ml leupeptin, 0.1 mM sodium orthovanadate (pH 7.4)). After sonication and centrifugation at 13 000 g for 20 min; the resulting supernatant was centrifuged at 100 000 g for 20 min to yield a pellet of total cellular membranes and a supernatant representing the cytosolic fraction. The pellet was resuspended (30 strokes in a glass Dounce homogenizer) in buffer B (20 mM HEPES, 137 mM NaCl, 1.5 mM KH₂PO₄, 8 mM Na₂HPO₄ (pH 7.4)), adipocytes were scraped in ice-cold buffer A (20 mM HEPES, 250 mM sucrose, 1 mM EDTA (pH 7.4)) containing protease inhibitor cocktail (10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM PMSF), and subsequently homogenized with 30 strokes in a glass Dounce homogenizer at 4 °C. All subsequent procedures were performed at 4 °C. After centrifugation at 100 000 g for 10 min to remove large cell debris and unbroken cells, the supernatant was then centrifuged at 8000 g for 20 min to yield a pellet of total cellular membranes and a supernatant representing the cytosolic fraction. The pellet was resuspended (30 strokes in a glass Dounce homogenizer) in buffer B (20 mM HEPES, 250 mM sucrose, 1 mM EDTA (pH 7.4)) containing protease inhibitor cocktail and layered on 1.12 M sucrose cushion. Following 1 h centrifugation at 16 000 g, the PM layer at the interface was collected by centrifugation, and resuspended in buffer B. The 16 000 g supernatant was centrifuged at 41 000 g for 20 min; the resulting supernatant was centrifuged for 75 min at 180 000 g to pellet the low-density membrane (LDM) fraction, which was resuspended in buffer B. The protein concentrations of PM and LDM were determined with BCA protein assay kit.

**Immunoprecipitation and assay of PI3-kinase activity**

PI3-kinase activity was measured as described previously (Imamura et al. 1999). The 3T3-L1 adipocytes were washed twice with ice-cold buffer A (137 mM NaCl; 20 mM Tris–HCl (pH 7-4) 1 mM CaCl₂, 1 mM MgCl₂, and 0-1 mM sodium orthovanadate) and were lysed with ice-cold lysis buffer (buffer A containing 1% NP-40 and 1 mM PMSF). The samples were centrifuged for 10 min at 13 000 g at 4 °C to sediment insoluble material; the supernatant fractions were subjected to immunoprecipitation with 4 μg anti-P85 antibody overnight at 4 °C. Immunocomplexes were then precipitated from the supernatant with protein A/G PLUS-agarose (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and washed. The washed immunocomplexes were incubated with phosphatidylinositol (Sigma) and γ-32P ATP for 10 min at 37 °C. After reactions were stopped by 6 M HCl, the radiolabeled lipid was extracted with a mixture of CHCl₃:MeOH (1:1), and separated by thin layer chromatography. The phosphorylated products were then visualized by autoradiography.

**Transduction of GLUT4-green fluorescence protein (GFP) in CHO cells**

Chinese Hamster Ovary cells (CHO, obtained from ATCC) were maintained in 10% FCS–DMEM and plated. On the next day, the 95% confluent cells were transfected with GLUT4-eGFP plasmid (kindly provided by Alan R Saltiel) (Inoue et al. 2003) using Lipofectamine 2000 according to the manufacturers’ protocol. At 48 h post-transfection, the cells were serum starved for 8 h and treated with Rb₁ for 3 h or insulin for 30 min. The translocation of GLUT4 was observed under fluorescence microscopy (Leica, Wetzlar, Germany).

**Statistical analyses**

Quantitative data were presented as the mean ± S.E.M. Statistical analysis was performed using Student’s t-test or ANOVA with SPSS software (Chicago, IL, USA). The significance of differences was set at P<0.05.

**Results**

**Ginsenoside Rb₁ stimulates basal and insulin-mediated glucose uptake in 3T3-L1 adipocytes and C2C12 myotubes**

To observe the effect of Rb₁ on glucose transport, 3T3-L1 cells were induced to mature adipocytes and then incubated with various concentrations of Rb₁ for different lengths of time, after which the rate of glucose uptake was measured. Rb₁ induced a time- and dose-dependent increase in glucose uptake rates in 3T3-L1 adipocytes and C2C12 myotubes. Rb₁ exerted an initial significant effect at 0-01 μM (168% of Con; P<0.05) and maximal effect at 1 μM in glucose uptake (185% of Con; P<0.05; Fig. 1A). However, the effect of Rb₁ was less potent than that of insulin (P<0.05, Fig. 1C). With 1 μM Rb₁,
glucose uptake was augmented maximally at 3 h of treatment with Rb1 (P<0.05; Fig. 1B). Furthermore, after 3 h of incubation with 1 μM and 10 μM Rb1, 100 nM insulin-mediated glucose uptake was increased significantly (119 and 118% of Con with insulin, P<0.05; Fig. 1C). Accordingly, Rb1 was used in the following experiments at a concentration of 1 μM for 3 h.

The effect of Rb1 on glucose transport was also investigated in C2C12 myotubes. The potency of Rb1 was less than that in adipocytes, but comparative with that of 100 nM insulin in C2C12 myotubes (P>0.05; Fig. 2C). Rb1 also induced a significant increase in insulin-mediated glucose uptake, 43% at a concentration of 1 μM and a time of 3 h (P<0.05; Fig. 2C).

**Figure 1** Ginsenoside Rb1 stimulated basal and insulin-mediated glucose uptake in 3T3-L1 adipocytes. (A) Adipocytes were incubated with the indicated concentrations of Rb1 for 3 h or (B) with 1 μM Rb1 for the indicated times before 2-DOG uptake measurements. (C) After incubation with the indicated concentrations of Rb1 for 3 h, 2-DOG uptake in adipocytes were measured in the absence or presence of 100 nM insulin (Ins) for 30 min. Data represent mean±S.E.M. of three to four independent experiments. *P<0.05 vs 0 μM or 0 h; **P<0.05 vs 0 μM with insulin.

**Figure 2** Ginsenoside Rb1 stimulated basal and insulin-mediated glucose uptake in C2C12 myotubes. (A) Myotubes were incubated with the indicated concentrations of Rb1 for 3 h or with 100 nM insulin (Ins) for 30 min or (B) with 1 μM Rb1 for the indicated times before 2-DOG uptake measurements. (C) After incubation with 1 μM Rb1 for 3 h, 2-DOG uptake rates in myotubes were measured in the absence or presence of 100 nM insulin (Ins) for 30 min. Data represent mean±S.E.M. of two independent experiments. *P<0.05 vs 0 μM Rb1 or 0 h; **P<0.05 vs 0 μM with insulin.
Ginsenoside Rb1 promotes translocations of GLUT1 and GLUT4 in 3T3-L1 adipocytes

For assessing whether the increased glucose transport induced by Rb1 in 3T3-L1 adipocytes was due to the translocations of GLUT1 and GLUT4, which are two predominant forms of glucose transporters expressed in 3T3-L1 adipocytes, the amounts of glucose transporter protein in LDM and PM fractions in the basal state, and after treatment with Rb1 or insulin were measured. Rb1 induced a significant increase of GLUT1 and GLUT4 protein in the PM content (P < 0.05, respectively, Fig. 3A and B). In addition, a significant decrease of GLUT4 protein and a modest but no significant decrease of GLUT1 in the LDM fraction was observed after insulin treatment (P < 0.05; P > 0.05 respectively, Fig. 3A and B). However, the levels of GLUT1 and GLUT4 in the LDM fraction did not show significant changes in response to Rb1 (Fig. 3A and B). The experiment in CHO cells transfected with GLUT4-eGFP demonstrated that Rb1 induced translocation of GLUT4 to the cell surface (Fig. 3C).

Ginsenoside Rb1 activates IRS-1, PI3K, and AKT in adipocytes

To observe the effects of Rb1 on the insulin signaling pathway, the activations of IR, IRS-1, PI3K, and AKT were measured. As expected, insulin stimulation resulted in augmentation of IR-β (Y1150/1151), IRS-1 (Y612) tyrosine phosphorylation, and AKT (Ser473) serine and threonine (Thr308) phosphorylation (Fig. 4A and B) and increased the activity of PI3K (Fig. 4C). Rb1 also significantly enhanced IRS-1 (Y612), AKT (Thr308) phosphorylation (P < 0.05 respectively; Fig. 4A and B) and stimulated the activity of PI3K (Fig. 4C). However, Rb1 treatment did not produce detectable change in tyrosine phosphorylation of IR-β (Fig. 4A). Thus, we concluded that Rb1 activates IRS-1 in the absence of the activation of IR, while activating PI3K and AKT.

Figure 3  Ginsenoside Rb1 promoted the translocations of GLUT1 and GLUT4 in 3T3-L1 adipocytes. (A) Adipocytes were incubated with 1 μM Rb1 for 3 h or 100 nM insulin for 30 min, and then subcellular membrane fractions were extracted and subjected to immunoblot analysis. (B) The immunoblotting signals of GLUT1 and GLUT4 in LDM and PM fractions from five independent experiments were quantified using densitometer. Data represent mean ± S.E.M. *P < 0.05 versus LDM of Con, # P < 0.05 versus PM of Con. (C) GLUT4-eGFP transfected CHO cells were treated with 1 μM Rb1 for 3 h or 100 nM insulin for 30 min, and then the translocation of GLUT4 was photographed under fluorescence microscopy. The arrowhead points to GLUT4-eGFP accumulated at cell membrane.

Figure 4  Effects of ginsenoside Rb1 on the insulin signaling pathway in 3T3-L1 adipocytes. (A) Adipocytes were treated with 1 μM Rb1 for 3 h or 100 nM insulin for 30 min; total protein was extracted and subjected to immunoblot analysis with the indicated antibodies. (B) The immunoblotting signals of the indicated proteins for Rb1 and insulin from three independent experiments were quantified using densitometer. Data represent mean ± S.E.M. *P < 0.05 versus Con. (C) Adipocytes were treated with 1 μM Rb1 for 3 h or 100 nM insulin for 30 min, and then total protein was extracted for assay of PI3K activity and immunoblotting with anti-p85 antibody.

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Inhibition of PI3K abrogates glucose uptake and translocations of GLUT1 and GLUT4 induced by ginsenoside Rb1

Whether enhancing glucose transport induced by Rb1 is associated with the activation of PI3K was determined next. Pretreatment with HNMPA-(AM)3 (specific insulin receptor tyrosine kinase inhibitor), and wortmannin and LY294004 (specific PI3K inhibitors) markedly inhibit insulin-mediated glucose uptake in adipocytes ($P<0.05$ versus insulin control, Fig. 5), as previously reported (Li et al. 2005). Increased glucose uptake induced by Rb1 was inhibited by both wortmannin and LY294004 ($P<0.05$ versus Rb1 control, Fig. 5); however, this was not significantly affected by HNMPA-(AM)3. This result was consistent with that of Rb1-activated IRS-1 independent of IR (Fig. 4). To assess the association of Rb1-induced translocations of GLUT1 and GLUT4 with the enhanced activity of PI3K, GLUT1 and GLUT4 in the LDM and PM were measured in adipocytes pretreated with wortmannin. Wortmannin prevented the translocation of GLUT4 from the LDM to PM fractions induced by Rb1 or insulin ($P<0.05$ or 0.01 respectively; Fig. 6A and C). GLUT1 in PM also showed a tendency to decline in PM in the presence of wortmannin, but it was not statistically significant (Fig. 6A and B). Therefore, we concluded that the activation of the insulin signaling cascade, including IRS-1, PI3K, and AKT, is required for Rb1-induced glucose uptake and translocations of GLUT4, and to some extent, of GLUT1.

Discussion

In the present study, we observed that ginsenoside Rb1 stimulated basal and insulin-mediated glucose uptake in insulin-sensitive cells, including adipocytes and muscle cells, although the potency of Rb1 was much less than that of insulin. Previous studies have shown that ginseng was able to reduce blood glucose level in type 2 diabetes patients (Vuksan et al. 2008), normal people (Reay et al. 2006), and diabetic mice (Attele et al. 2002, Xie et al. 2005).
uptake in target tissues is a critical step in maintaining glucose homeostasis and in clearing the postprandial glucose load (Herman & Kahn 2006), our results may explain partially the mechanism of ginseng’s hypoglycemic effects.

To understand the mechanism underlying the stimulation of glucose transport by Rb1, we examined the effect of this compound on GLUT1 and GLUT4 redistribution and the insulin signaling pathway in 3T3-L1 adipocytes. Both GLUT1 and GLUT4 are essential transporters of glucose uptake in adipocytes (Herman & Kahn 2006, Liao et al. 2006). In the present study, we showed that Rb1 was able to stimulate basal glucose uptake in adipocytes simultaneously accompanied by promoted translocations of the GLUT1 and GLUT4 protein. Previous literature has documented that the PI3K pathway plays an important role in the insulin signaling cascade leading to glucose transport translocation (Khan & Pessin 2002, Watson et al. 2004). The inhibition of PI3K activity blocks the translocation of both GLUT1 and GLUT4 in adipocytes (Clarke et al. 1994, Perrini et al. 2004). In our study, GLUT1 and GLUT4 translocations induced by Rb1 was associated with the activation of PI3K. Pretreatment of adipocytes with a specific PI3K inhibitor, wortmannin, abrogated Rb1-induced GLUT1 and GLUT4 translocations as well as glucose uptake. Therefore, glucose transport and transporter translocation induced by Rb1 is dependent on the involvement of PI3K. A recent study found that ginsenoside Re, which is another kind of ginsenosides, protopanaxatriol, also activated insulin signaling pathway independent of the insulin receptor and stimulated glucose uptake in adipocytes (Zhang et al. 2008). These results may indicate that insulin-like property is the underlying mechanism of action of ginsenosides.

In the insulin pathway, the activation of PI3K results from the autophosphorylation of intracellular β subunit tyrosine residues of the insulin receptor, subsequent activation of downstream signaling molecules, including IRS-1, and then leads to phosphorylation of AKT which is downstream from PI3K (Khan & Pessin 2002, Watson et al. 2004). To determine the engagement of other proteins in the insulin signaling cascade, we examined several upstream and downstream elements of PI3K. In 3T3-L1 adipocytes, Rb1 was found to enhance the phosphorylation of IRS-1 at position 612 and AKT at position 308 without a detectable activation of IR-β. Although we did not directly measure the IRS-1-associated PI3K activity, because the tyrosine at position 612 is important for IRS-1 to activate PI3K and mediate the translocation of GLUT4 (Esposito et al. 2001), our results suggest that Rb1 stimulates PI3K by a similar mechanism involving IRS-1 as that in insulin action. However, unlike insulin action, Rb1 stimulated IRS-1 independent of insulin receptor activation. This conclusion was confirmed by the fact that the inhibition of IR with specific IR inhibitor did not significantly affect Rb1-induced glucose transport. It is not clear how Rb1 initiate the activation of the IRS/PI3K/AKT pathway. A distinct mechanism may be involved in the activation of insulin signaling cascade induced by Rb1. Although the activation of IR is necessary for insulin to stimulate the insulin signaling pathway (Khan & Pessin 2002), actually insulin’s action could be mimicked independent of insulin receptor activation in some cases. For example, dehydroepiandrosterone (DHEA), one of the steroid hormone, activated IRS/PI3K to result in the translocations of GLUT1 and GLUT4 and the augmentation of glucose transport in adipocytes in the absence of the activated insulin receptor. The authors suggested that DHEA might bind to specific cell-surface G-protein receptors to activate the IRS/PI3K pathway. Another report indicated that selenium promoted glucose transport and anti-lypysis in an IRS-1/PI3K-dependent manner with no activation of the insulin receptor (Heart & Sung 2003). JAK2, as a growth hormone (GH) receptor-associated, GH-activated tyrosine kinase has been shown to interact with IRS-1 and stimulate binding of IRS-1 to the 85 kDa regulatory subunit of PI3K, which provides a biochemical basis for the insulin-like metabolic effects of GH (Argetsinger et al. 1995). Owing to their structural features of steroid skeleton and lipid-soluble ability, ginsenosides traverse the PM and initiate genomic effects; on the other hand, they might interact with some membrane receptor (Attele et al. 1999). Therefore, we speculate that Rb1 may initiate insulin signaling pathway at IRS-1 responsive for the translocation of glucose transporters by other membrane receptors, which remain further clarified.

Our previous studies have shown that Rb1 could bind to PPARγ and regulate its expression to promote adipogenesis in adipocytes, suggesting that Rb1 might be a PPARγ ligand (Shang et al. 2007a,b). In this study, we found that besides promoting adipocytes differentiation, Rb1 is also able to stimulate glucose transport in mature adipocytes through activating some elements of the insulin signaling pathway. It has been shown that troglitazone acutely stimulated glucose transport by activating PI3K in skeletal muscle (Kausch et al. 2001) and promoting GLUT4 translocation in adipocytes and myotubes (Shintani et al. 2001, Yonemitsu et al. 2001). These results imply that independent of activating PPARγ, ginsenoside Rb1 has a direct effect on insulin signaling pathway, similar to the effect pattern of troglitazone on adipose tissue.

In conclusion, we have demonstrated that ginsenoside Rb1 stimulates basal and insulin-mediated glucose uptake in adipocytes and muscle cells. Rb1 increases basal glucose transport and the translocations of GLUT1 and GLUT4 in adipocytes through an insulin-like signaling pathway. These results help to clarify the glucose-lowering action and anti-diabetic properties of ginseng and ginsenosides and further support their therapeutic importance in the treatment of diabetes.

Declaration of interest

The authors state no conflict of interest.

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