Effects of PKGI-dependent pathway on glucose uptake in rat cultured podocytes.

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INTRODUCTION AND OBJECTIVES

Podocyte are highly specialized cells that cover the glomerular capillaries, and they are a key part of the glomerular filtration barrier by contributing to the formation of the slit diaphragm (1). Podocytes are uniquely sensitive to insulin, they have demonstrated similarities to skeletal muscle and fat cells with respect to insulin stimulated glucose uptake kinetics and the expression of glucose transporters (GLUT) (2). Insulin signaling is regulated by oxidative stress and intracellular energy levels (3). We demonstrated recently that superoxide anion generation or insulin increases dimerization of protein kinase G type Iα (PKGIα) subunits, leading to podocyte dysfunction (4,5). Here we investigated whether PKGI-dependent pathway can modulate the insulin signaling and glucose transport system in rat cultured podocytes.

METHODS

To determine whether cGMP-dependent protein kinase is involved in the insulin regulation of glucose transport, we measured insulin-dependent glucose uptake into cultured rat podocytes under conditions of modified PKG activity using pharmacological (PKG activator or inhibitor) and biochemical (siRNA PKGIα, siRNA insulin receptor) means. Protein expression was measured with Western blot and immunofluorescence. Glucose uptake was measured with radioactive method.

RESULTS

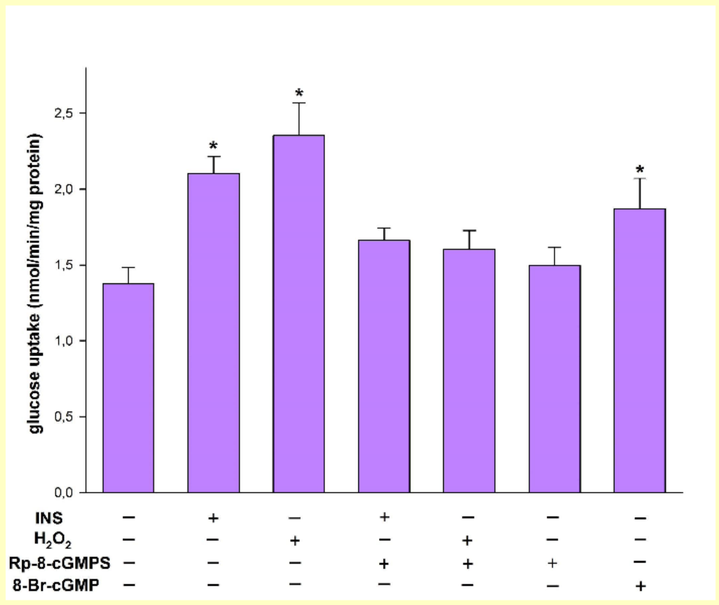

Figure 1. Effect of PKGI inhibitor on insulin-stimulated glucose uptake into cultured rat podocytes.
Cells were pre-incubated with Rp-8-cGMPS (PKGI inhibitor) or buffer for 20 min. Uptake was measured after the addition of 1μCi of [1,2-^{3}H]-deoxy-D-glucose diluted in non-radioactive glucose to final concentration of 50 μM and 300 nM insulin, 100 μM H_2O_2 or 100 μM 8-Br-cGMP (PKGI activator) for 3 minutes. Values are the mean ± SEM (n=4), *P<0.05 compared to control.

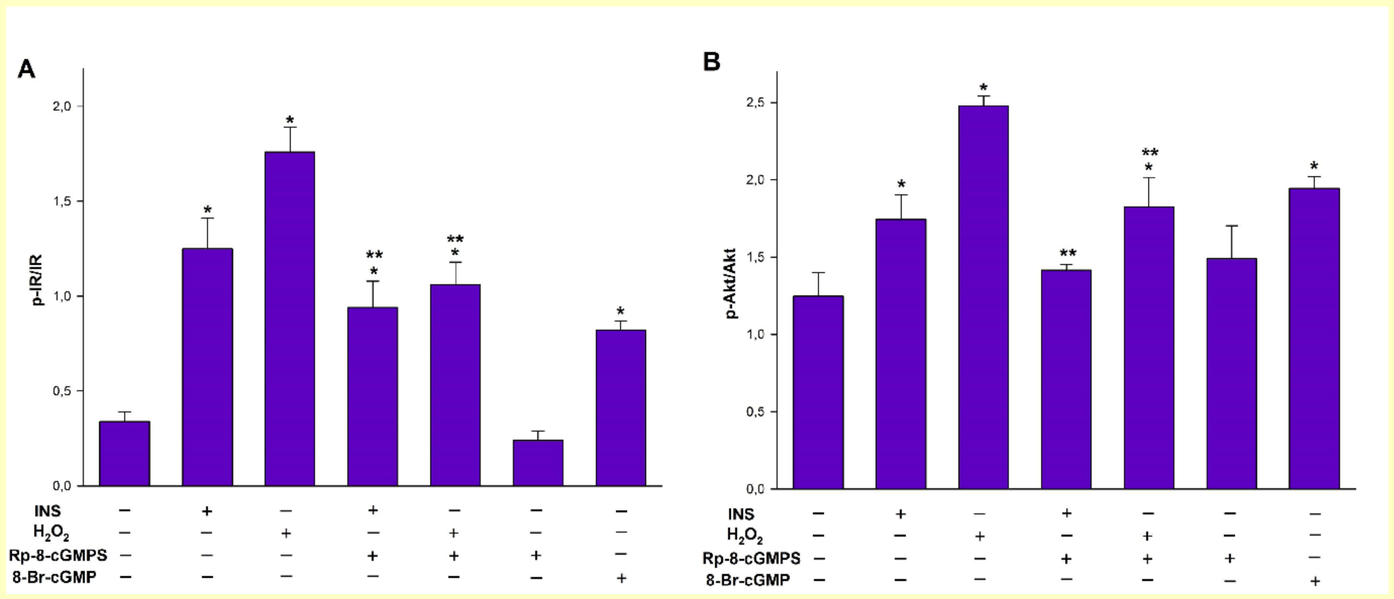

Figure 2. Insulin enhances insulin signaling pathways via PKG activation.
Cells were pre-incubated with Rp-8-cGMPS (100 μM for 20 min), and then incubated with insulin (300 nM, 3 min), H_2O_2 (100 μM, 3 min) or 8-Br-cGMP (100 μM, 3 min) The cell lysates (20 μg) were analyzed by Western blotting. Quantitative densitometric analysis was used to determine the ratio of (A) p-IRβ (Tyr1150/1151) to IRβ and (B) p-Akt1/2/3 (Ser473) to Akt1/2/3. Values are the mean ± SEM (n=4), *P<0.05 vs. control, **P<0.05 vs. the appropriate control with insulin or H_2O_2.

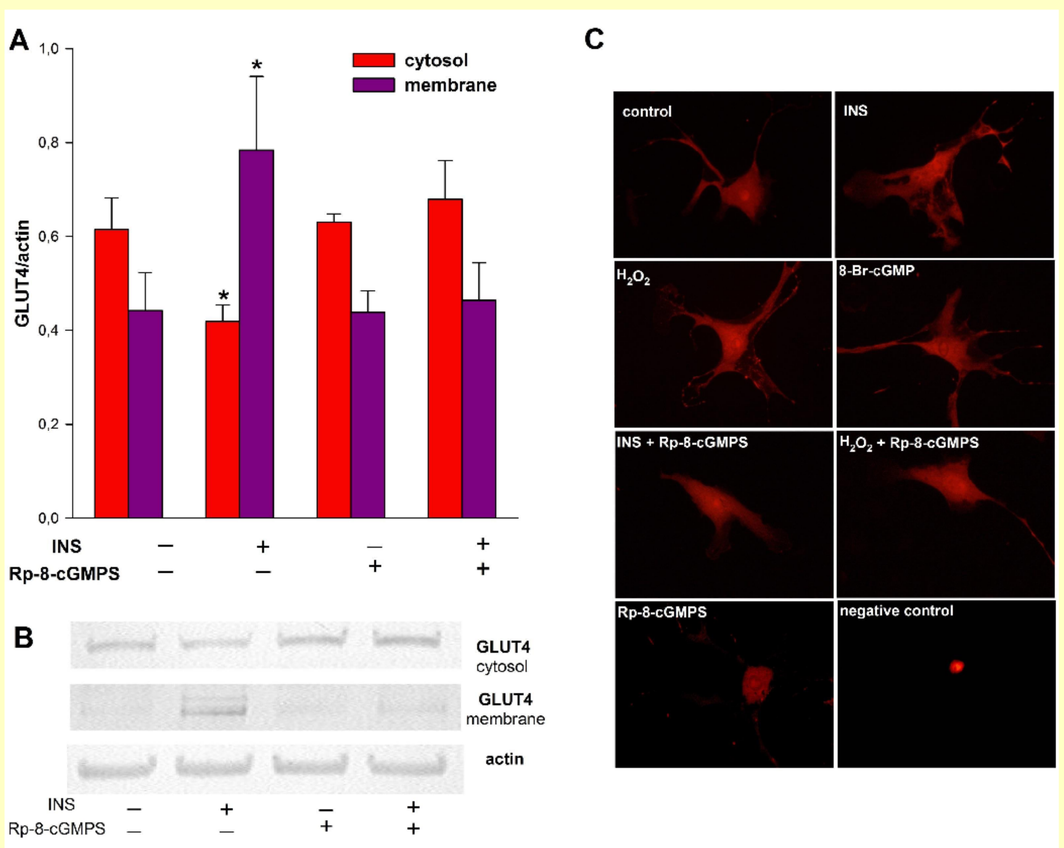

Figure 3. The effect of PKGI signaling pathways on expression and translocation of the GLUT4 transporter.
Results of Western blot analysis of cytosolic and membrane fractions. Values are the means ±SEM (n=4). *P < 0.05 vs. control (A).Representative immunoblots for cytosolic and membrane fractions (B). Rat podocytes seeded onto coverslips were preincubated with Rp-8-cGMPS (100 μM for 20 min, PKGI inhibitor) and then incubated for 3 min in the absence or presence of 300 nM insulin, 100 μM H_2O_2 or 100 μM 8-Br-cGMP (PKGI activator). Cells were then immunostained with anti-GLUT4 antibody (C).

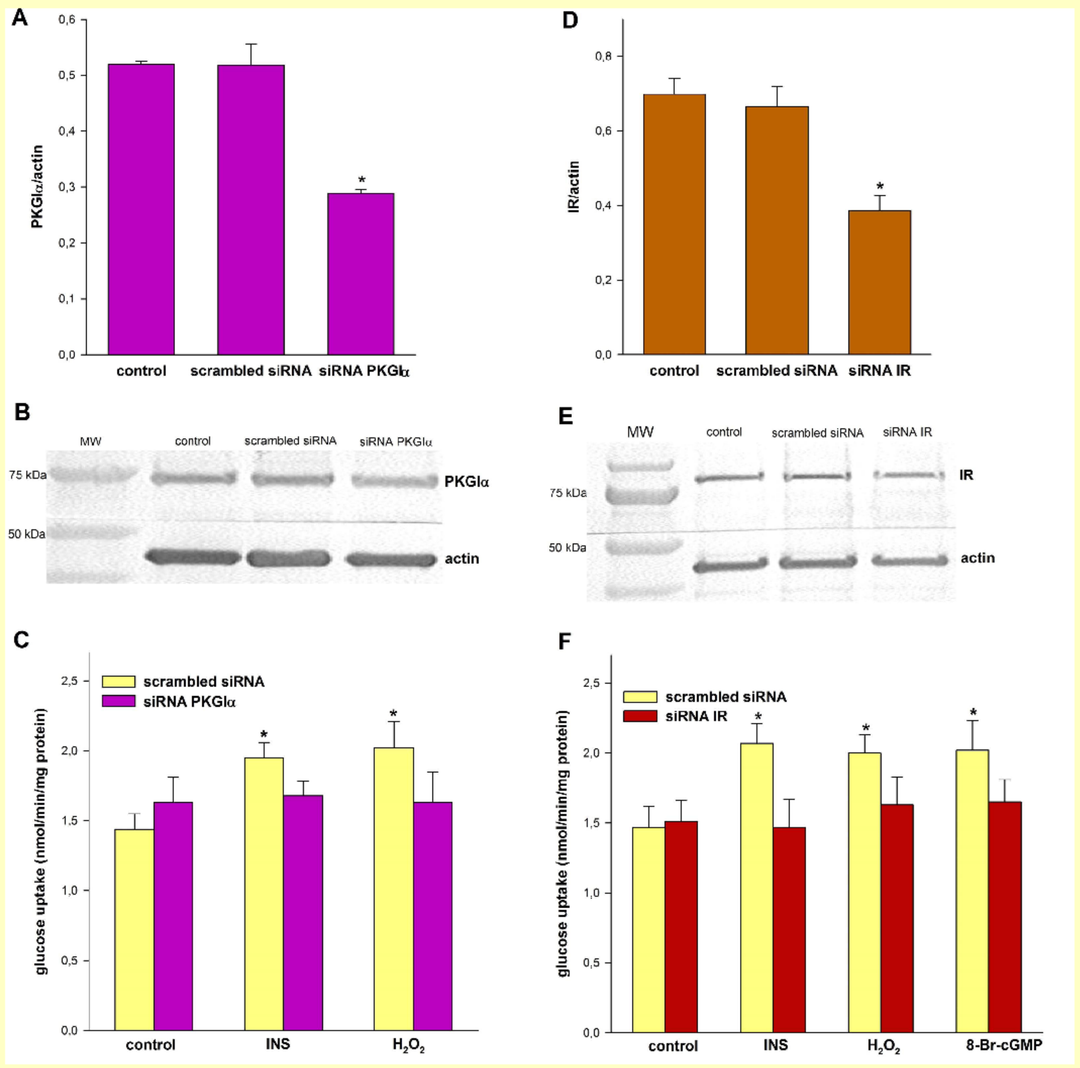

Figure 4. The effect of PKGIα and IRβ gene silencing on insulin-stimulated glucose uptake into cultured rat podocytes.
The effect of PKGIα or IRβ small interfering RNA (siRNA) and scrambled siRNA on PKGIα (A) and IRβ (D) proteins expression. Densitometry of the PKGIα and IRβ bands were normalized to the actin band. Values are the mean±SEM of four independent experiments. *P<0.05 versus transfection with scrambled siRNA or non-transfected podocytes (control). Representative immunoblots show PKGIα, IRβ and actin expression in homogenates from transfected and non-transfected podocytes (B,E). The effect of downregulation of PKGIα (C) or IRβ (F) on glucose uptake. Uptake was measured after the addition of 1μCi of [1,2-^{3}H]-deoxy-D-glucose diluted in non-radioactive glucose to a final concentration of 50 μM and 300 nM insulin, 100 μM H_2O_2 or 100 μM 8-Br-cGMP for 3 minutes. The values are the mean±SEM of three independent experiments. *P<0.05 compared to control.

CONCLUSIONS

The overall conclusion of our present observations is a novel mechanism in which insulin significantly increases glucose uptake into podocytes *via* the activation of protein kinase G type Iα. This signaling may play a potential role in the prevention of insulin resistance under conditions associated with oxidative stress.

References:
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