Effect of insulin on PKGIα-dependent activation of BK_{Ca} in podocytes; implications for the regulation of actin cytoskeleton and filtration barrier permeability.

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Abstract

Podocytes are dynamic polarized cells that lie on the surface of glomerular capillaries and comprise an essential component of the glomerular filtration barrier. The insulin signaling to podocytes is essential for normal glomerular function. Insulin signaling is regulated by oxidative stress and intracellular energy levels. We demonstrated recently that insulin increases activation of protein kinase G type Iα (PKGIα) subunits, leading to podocyte dysfunction. Here we investigated whether large-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) is involved in insulin-dependent regulation of filtration barrier permeability in PKGIα-dependent manner.

We assessed changes in insulin-induced glomerular permeability by measuring glomerular capillary permeability to albumin in isolated glomeruli from Wistar rats and transmembrane albumin flux in cultured rat podocytes. Expression of BK_{Ca}, PKGIα and upstream proteins was confirmed in the podocytes using RT-PCR, Western blot and immunofluorescence.

The RT-PCR showed the presence of mRNAs encoding the pore forming α subumit and four accessory β subunits of BKca in primary cultured rat podocyte. The BKca inhibitor iberiotoxin (ibTX) abolished insulin-dependent glomerular albumin permeability in Wistar rats and PKGI-dependent transmembrane albumin flux in cultured podocytes. We confimed the role of BKca in insulin-evoked increases in albumin permeability in podocytes with BKca siRNA. Moreover, the ibTX abolished insulin induce changes in the phosphorylation of the PKG target proteins MYPT1 and RhoA, and disruption of the actin cytoskeleton.

The results indicate that insulin increases filtration barrier permeability through activation of BK_{Ca} channels *via* PKGIα in podocytes. The experimental results suggest a molecular mechanism that could explain podocyte injury.

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