Supplementary Methods

Materials

Polyclonal anti-total-Akt and anti-phospho-Akt (Ser-473) antibodies were from Cell Signaling Technology. Polyclonal anti-GRK2 (C-15) and polyclonal anti-GRK3 (C-14) antibodies were from Santa Cruz Biotechnology. GRK 2/3 monoclonal antibody was from Upstate Cell Signaling Solutions. Polyclonal anti-GRK5 and anti-GRK6 antibody were from Santa Cruz Biotechnology. Polyclonal anti-phospho-eNOS (Ser-1179) and monoclonal anti-eNOS antibodies were from BD Transduction Laboratories. Anti-rabbit and anti-mouse IgG/horseradish peroxidase conjugated antibodies were from Promega. GRK 2 -/+ and +/+ mice \(^{13}\) were the kind gift of Dr. Julia Walker and Dr. Marc Caron (Duke University Medical Center). Recombinant Akt (active and inactive) was from Upstate Cell Signaling Solutions. Recombinant GRK2 was kindly provided by Darrel Capel and Dr. Robert Lefkowitz (Duke University Medical Center).

Cell isolation and culture

The method for isolation of sinusoidal endothelial cells is as follows. After in situ perfusion of the liver with 20 mg% pronase (Roche Molecular Biochemicals), followed by collagenase (Crescent Chemical), dispersed cell suspensions were layered on a discontinuous density gradient of 8.2% and 15.6% Accudenz (Accurate Chemical and Scientific). Endothelial cells, present in the lower layer, were further purified by centrifugal elutriation (18 ml/min flow) and were grown on collagen coated dishes in medium containing 20% serum (10% horse/10% calf). Cell isolation from genetically altered GRK2 and littermate control wild-type mice were as for rats, with the exception that flow rates and enzyme concentrations were reduced in proportion to total body weight. The purity of primary endothelial cell isolates was greater than 95%.

Animal model of portal hypertension and gene transfer in vivo

Liver injury with portal hypertension was induced by performing bile duct ligation (BDL) in 450-500g male retired breeder Sprague-Dawley rats as described \(^{14}\). In brief, rats were anesthetized, laparotomy was performed under sterile technique, and the common bile duct was isolated and ligated. In sham animals, surgery was identical, except ties were not placed and the duct was not ligated. In some experiments, adenovirus was administered. In these experiments, 6 days after bile duct ligation surgery, solutions of saline containing adenoviral constructs (titers were \(1 \times 10^9\) pfu/kg for Ad-EV, Ad-myr.Akt for all rats) were administered via the femoral vein. Four days later, portal pressure was measured (as below) and cell isolations (as above) were performed. For experiments with Ad-GRK2, this virus or a control virus was administered via the femoral vein (at a concentration
of 1 x 10^{10} pfu/kg) to normal rats. In genetically altered GRK2 and control wild-type mice, portal pressure (as below) was measured 10 days after bile duct ligation or sham operation.

**Adenovirus**

Viral titers were measured by standard plaque assay using 293 cells, and virus was stored in 40% glycerol at -80°C. Myristoylated constitutively active Akt (Ad.myr-Akt), and a dominant negative Akt construct (Ad-dn.Akt) containing mutations at amino acid 308 (T→A) and amino acid 473 (S→A) (provided by Dr. Ken Walsh, Boston University Medical Center) or an identical adenovirus without insert DNA (Ad-EV) were utilized. Adenoviruses encoding full length bovine GRK2 were provided by Dr. Walter Koch and Dr. Karsten Peppel (Duke University Medical Center). For cell culture experiments, viruses were typically used at a multiplicity of infection (m.o.i.) of 250. For *in vivo* experiments, viral titers were 1 x 10^9 to 1 x 10^{10} pfu/kg.

**Immunoprecipitation**

Sinusoidal endothelial cells were rinsed in PBS, and lysed in buffer (137 mM NaCl, 2 mM EDTA, 10% glycerol, 1% Triton X-100, 20mM Tris-HCl, pH8.0) containing protease inhibitors (1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 mM NaF, 1 mM leupeptin, 1 g/ml pepstatin). Lysates were centrifuged at 11,000 G at 4°C for 10 min. Supernatants (100 µg to 200 µg protein) were incubated with 5 µg of the specified antibody overnight at 4°C followed by 50 µl of protein A-beads for 4 hours at 4°C with gentle rotation. The beads were then pelleted at 7,000 G for 2 min and washed 3 times in 1 ml ice-cold buffer (5mM Tris-HCl, pH 7.4, 20mM NaCl, 0.5% v/v Triton X-100 containing 1mM PMSF, and 50 g/ml aprotinin). Antibody-protein conjugates were removed from beads by boiling (5 minutes) and samples were then subjected to SDS-PAGE and immunoblotting as below.

**siRNA**

The sequences designed to target rat GRK2 were as follows: siRNA 1 sense sequence - 5'-GGAAUACGAGAAACUGGACUU-3’ and antisense sequence - 5'-GUCCAGUUUCUCGUAAUCCUU-3’; siRNA 2 sense sequence - 5'-GCAAGUGUCUCGUAAUAAUU-3’ and antisense sequence - 5’-PUUAAGCAGAGACACUUGCUU-3’ using Ambion web-based criteria. Control siRNA included nonspecific siRNA duplexes in a scrambled format as described (Dharmacon Research). Sequences were as follows: sense sequence 5’-UAGCGACUAACACAUCAAUU-3’; antisense sequence: 5’-UUGAUGGUUUAGUGCUUAAU-3’). siRNAs were synthesized by a transcription-based method using the Silencer siRNA construction Kit (Ambion) according to manufacturer’s instructions.