Insulin Receptor (IR) and Glucose Transporter 2 (GLUT2) Proteins Form a Complex on the Rat Hepatocyte Membrane

Michael L. Eisenberg¹, Ajay V. Maker¹, Lori A. Slezak¹, Jaimie D. Nathan¹, Kumudesh C. Sritharan^{1,2}, Bhanu P. Jena¹, John P. Geibel^{1,2} and Dana K. Andersen¹

Departments of ¹Surgery and ²Physiology Yale University School of Medicine

Key Words

Insulin • GLUT2 • Receptor-Transporter Complex • Hepatic Glucose Production • Hepatocytes

which forms a mechanism of insulin-mediated hepatic glucose regulation.

Copyright © 2005 S. Karger AG, Basel

Abstract

The hepatic glucose transporter, GLUT2, facilitates bidirectional glucose transport across the hepatocyte plasma membrane under insulin regulation. We studied the interactions of IR and GLUT2 proteins to determine whether they are physically coupled in a receptor-transporter complex. By comparing endosome and plasma membrane IR and GLUT2 ratios before and after feeding, it was determined that IR and GLUT2 are internalized in a fixed ratio. When solubilized hepatocytes were immunoprecipitated with antibodies against either IR or GLUT2, both proteins co-precipitated. The association of IR and GLUT2 was further assessed by confocal microscopy. Sections of fed liver were incubated with fluorescein-tagged anti-GLUT2 or Texas Red-tagged anti-IR. Colocalization was observed both at the plasma membrane and in the cytosol. Fluorescence-resonance energy transfer studies further confirmed this association. We conclude that IR and GLUT2 form a receptor-transporter complex in hepatocytes,

Introduction

Hepatic glucose production is essential to maintaining normal circulating glucose levels. During low glucose states, the liver serves as the primary site for glucose production through glycogenolysis and gluconeogenesis. When glucose levels rise, however, as in the post-prandial state, hepatic glucose production is rapidly reduced to low levels in response to elevated circulating insulin [1-6].

Glucose transporter 2 (GLUT2), the primary hepatic liver transporter, is a low affinity, high capacity transporter expressed in high levels on the sinusoidal membranes of hepatocytes, the basolateral membrane of intestinal epithelial cells, renal proximal tubule cells, and pancreatic beta-cells [7-10]. In non-fed, low blood glucose states, GLUT2 is present in large amounts on the hepatocyte plasma membrane, and facilitates the net hepatic efflux

of glucose. Insulin perfused livers, however, show a marked decrease in the presence of GLUT2 in the cell membrane fraction which is dose-dependent, and parallels the insulin-mediated reduction in hepatic glucose production [11]. Subsequent studies in our laboratory have shown this disappearance to be mediated by an endocytotic process [12].

Insulin facilitates glucose clearance from the blood by activating glucose uptake into peripheral tissues as well as through the inhibition of hepatic glucose production [1, 13]. The insulin receptor (IR) exists as a heterotetrameric ($\alpha_2\beta_2$) structure with the α -subunits containing the insulin binding site and the β -subunits holding the transmembrane domain and tyrosine kinase activity. [14, 15]. Insulin binding to the extracellular portion of IR leads to autophosphorylation of specific intracellular tyrosine residues. Once phosphorylated, IR is able to phosphorylate and activate intermediate docking proteins such as IRS-1, -2, -3, and -4 which initiates internalization, and elaborate intracellular signaling cascades [16, 17]. Of note, insulin binding stimulates the internalization of IR in a manner that parallels the plasma membrane regulation of GLUT2 [18].

The precise role that IR plays in the regulation of GLUT2, however, remains unclear. Previous studies have suggested GLUT2 transcriptional regulation as the key signaling event that regulates GLUT2 [19-22], while other studies have explored the role of GLUT2 phosphorylation [23]. However, because both IR and GLUT2 are internalized from the hepatocyte plasma membrane upon insulin binding, the possibility exists that the two transmembrane proteins are physically linked and regulate hepatic glucose production as a complex.

The aim of this study was to establish whether a physical link between IR and GLUT2 exists that serves as a mechanism of insulin regulation of hepatic glucose production. We hypothesized that as IR enters the endocytic compartment after insulin binding, GLUT2 is simultaneously removed from the plasma membrane due to a physical link with IR. Our membrane fractionation studies show not only that IR and GLUT2 are transferred from the plasma membrane to the endosomal compartment, but also that endocytosis occurs in a similar ratio between the two proteins. In addition, immunofluorescence and fluorescence resonance energy transfer (FRET) show that the proteins colocalize on the hepatocyte membrane and are separated by 20-60 Å [24, 25]. Immunoprecipitation studies further show that IR and GLUT2 are physically linked. These data establish the existence of a physical coupling of IR and GLUT2 on

the hepatocyte membrane, and suggest that this forms a novel mechanism for the regulation of hepatic glucose production.

Materials and Methods

Animal Protocol for Internalization Studies

Sprague-Dawley rats ($n = 35$) weighing 150-200 mg were anesthetized by intraperitoneal injection of either sodium thiopental (1 mL/kg) or phenobarbital (0.07 mg/g). A ventral midline incision was made to expose the viscera. For the fractionation studies, prior to liver excision, a 2-mm gastrotomy was made in the anterior gastric wall 1 cm proximal to the pylorus, and the proximal duodenum was intubated with a rubber catheter. A duodenal feeding bolus of 0.5 mL/100 g of a liquid meal (1 kcal/mL; 14% protein, 53% carbohydrate, 33% fat; NuBasics, Clintek, Deerfield, IL, USA) was administered. Forty-five minutes after duodenal feeding or intubation alone (fasting), livers were harvested. All animal protocols were reviewed and approved by the Yale Animal Care and Use Committee.

Liver harvest and tissue preparation for confocal studies

At laparotomy, the distal aspect of the right main lobe of the liver from fed animals was excised, rinsed in Ringers lactate solution, and promptly minced at 4°C. Samples were processed in 72% sucrose solution and re-equilibrated in 1XPBS at the time of sectioning. Tissue was snap frozen in blocks for sectioning on a sliding cryostat and cut into 5 micron sections. Sections were fixed in cold acetone and rehydrated in 120 mM phosphate solution for 40 minutes. Samples were then blocked with 8% bovine serum albumin in 120 mM phosphate solution and 0.7% Triton-X for one hour.

Fractionation of liver specimens

The subcellular fractionation protocol utilized was developed by the Department of Cell Biology at Yale University School of Medicine [26-28]. Briefly, livers ($n=16$) were harvested, minced, and homogenized in homogenization buffer (0.25 M sucrose, 10 mM triethanolamine, 10 mM acetic acid pH=7.4, 1 mM EDTA, 1 mM PMSF, 1 mM benzamidinium, 1 mM DTT). The crude homogenate was then centrifuged at 1,000 g at 4°C for 10 minutes. The pellet was discarded and the supernatant was layered onto a sucrose cushion (1.0 M sucrose in TEA buffer) and centrifuged at 100,000 g at 4°C for 45 minutes. The pellet was resuspended with sucrose/TEA buffer to a final concentration of 1.3 M. The solution was then overlaid with 1.1 M sucrose and 0.6 M sucrose (both in TEA buffer) and centrifuged at 133,000g at 4°C for 60 minutes. The endosome fraction was collected from the 1.1 M/0.6 M interface and the plasma membrane fraction was collected from the 1.3 M/1.1 M sucrose interface.

Hepatocyte Isolation for Immunoprecipitation Studies

Following liver exposure, the portal vein was isolated. Heparin (500 units/100 g body weight) was injected into the inferior vena cava and allowed to circulate for 1 minute. The

portal vein was cannulated, and the suprahepatic IVC was cut to allow perfusate to escape. The liver was perfused with Hanks A Solution (120 mM NaCl, 5 mM KCl, 0.4 mM KH_2PO_4 , 0.2 mM Na_2PO_4 , 25 mM NaHCO_3 , 0.5 mM EGTA, 0.1% D-Glucose) for 3–4 minutes. Next, the liver was perfused with Hanks B Solution (120 mM NaCl, 5 mM KCl, 0.4 mM KH_2PO_4 , 0.2 mM Na_2PO_4 , 25 mM NaHCO_3 , 0.4 mM MgSO_4 , 0.5 mM MgCl_2 , 3 mM CaCl_2 , 0.1% D-Glucose) with collagenase (0.05%/400 ml Hanks B) until liver digestion became visible. The liver was then removed and rinsed with L-15 in a culture dish. The capsule was then detached, and the hepatocytes were removed. The suspension was then filtered through an 80 μm mesh followed by a 45 μm mesh with L-15. The suspension was then brought up to 14 ml with L-15 in volume and centrifuged at 4°C for 2 minutes at 400 rpm. The supernatant was aspirated, and the pellet was resuspended in 14 ml of L-15 and centrifuged for 2 minutes at 400 rpm. The supernatant was aspirated off, and the pellet was resuspended in 7 ml of L-15.

Immunoprecipitations

4.5 $\times 10^5$ rat hepatocytes isolated from each of eleven fasted livers were vortexed in Lysis Buffer (140 mM NaCl, 10 mM KCl, 8.2 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , 1mM DTT, 1mM PMSF, 1 mM Benzamidine, 1% Triton, pH=7.5) in a final volume of 1150 μL . The lysates were cleared of insoluble material by centrifugation at 14,000 rpm for 10 min at 4°C, and the supernatant was precleared with 20 μL of 1:1 slurry of Protein G-Agarose (Sigma-Aldrich Co., St. Louis, MO) for 1 hr at 4°C. The supernatant was separated by centrifugation at 1,500 rpm for 1 min at 4°C and then precipitated by adding 20 μL of Protein G beads (previously incubated overnight at 4°C with 1 μg of primary antibody) for 2 hrs at 4°C. The beads were collected by centrifugation for 6 min at 4°C. The beads were washed two times with Wash I (400 mM NaCl, 50 mM Tris/HCl, 0.1% Triton, 0.02% NaN_3) and collected by centrifugation at 6,000 rpm for 5 min at 4°C. The beads were washed two additional times with Wash II (500 mM NaCl, 10 mM Tris/HCl, 0.02% NaN_3) and collected by centrifugation at 6,000 rpm for 4 min at 4°C. The proteins were solubilized in 60 μL of Sample Prep Buffer (1X diluted from 5X; 1.5 M Tris-HCl pH 6.8, 50% glycerol, 5% β -mercaptoethanol, 20% SDS, 0.002% bromophenol blue), boiled for 3 minutes, and centrifuged at 6,000 rpm for 1 min at 4°C. The samples were then transferred to a fresh tube and stored at -80°C.

Western Blot

Immunoprecipitation samples were separated by SDS-PAGE (7.5% acrylamide, Bio-Rad Laboratories, Hercules, CA) and electrotransferred to a Hybond-ECL nitrocellulose membrane (Bio-Rad Laboratories). The membrane probed for IR was blocked with PBS-Tween containing 3% skim milk for 1 hour, while the membrane probed for GLUT2 was blocked with PBS containing 5% BSA for 1 hour. After thorough washing with PBS-Tween (1X PBS with 1% Tween 20), the membranes were incubated with rabbit anti-IR (1:250, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) in PBS-Tween or donkey anti-GLUT2 (1:500, Santa Cruz Biotechnology, Inc.) in PBS-Tween for 1 hour. The membranes were then washed again in

PBS-Tween, incubated in donkey anti-rabbit (1:1,000, Amersham Corp, Arlington Heights, IL) in PBS-Tween or rabbit anti-goat (1:10,000, Pierce Chemical Co.) in PBS-Tween for 1 hour. After that, the membranes were thoroughly washed and developed with an ECL-Western Blot Kit (Amersham Corp).

Blots of fractionation study samples were performed in a similar manner except 12.5% SDS-polyacrylamide gels were used. In addition, a rabbit anti-rat GLUT2 antibody (1:50,000, Biogenesis, Sandown, NH, U.S.A.) was used as a primary antibody.

Immunostaining for confocal studies

Primary antibodies to GLUT2 (goat anti-rat, Santa Cruz Biotechnology, Inc.) and IR (rabbit anti-rat, Santa Cruz Biotechnology, Inc.) were prepared in a 1:20 dilution in 1% BSA-120 mM phosphate, and incubated overnight at 4°C. Slides were then washed and incubated with donkey anti-goat IgG fluorescein (FITC) tagged antibody (1:100, Santa Cruz Biotechnology, Inc.) and donkey anti-rabbit Texas Red tagged antibody (1:100, Santa Cruz Biotechnology, Inc.) in the dark for three hours at room temperature. Slides were then washed, mounted with glycerol-PBS, and placed at 4°C until imaged. Specimens were viewed on a Zeiss LSM 410 confocal microscope with a 40x oil immersion lens, images were processed using Adobe Photoshop v. 6.0. FITC was excited at 488 nm and emission collected with a 515–565 nm filter system. Texas Red was excited at 568 nm and emission collected at 670–810 nm to avoid overlap in emission spectra. Fluorescent resonance energy transfer (FRET) was conducted with a 488 nm excitation with a 670–810 nm emission. All background autofluorescence was corrected on all slides for all wavelengths.

Scanning Densitometry

Densitometric scanning analysis of developed films was performed using a Personal Densitometer and ImageQuaNT™ software (Molecular Dynamics, Sunnyvale, CA). The expected molecular weight of IR was taken to be 95 kDa, and for GLUT2 was 52 kDa. Volume analysis was performed by measuring a rectangular-shaped area of interest over the relevant band. The data was then normalized to an internal control of total cell lysate run on each gel.

Antibodies

For insulin receptor, a rabbit antibody against the carboxy terminus of the β -subunit of IR (Santa Cruz Biotechnology, Inc.) was used for immunoprecipitation and Western Blot. Immunoprecipitations of GLUT2 used a rabbit antibody against a portion of the extracellular domain of GLUT2 (Santa Cruz Biotechnology, Inc.). For Western blot of fractionation studies, rabbit anti-rat GLUT2 antibody was used. For Western Blot, a goat antibody against a portion of the carboxy terminus of the cytoplasmic domain of GLUT2 (Santa Cruz Biotechnology, Inc.) was used for immunoprecipitation and chemical cross-linking studies. Rabbit anti-rat GLUT2 antibody (1:50,000; Biogenesis) was used for fractionation studies. For development of the Western Blot, peroxidase labeled donkey anti-rabbit antibody (Amersham Corp) and rabbit anti-goat antibody (Pierce Chemical Co.) was used. As a control, we used a mouse antibody

Fig 1. Fractionation of hepatocytes showing compartmentalization of IR and GLUT2 before and after feeding. Representative Western blots used to quantify GLUT2 and IR in endosome and plasma membrane liver fractions from rats in fasted and fed states. Both transmembrane proteins displayed a similar membrane disappearance after feeding.

Fig. 2. Rat hepatocyte IR and GLUT2 are internalized in a similar ratio. Endosome (E) to plasma membrane (PM) ratio of GLUT2 and IR in fasted and fed rat livers are graphed simultaneously. A linear relationship between the two membrane proteins ($r=0.81$, $p<0.0002$) becomes apparent, implying a coupling between the internalization of IR and GLUT2.

against Na,K ATPase (a gift from Dr. Michael Kashgarian, Department of Pathology, Yale University School of Medicine, New Haven, CT).

Statistical Analysis

Assays were performed on duplicate samples from 16 livers in the fractionation studies, and 11 livers in the immunoprecipitation and confocal studies. All results are expressed as mean $\pm$ standard error. Statistical comparisons between groups for the immunoprecipitation studies used the Student unpaired t test on Microsoft Excel 2000. Linear regression analysis for the fractionation studies was performed on Excel and graphed on SPSS for Windows 10.1. Statistical significance was taken at $p<0.05$.

Results

Fractionation

Representative Western blots used to quantify IR and GLUT2 endosome and plasma membrane fractions from fasted and fed rats are shown in Fig. 1. As demonstrated, an IR band was found at 95 kDa and a GLUT2 band was found at 52 kDa. Scanning densitometry and volume analysis determined the mean ratio of endosome:plasma membrane IR was 0.65 ± 0.17 in fasted rats. The mean ratio increased to 1.10 ± 0.05 following feeding ($p<0.02$). Similarly, the mean ratio of

endosome:plasma membrane GLUT2 was 0.68 ± 0.11 in fasted rats. The mean ratio of endosome:plasma membrane GLUT2, as previously shown [12], increased to 1.04 ± 0.09 after feeding ($p<0.02$).

Figure 2 shows a simultaneous graph of IR endosome:plasma membrane content vs. GLUT2 endosome:plasma membrane content. A linear correlation is clearly observed with $r=0.81$ ($p<0.0002$).

Immunofluorescence

The cellular localization of GLUT2 and IR in the adult fed rat liver is shown in figures 3A, 3B, respectively. Numerous hepatocytes show positive cell membrane staining with punctate low signal cytoplasmic staining and clearly visualized nuclei.

The same sections of hepatic tissue used to localize GLUT2 and IR independently are superimposed upon each other and viewed in pseudocolor to look for colocalization of the proteins; areas in yellow are regions of colocalization. The strongest signals present are on the hepatocyte cell membrane. The majority of cells display a yellow pseudocolor which signifies IR colocalization of FITC-labeled GLUT2 and Texas Red-labeled IR (Fig. 3C).

Samples stimulated in the FITC frequency at 488 nm and collected in the Texas Red emission spectra

Fig. 3. Colocalization of IR and GLUT2 by confocal microscopy. 5 μm sections of fed rat liver were stained for IR, GLUT, or both. Numerous hepatocytes show positive cell membrane staining with punctate low signal cytoplasmic staining. The nucleus is clearly visualized. A. Sectioned liver stained with anti-GLUT2 antibody. B. Rat liver stained with anti-IR antibody. C. Liver section stained with antibodies for both IR and GLUT2. The majority of cells are seen to display a yellow halo effect in areas where pixels of FITC labeled GLUT2 and Texas Red labeled IR colocalize on the periphery of the cells. D. Fluorescence Resonance Energy Transfer (FRET) with stimulation at 488nm (within the absorption range of fluorescein) and collection at 670-810nm (within the emission range of Texas Red) showed markedly increased fluorescence compared to controls implying that GLUT2 and IR are separated by a distance of 20-60 $\text{\AA}$.

Fig. 4. Co-immunoprecipitation of GLUT2 and IR from lysed fasted rat hepatocytes. Equal numbers of rat hepatocytes were solubilized in Triton and immunoprecipitated with 1 μg of primary antibody and immunoblotted for either GLUT2 or IR. A. Immunoblotted with anti-GLUT2 antibody. Staining for GLUT2 is present when immunoprecipitating with primary antibody against either GLUT2 or IR. The band is absent when using a primary antibody against Na,K ATPase as a control, or in the absence of precipitating antibody. B. Immunoblotted with anti-IR antibody. Staining for IR is present when immunoprecipitating with primary antibody against either GLUT2 or IR. The band is absent when using a primary antibody against Na,K ATPase as a control or in the absence of precipitating antibody.

at 670-810 nm reveal immunofluorescence on the hepatocyte membrane and diffuse staining throughout the cytoplasm, suggesting a close association between IR and GLUT2 of 20-60 $\text{\AA}$ (Fig. 3D).

There was an absence of signal when samples were stimulated for either FITC or Texas Red and emission was collected in both spectra for a) blocking solution only, b) blocking solution with both secondary antibodies independently and together, c) primary antibodies independently and together, d) rabbit anti-IR primary and anti-goat FITC, and e) goat anti-GLUT2 primary and anti-rabbit Texas Red.

Immunoprecipitation

We studied the association between GLUT2 and IR in vivo by immunoprecipitation of solubilized fasting rat hepatocytes. Lysates were immunoprecipitated with anti-IR and anti-GLUT2 antibodies, and the precipitates were blotted with both anti-IR and anti-GLUT2 antibodies. Blots showed that the transmembrane proteins co-immunoprecipitated and were associated in vivo (Fig. 4).

Discussion

The current study builds upon our previous work to elucidate the role of IR and GLUT2 in regulating hepatic glucose production. Our findings indicate that the two transmembrane proteins are physically linked on the hepatocyte membrane. This suggests a mechanism which obligates a change in glucose transport after insulin binding to IR, independent of IR-mediated changes in intracellular glucose or glycogen metabolism.

Fractionation studies showed that both IR and GLUT2 are removed from the plasma membrane and internalized to endosomal compartments upon insulin binding. The internalization serves to remove the glucose transporter from the hepatocyte plasma membrane and slow the release of hepatic glucose during hyperglycemic, hyperinsulinemic states. It is interesting to note that not all of the GLUT2 is internalized in the normal hepatic response to the post-prandial state. Our previous data showed that after an intraduodenal feeding bolus, which stimulates the release of insulin and the suppression of hepatic glucose production, a substantial fraction (15-53%) of GLUT2 endocytosis occurs [12]. A prior study showed that this same effect was seen during *in vitro* liver perfusion in response to graduated doses of insulin [11]. The current study shows that the internalization of the two transmembrane proteins occurs in a similar ratio with a correlation coefficient of 0.81 ($p < 0.0002$), which suggests a possible direct connection between the two proteins.

To substantiate *in vivo* colocalization of IR and GLUT2, double staining with confocal microscopy showed colocalization of the proteins within the plasma membrane region and cytosol in hepatocytes taken from fed animals. FRET studies further suggest that the intra-protein distance between IR and GLUT2 is within 20-60 Å. Such a close localization between IR and GLUT2 in sectioned liver strongly suggests a physical link between the two proteins. To corroborate these findings, coimmunoprecipitation studies were performed, and these results support the physical interaction between IR and GLUT2.

Several other models of receptor-transporter complexes that function by means of a direct protein-protein interaction have recently been elucidated. The pancreatic beta-cell, inwardly rectifying K channel has been shown to associate with the ATP binding cassette protein, or sulfonylurea receptor, which results in the

regulation of the cellular distribution and function of the transporter [29, 30]. In addition, hereditary hemochromatosis protein (HPE), related to the major histocompatibility complex class I family, detects serum iron through an unknown mechanism and then affects cellular iron uptake by means of a direct interaction with the transferrin receptor in enterocytes [31, 32]. The GABA_A-ligand-gated channels form heteromers with the dopamine D5 receptor enabling mutually inhibitory functional interactions upon neurotransmitter binding [33]. The sigma receptor, a receptor for psychotropic drugs, was found to modulate voltage-gated K⁺ channels (Kv1.4 or Kv1.5) by means of direct protein-protein interactions [34]. In addition, the β2-adrenergic receptor was found to associate directly with the L-type calcium channel in rat neurons, thus ensuring specific and rapid signaling [35]. Also, the metabotropic Glutamate Receptor Subtype 1 regulates the voltage sensitive Ca_v2.1 Ca²⁺ channel by means of a direct interaction [36]. With this growing list of direct receptor-transporter interactions, it is feasible that a similar physical link forms the basis of the insulin receptor regulation of the hepatic glucose transporter. Such a coupling could serve as a mechanism of hepatic glucose regulation, by the reduction of glucose efflux from the hepatocyte through the internalization of a portal for glucose release. The current study does not address the exact nature of the complex and link which leaves open the possibility of a linker protein mediating the interaction.

Several recent knockout studies have emphasized the critical role that both GLUT2 and hepatic IR play in growth and development. While GLUT4 knockout mice display a nearly normal phenotype [37], GLUT2 knockouts develop growth retardation and death by 2-3 weeks [38]. These mice display altered glucose tolerance suggesting a central role of GLUT2 in glucose metabolism. While recent studies from GLUT2 null murine hepatocytes fail to show impairment of hepatic glucose output in the absence of the hepatic glucose transporter, they suggest multiple compensatory and redundant pathways that accomplish the vital task glucose membrane transport [39, 40]. Given the importance of intracellular carbohydrate regulation and the devastating phenotypes associated with deficiencies in carbohydrate metabolic and transport pathways [41-44], it is not surprising that redundant pathways would be present.

Similar to the vital importance of GLUT2, studies with tissue specific IR knockout mice show that muscle IR null mice display normal glucose tolerance [45], while

hepatic IR specific knockouts show altered glucose homeostasis [46, 47]. Since both hepatic IR and GLUT2 remain important determinates of body glucose levels, the IR:GLUT2 complex may represent an important form of regulation.

It has been shown that insulin exerts several mechanisms for controlling hepatic glucose production. Hepatic protein function is regulated at the transcriptional level for the hepatic enzymes of glucose metabolism [13], and GLUT2 expression is suppressed by elevated insulin and glucose levels [19-22]. In addition, in the post prandial state, insulin inhibits many key enzymes of hepatic gluconeogenesis and glycogenolysis, while activating enzymes involved in glycogen synthesis [2-6, 13]. Although our data do not contradict these established mechanisms of insulin-mediated regulation of hepatic glucose metabolism, our findings of an IR:GLUT2 receptor-transporter complex do suggest a direct link between

insulin binding and the suppression of hepatic glucose efflux, which possibly comprises a direct, rapid pathway.

Acknowledgements

These studies were supported by grants from the National Institutes of Health, DK56212 and NS39918 (BPJ), DK50230 (JPG), and DK30995 (DKA). The data were presented in preliminary form at the American College of Surgeons Surgical Forum, Orlando, Florida, October 1998, and the American Gastroenterological Association, Atlanta, Georgia, May 2001. Co-authors Eisenberg and Maker contributed equally to these studies. Current address of Dr. Bhanu P. Jena is: Department of Physiology Wayne State University School of Medicine Detroit, MI. 48201.

References

- 1 Shepherd PR, Kahn BB: Glucose transporters and insulin action - implications for insulin resistance and diabetes mellitus. *N Engl J Med* 1999;341:248-257.
- 2 Roden M, Bernroider E: Hepatic glucose metabolism in humans - its role in health and disease. *Best Pract Res Clin Endocrinol Metab* 2003;17:365-383.
- 3 Moore MC, Cherrington AD, Wasserman DH: Regulation of hepatic and peripheral glucose disposal. *Best Pract Res Clin Endocrinol Metab* 2003;17:343-364.
- 4 Barthel A, Schmoll D: Novel concepts in insulin regulation of hepatic gluconeogenesis *Am J Physiol Endocrinol Metab* 2003;285:E685-692.
- 5 Cherrington AD: Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. *Diabetes* 1999;48:1198-1214.
- 6 Nordlie RC, Foster JD, Lange AJ: Regulation of glucose production by the liver. *Annu Rev Nutr* 1999;19:379-406.
- 7 Thorens B: Glucose transporters in the regulation of intestinal, renal, and liver glucose fluxes. *Am J Physiol* 1996;270:G541-553.
- 8 Mueckler M: Facilitative glucose transporters. *Eur J Biochem* 1994;219:713-725.
- 9 Pederson O: Glucose Transporters and Diabetes Mellitus. Amsterdam: Elsevier, 1993, pp 30-54.
- 10 Olson AL, Pessin JE: Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. *Annu Rev Nutr* 1996;16:235-256.
- 11 Andersen DK, Ruiz CL, Burant CF: Insulin regulation of hepatic glucose transporter protein is impaired in chronic pancreatitis. *Ann Surg* 1994;219:679-686; discussion 686-677.
- 12 Nathan JD, Zdankiewicz PD, Wang J, Spector SA, Aspelund G, Jena BP, Seymour NE, Geibel JP, Andersen DK: Impaired hepatocyte glucose transport protein (GLUT2) internalization in chronic pancreatitis. *Pancreas* 2001;22:172-178.
- 13 Pilkis SJ, Granner DK: Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu Rev Physiol* 1992;54:885-909.
- 14 Ebina Y, Edery M, Ellis L, Standring D, Beaudoin J, Roth RA, Rutter WJ: Expression of a functional human insulin receptor from a cloned cDNA in Chinese hamster ovary cells. *Proc Natl Acad Sci USA* 1985;82:8014-8018.
- 15 Haring H, Obermair-Kusser B: The Insulin Receptor: It's Role in Insulin Action and in the Pathogenesis of Insulin Resistance. Amsterdam: Elsevier 1990;537-567.
- 16 Di Guglielmo GM, Drake PG, Bass PC, Authier F, Posner BI, Bergeron JJ: Insulin receptor internalization and signalling. *Mol Cell Biochem* 1998;182:59-63.
- 17 Bergeron JJ, Cruz J, Khan MN, Posner BI: Uptake of insulin and other ligands into receptor-rich endocytic components of target cells: the endosomal apparatus. *Annu Rev Physiol* 1985;47:383-403.
- 18 Slezak LA, Nathan JD, Zdankiewicz PD, Spector SA, Wang JP, Jena BP, Geibel JP, Seymour NE, Andersen DK: Impaired Hepatocyte Insulin Receptor Internalization in Chronic Pancreatitis. *Surg Forum* 1998;49:152-153.
- 19 Thorens B, Flier JS, Lodish HF, Kahn BB: Differential regulation of two glucose transporters in rat liver by fasting and refeeding and by diabetes and insulin treatment. *Diabetes* 1990;39:712-719.
- 20 Burcelin R, Eddouks M, Kande J, Assan R, Girard J: Evidence that GLUT-2 mRNA and protein concentrations are decreased by hyperinsulinaemia and increased by hyperglycaemia in liver of diabetic rats. *Biochem J* 1992;288:675-679.
- 21 Postic C, Burcelin R, Rencurel F, Pegorier JP, Loizeau M, Girard J, Leturque A: Evidence for a transient inhibitory effect of insulin on GLUT2 expression in the liver: studies in vivo and in vitro. *Biochem J* 1993;293:119-124.

- 22 Rencurel F, Waeber G, Antoine B, Rocchiccioli F, Maulard P, Girard J, Leturque A: Requirement of glucose metabolism for regulation of glucose transporter type 2 (GLUT2) gene expression in liver. *Biochem J* 1996;314(Pt 3):903-909.
- 23 Maker AV, Slezak LA, Aspelund G, Sritharan KC, Jena BP, Andersen DK: Impaired phosphorylation of hepatic insulin receptor and glucose transporter proteins in chronic pancreatitis. *Surg Forum* 1999;50:14-16.
- 24 Kenworthy AK: Imaging protein-protein interactions using fluorescence resonance energy transfer microscopy. *Methods* 2001;24:289-296.
- 25 Selvin PR: The renaissance of fluorescence resonance energy transfer. *Nat Struct Biol* 2000;7:730-734.
- 26 Marsh M, Schmid S, Kern H, Harms E, Male P, Mellman I, Helenius A: Rapid analytical and preparative isolation of functional endosomes by free flow electrophoresis. *J Cell Biol* 1987;104:875-886.
- 27 Schmid SL, Fuchs R, Male P, Mellman I: Two distinct subpopulations of endosomes involved in membrane recycling and transport to lysosomes. *Cell* 1988;52:73-83.
- 28 Whitney JA, Gomez M, Sheff D, Kreis TE, Mellman I: Cytoplasmic coat proteins involved in endosome function. *Cell* 1995;83:703-713.
- 29 Inagaki N, Gonoi T, Clement JPt, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J: Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 1995;270:1166-1170.
- 30 Lorenz E, Alekseev AE, Krapivinsky GB, Carrasco AJ, Clapham DE, Terzic A: Evidence for direct physical association between a K⁺ channel (Kir6.2) and an ATP-binding cassette protein (SUR1) which affects cellular distribution and kinetic behavior of an ATP-sensitive K⁺ channel. *Mol Cell Biol* 1998;18:1652-1659.
- 31 Gross CN, Irrinki A, Feder JN, Enns CA: Co-trafficking of HFE, a nonclassical major histocompatibility complex class I protein, with the transferrin receptor implies a role in intracellular iron regulation. *J Biol Chem* 1998;273:22068-22074.
- 32 Bennett MJ, Lebron JA, Bjorkman PJ: Crystal structure of the hereditary haemochromatosis protein HFE complexed with transferrin receptor. *Nature* 2000;403:46-53.
- 33 Liu F, Wan Q, Pristupa ZB, Yu XM, Wang YT, Niznik HB: Direct protein-protein coupling enables cross-talk between dopamine D5 and gamma-aminobutyric acid A receptors. *Nature* 2000;403:274-280.
- 34 Aydar E, Palmer CP, Klyachko VA, Jackson MB: The sigma receptor as a ligand-regulated auxiliary potassium channel subunit. *Neuron* 2002;34:399-410.
- 35 Davare MA, Avdonin V, Hall DD, Peden EM, Burette A, Weinberg RJ, Horne MC, Hoshi T, Hell JW: A beta2 adrenergic receptor signaling complex assembled with the Ca²⁺ channel Cav1.2. *Science* 2001;293:98-101.
- 36 Kitano J, Nishida M, Itsukaichi Y, Minami I, Ogawa M, Hirano T, Mori Y, Nakanishi S: Direct interaction and functional coupling between metabotropic glutamate receptor subtype 1 and voltage-sensitive Cav2.1 Ca²⁺ channel. *J Biol Chem* 2003;278:25101-25108.
- 37 Katz EB, Stenbit AE, Hatton K, DePinho R, Charron MJ: Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. *Nature* 1995;377:151-155.
- 38 Guillam MT, Hummler E, Schaerer E, Yeh JJ, Birnbaum MJ, Beermann F, Schmidt A, Deriaz N, Thorens B, Wu JY: Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2. *Nat Genet* 1997;17:327-330.
- 39 Hosokawa M, Thorens B: Glucose release from GLUT2-null hepatocytes: characterization of a major and a minor pathway. *Am J Physiol Endocrinol Metab* 2002;282:E794-801.
- 40 Guillam MT, Burcelin R, Thorens B: Normal hepatic glucose production in the absence of GLUT2 reveals an alternative pathway for glucose release from hepatocytes. *Proc Natl Acad Sci USA* 1998;95:12317-12321.
- 41 Wolfsdorf JJ, Weinstein DA: Glycogen storage diseases. *Rev Endocr Metab Disord* 2003;4:95-102.
- 42 Talente GM, Coleman RA, Alter C, Baker L, Brown BI, Cannon RA, Chen YT, Crigler JF Jr, Ferreira P, Haworth JC, et al.: Glycogen storage disease in adults. *Ann Intern Med* 1994;120:218-226.
- 43 Longo N, Elsas LJ: Human glucose transporters. *Adv Pediatr* 1998;45:293-313.
- 44 Brown GK: Glucose transporters: structure, function and consequences of deficiency. *J Inherit Metab Dis* 2000;23:237-246.
- 45 Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, Kahn CR: A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* 1998;2:559-569.
- 46 Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR: Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* 2000;6:87-97.
- 47 Fisher SJ, Kahn CR: Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. *J Clin Invest* 2003;111:463-468.