Cell. Mol. Life Sci. (2009) 66:2721–2732
DOI 10.1007/s00018-009-0065-8

Cellular and Molecular Life Sciences

RESEARCH ARTICLE

Glucose binds to the insulin receptor affecting the mutual affinity of insulin and its receptor

Robert Root-Bernstein · Jessica Vonck

Received: 10 March 2009 / Revised: 1 June 2009 / Accepted: 4 June 2009 / Published online: 25 June 2009

Abstract Insulin activity is sensitive to glucose concentration but the mechanisms are still unclear. An unexamined possibility is that the insulin receptor (IR) is sensitive to glucose concentration. We demonstrate here that insulin-like peptides derived from the IR bind glucose at low millimolar, and cytochalasin B at low micromolar, concentrations; several insulin-like IR peptides bind insulin at nanomolar Kd; and this binding is antagonized by increasing glucose concentrations. In addition, glucose and cytochalasin B bind to IR isolated from rat liver and increasing glucose decreases insulin binding to this IR preparation. The presence of GLUT 1 in our IR preparation suggests the possibility of additional glucose-mediated allosteric control. We propose a model in which glucose binds to insulin, the IR, and GLUT; insulin binds to the IR; and the IR binds to GLUT. This set of interactions produces an integrated system of insulin-dependent interactions that is highly sensitive to glucose concentration.

Note on insulin receptor numbering: All IR sequences provided are in the SwissProt numbering system (http://www.expasy.ch) that begins with the pre-pro sequence. Many papers on the IR use a numbering system that begins with the edited transcript and thus have 27 amino acids subtracted from the number as compared with the SwissProt convention.

R. Root-Bernstein (✉)
Department of Physiology, Michigan State University,
2174 Biomedical and Physical Sciences Building, East Lansing,
MI 48824, USA
e-mail: rootbern@msu.edu

J. Vonck
College of Human Medicine, Michigan State University,
East Lansing, MI 48824, USA
e-mail: vonckjes@msu.edu

Keywords Glucose binding · Insulin receptor · Insulin binding · Glucose transport · Cytochalasin B · Allostery · Glucoreceptor · Glucosensor

Introduction

Cushman and Wardzala [1] and Suzuki and Kono [2] established that insulin increases uptake of glucose into cells by activating translocation of glucose transporters (GLUT) from intracellular storage to the cell surface in various tissues. Insulin itself is released from pancreatic beta cells by increasing glucose concentrations (reviewed in [3]). As one would expect of such a system, insulin resistance in diabetes is associated with decreased numbers of GLUT, and the resulting hyperglycemia produces increased release of insulin, but the insulin is less active (reviewed in [4]). But how can insulin activity, in addition to release, also be glucose-dependent? The most likely answer is that the system is significantly more complex than glucose releasing insulin to translocate GLUT to regulate glucose. Indeed, as long ago as 1979, Jarrett and Smith [5] discovered that cytochalasin B, a glucose-transporter antagonist, had direct effects on insulin activity, and they proposed that the insulin receptor (IR) may directly control the rate of glucose transport through GLUT. Garvey et al. [6] independently arrived at the same conclusion as a result of experiments demonstrating that glucose regulation is relatively insensitive to insulin or to glucose alone and requires a synergistic interaction between the two. Subsequent research has strengthened that proposal. Deletion of the IR from various tissues has been shown by a number of investigators to result in hyperglycemia, ketosis, and other symptoms of diabetes (reviewed in [7]). Nevado, Valverde, and Benito [8], for example, have demonstrated that the IR binds to and

acts as a co-transporter with glucose transporter types 1 and 2 in neonatal hepatocytes, such that deletion of either the IR or GLUT results in loss of glucose regulation. Kulkarni et al. [9] have similarly demonstrated that tissue-specific knock-out of the IR in pancreatic beta cells results in loss of glucose-stimulated insulin release even though GLUT expression and function in these cells is unaltered. Conversely, deletion of GLUT in muscle results in insulin resistance, even when IR are expressed and fully functional [7]. How the IR acts as a co-transporter with GLUT or can respond to glucose concentration in an IR-GLUT system remains unexplained by current models of glucose regulation.

We make the novel proposal that the IR acts, in part, as a glucose sensor that allosterically regulates GLUT function. This hypothesis is based on the observations summarized above as well as additional evidence that has remained tangential to most discussions of glucose regulation. First, insulin itself contains two high-affinity (ca. 1×10^{-3} M) and four low-affinity (ca. 6×10^{-2} M) binding sites for glucose [10–14]. Ultraviolet spectroscopy studies show that insulin structure is modified in the presence of glucose [12]. It follows that binding of insulin to the IR may be sensitive to glucose concentration. In addition, the IR contains several highly conserved extracellular sequences that mimic insulin [15, 16], and these may also act as glucose-binding sites resulting in allosteric modification of receptor activity. This possibility is bolstered by the fact that each insulin-like sequence in the receptor is associated with an insulin-binding region [15, 16] so that receptor affinity for insulin may be glucose-sensitive. Finally, Nevado et al. and Eisenberg et al. have demonstrated that the IR forms a complex with GLUT [7, 17, 18] so there is a possibility of allosteric interaction between the receptor and the transporter. If glucose modifies the structures of the receptor, the transporter, or both, and does so in a glucose-dependent manner, then allosteric regulation of glucose transport by the IR would be possible, as well as GLUT allosteric regulation of IR sensitivity. In addition, if glucose alters the nature of insulin binding to its receptor (whether by binding to insulin, the receptor, or both) in a manner that allosterically alters glucose transport, then the IR could act not only as a glucose sensor but also as an allosteric glucose transport regulator.

In this paper, we test one critical aspect of the hypothesis that the IR may act as a glucose sensor and regulator, which is whether the IR is a glucose-binding protein. We examine whether the IR alters its structure in the presence of glucose, whether it alters its affinity for insulin in a glucose-dependent manner, whether insulin-like peptides derived from the receptor bind glucose, and whether the binding of insulin to these peptides is insulin sensitive. We also test whether the IR and its insulin-like regions bind the high-affinity glucose antagonist, cytochalasin B. And finally, we test our IR preparation for the presence of GLUT. Because glucose may bind to both insulin and to the IR in these experiments, and because GLUT may be present as well, the interpretation of some of the individual experiments is necessarily ambiguous, but the logic of the set of experiments leads to an unambiguous conclusion that the IR does act as a glucose-binding protein.

Materials and methods

Glucose binding to insulin and IR peptides

Initial experiments were designed to determine binding of glucose and cytochalasin B to insulin (bovine, Sigma-Aldrich Chemical) and individual peptides derived from the IR. Peptide regions previously identified [15, 16] as being either insulin-like (IL) or glucagon-like (GL) regions of the IR (see Table 1) were synthesized by the Mass Spectrometry, Synthesis and Sequencing Facility of the Department of Biochemistry at Michigan State University and purified to >99% purity. Insulin (2.5 mg/ml) and IR peptides (1.0 mg/ml) were dissolved as solutions of ca. 0.5 mM in pH 7.4 phosphate buffer (Fisher Scientific). A 1.0 M D-glucose (Sigma-Aldrich) solution was made as a stock solution in the same buffer and diluted by thirds. Then 100 µl of each glucose concentration plus 100 µl of buffer, and mixtures of 100 µl of each peptide with 100 µl of buffer or with 100 µl of each glucose concentration were made in a crystal 96-well plate and a complete spectrum of each well was recorded on a Spectromax Plus spectrophotometer. Data were collected using SoftMax Pro 4.0 software. Every combination was done in triplicate and the results averaged. The curves were examined for the absorbance at which the greatest spectral shifts occurred (usually between 195 and 205 nm) and the data were analyzed to produce binding constants (Kd) determined by the difference in absorbance between the absorbance obtained from the mixture of the peptide with glucose and the sums of the peptides (plus buffer) and glucose (plus buffer) individually.

Cytochalasin B binding to insulin and IR peptides

Following a procedure described by us previously for measuring binding to receptors [19], UV spectrophotometry was also used to measure cytochalasin B (Sigma-Aldrich) binding to insulin and to the IR peptides, but a different protocol was followed since cytochalasin B is not

Table 1 Kd of glucose, cytochalasin B, and insulin binding to insulin, insulin-like (IL), and glucagon-like (GL) peptides derived from the insulin receptor (IR) demonstrating that glucose and insulin binding to IR regions is co-localized

IR peptides		Glucose	Cyto B	Ins-HRP
INSULIN		250 μM and 30 mM	13 μM	nd
95–113 (IL) Insulin B chain	GLES LKDLF PNLTVIRGSR HLCGSH-LVEALYLVCGER	11 mM	75 μM	21 nM
157–166 (GL) Glucagon	T IDWSRILDS TSDYSKYLDS	57 mM	112 μM	30 nM
233–248 (IL) Insulin A chain	CCHSECLGNCSQPDD CCTSI CSLYQLENYCN	3 mM and 46 mM	nd	>1 μM
284–300 (GL) Glucagon	SFCQDLHHKCKNSRR-QG TFTS D-YSKYL DSRRAQD	0	0	8.5 nM
390–405 (GL) Glucagon	E ISGYLKIRRSYALVS DYSKYLDSRRAQDFV	30 mM	166 μM	150 nM
424–444 (GL) Glucagon	YSFYALDNQNLRQLWDWSKH SKY-LDS RRAQDFVNW	0	0	>1 μM
453–464 (IL) Insulin B chain	TQGKLFFHYNPK CGERGFF-YT PK	57 mM	nd	7.6 nM
660–679 (IL) Insulin B chain	ERQAEDSELFELDYCLKGLK NQHLCGSHLVEALYLVCGER	70 mM	117 μM	37 nM
897–916 (IL) Insulin B chain	HLCVSRKHFALERGCRLRGL HLCGSHLVEALYLVCGERGF	14 mM	25 μM	1.5 nM

Insulin-like (IL) and glucagon-like peptides were identified within the IR by similarity searching and shown to be associated with insulin-binding regions of the receptor [15, 16]. Some of these IL were synthesized and UV spectrometry used to determine binding constants to glucose and the glucose antagonist cytochalasin B. Insulin binding was determined by an ELISA-like protocol in which different concentrations of insulin conjugated to horseradish peroxidase were tested for binding to each peptide at 50 μM. IL peptides tend to bind glucose at low millimolar Kd, cytochalasin B at mid-micromolar Kd, and insulin at low nanomolar Kd. These data suggest that insulin- and glucose-binding sites overlap significantly within the IR. In contrast, glucagon-like peptides (GL) derived from the IR [15, 16] are less likely to bind glucose, cytochalasin B, or insulin. See Figs. 1, 2 and 3 for illustrative data

soluble in the buffer and was supplied in pure DMSO. Briefly, 10 mM cytochalasin B (DMSO) was serially diluted by thirds with phosphate buffer to produce eight dilutions. DMSO was similarly diluted to make solutions with matching DMSO concentrations. The same peptide solutions as above were utilized. Then 200 μl of a peptide in phosphate buffer was put in two wells of a crystal 96-well plate, and phosphate buffer alone in another two. Next 20 μl of the lowest concentration of cytochalasin B solution was added to one peptide well and one buffer well, and 20 μl of the lowest concentration of DMSO solution was added to the other peptide and buffer wells. The spectra of the wells were collected from 190 to 240 nm and saved. The process was repeated by adding the next higher concentration of cytochalasin B and DMSO, etc., until the highest concentration was added and the spectra recorded. In order to determine whether cytochalasin B bound to the peptide, the buffer plus DMSO dilutions data were subtracted from all corresponding spectra; then the spectra for the peptide and cytochalasin B wells were added to provide expected values and this was compared with the actual values obtained from the mixing of peptide and cytochalasin B. The clearest binding curves were found in the range of 200–210 nm.

Glucose and cytochalasin B binding to the IR

A similar protocol was used to measure the effects of glucose and of cytochalasin B on IR structure. IR from rat liver (Sigma-Aldrich) was diluted to a concentration of 401.8 units/ml. Then 200 μl of this solution was added to two wells in a crystal plate. Next 3.3 M glucose and 0.33 mM cytochalasin B in DMSO were serially diluted 1:3, and 20 μL of each dilution was added to two IR wells and to control wells. Finally 20 μL of buffer (or DMSO diluted in buffer) was added to two additional IR wells, and likewise to the second set of control wells. The plate was read in 15-min increments with increasingly concentrated solutions. An additional version of this experiment added bovine insulin (Sigma) with additional appropriate controls.

Insulin binding to peptides derived from the IR

A modified version of enzyme-linked adsorption assay (ELSA) was used to measure insulin binding to the peptides derived from the IR. First 0.5 mg of insulin-HRP [insulin conjugated to horseradish peroxidase (Sigma-Aldrich)] was diluted in 2.0 ml of pH 7.4 phosphate buffer

as a stock solution (5 μM). Serial dilutions of the insulin-HRP were then made from 1:100 to 1:333,333 by thirds, producing solutions that ranged in insulin-HRP concentration from 50 nM to 15 pM. Each IR peptide was dissolved in buffer to produce a 50 μM stock solution and 100 μl were then added to an ELISA plate (Costar), incubated for 1 h, and then triply washed with a 1% solution of TWEEN in phosphate buffer. Then 200 μl of a 2% polyvinylalcohol (PVA) blocking agent in phosphate buffer was added to each well, incubated for 1 h, and triply washed. Next 100 μl of each dilution of the insulin-HRP solution was added to a well, incubated for 1 h, and triply washed. Finally, 100 μl of ABTS single reagent (Chemicon) was added to each well, incubated for 30 min, and the plate read at 405 nm. All combinations and controls were run in duplicate and the resulting values averaged.

Glucose binding to the IR

A similar set of experiments was designed to determine whether insulin binding to the IR is glucose sensitive. First 50 μl of a 401.8 units/ml IR solution [isolated from rat liver (Sigma-Aldrich)] was diluted into 5.0 ml of pH 7.4 phosphate buffer (Fisher Scientific). Then 100-μl aliquots were plated on a 96-well ELISA plate (Costar). One set of wells was plated with no glucose, a second with 1 mM glucose, a third with 5 mM glucose, and a fourth with 25 mM glucose added to the buffer. These mixtures were incubated at room temperature for 2 h and then triply washed with 1% solution of TWEEN 20. Next 200 μl of 2% PVA blocking agent with the appropriate amount of glucose for each set of wells (0, 1, 5 or 25 mM) was added to each well, incubated for 1 h, and washed as above. Insulin-HRP (Sigma) was diluted from the stock solution above to 1:5,000 (1.0 nM) in phosphate buffer. This insulin-HRP solution was plated with appropriate concentrations of glucose for each set of wells (0, 1, 5, or 25 μl) and incubated for 1 h before being washed out as above. ABTS triple reagent (Chemicon) was then added (100 μl/well) and incubated for 30 min and the plate read at 405 nm.

Presence of GLUT in IR preparation

Finally, ELISA was used in order to determine whether the rat liver IR preparation used in these experiments (Sigma-Aldrich) contained glucose transporters (GLUT). IR was plated on an ELISA plate as described above, washed, PVA added, washed, and then 100 μl of 1/200 dilutions of GLUT antibodies were added, including goat anti-GLUT 1, rabbit anti GLUT 2, or rabbit anti-GLUT 4 (Santa Cruz Biotech, sc-7938). These antibodies were incubated for 1 h, triply washed, and 100 μl of a 1/1,000 dilution of a species-appropriate anti-IgG-HRP secondary antibody was added (Chemicon). The secondary antibody was incubated for 1 h and triply washed. Then 100 μl ABTS single reagent was added and the absorbance at 405 nm read after 30 min.

Data analysis

All data were analyzed and plotted using an Excel spreadsheet program.

Results

The first set of experiments involved UV spectroscopic studies of the binding of glucose and cytochalasin B to insulin and to individual peptides derived from the IR (Figs. 1, 2, Table 1). As previous studies have shown [10–14], insulin binds glucose in a two-step process involving a high-affinity set of sites (Kd in these experiments ca. 250 μM) and a set of low-affinity sites (Kd in these experiments ca. 15 mM). We demonstrate for the first time that insulin also binds to cytochalasin B (Kd ca. 13 μM). Similar binding with glucose or cytochalasin B also occurred with some of the peptides from the IR. These peptide regions of the IR were chosen because they mimic either insulin itself or glucagon [15, 16]. As Figs. 1c, 2, and Table 1 show, several insulin-like IR peptides bind glucose with Kd in the low millimolar range, as would be expected of IR regions sensitive to changes in normal blood glucose levels (ca. 5 mM). Additionally, each insulin-like IR peptide that bound glucose also bound cytochalasin B and did so with significantly higher affinity (mid-micromolar Kd).

The second set of experiments (Table 1, Figs. 1, 2) demonstrated that many of the insulin-like and one of the glucagon-like IR peptides have significant affinity for insulin-HRP (low nanomolar Kd). These peptides may represent parts of one or more larger, cooperative insulin-binding sites, or independent low-affinity sites. In either case, it is evident that several regions of the IR can bind insulin and that the same regions also bind glucose.

The third set of experiments examined the effects of altering the glucose concentration on insulin binding to IR peptides. As Fig. 2 demonstrates, insulin binding to IR peptide 897–916 is sensitive to glucose concentration, as well as to the presence of cytochalasin B. Notably, cytochalasin B appears to augment insulin binding (perhaps by cross-linking the molecules) while glucose antagonizes it. Similar data were obtained for other IR peptides, notably the 95–113 peptide (partial data shown in Fig. 1). Since glucose and cytochalasin B can bind to insulin itself, as well as to the insulin-receptor peptide, it is not clear from this set of experiments whether it is the glucose binding to

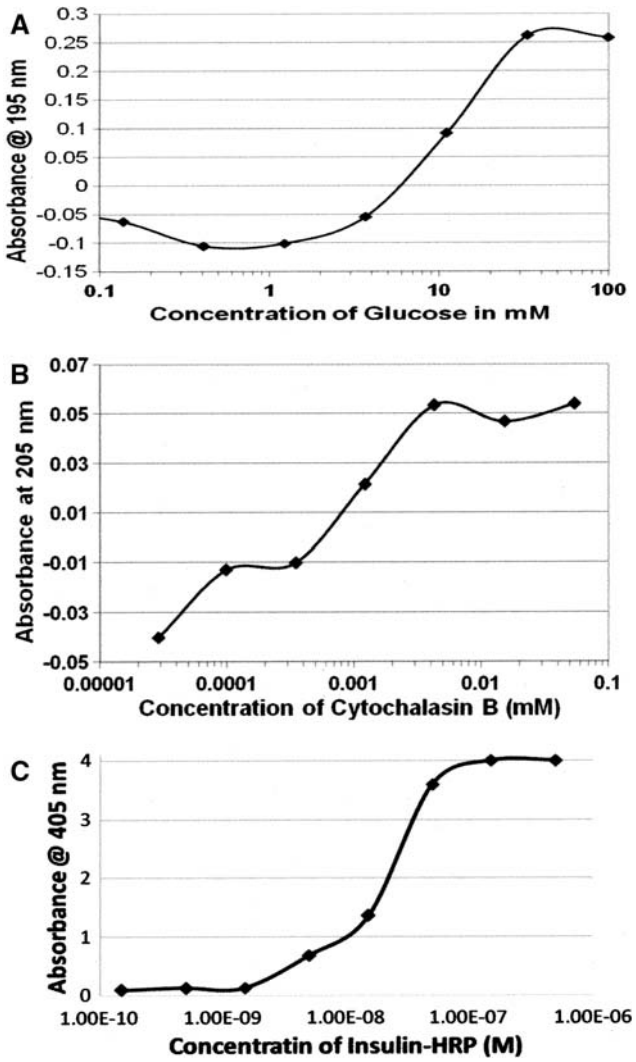

Fig. 1 An insulin-like peptide derived from the insulin receptor (IR) (using SwissProt numbering system for protein #95113) binds glucose, cytochalasin B, and insulin demonstrating that insulin-binding sites on the receptor may be glucose-sensitive. **a** A UV spectroscopic study of binding of glucose to IR 95–113 reveals a Kd of ca. 11 mM. **b** A similar study of cytochalasin B binding to the same IR peptide shows a Kd of ca. 75 μM. **c** Insulin-HRP binds to this IR peptide with a Kd of ca. 21 nM

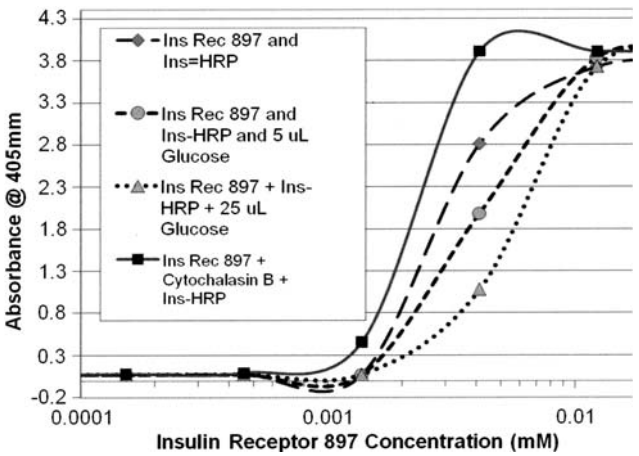

Fig. 2 Glucose and cytochalasin B alter insulin binding to the insulin receptor (IR)-derived peptide 897–916. The IR peptide was adsorbed onto an ELISA plate and insulin-HRP (2.5 nM) used to measure binding to the adsorbed peptide. Increasing concentrations of glucose decreased insulin-HRP binding to the IR peptide, while cytochalasin B increased binding. These observations are consistent with observations that cytochalasin B increases insulin binding to the IR [5, 20] and that supranormal glucose decreases it [21]

insulin, or to the IR peptides, or to both, that results in the modification of insulin binding to the peptides.

The fourth set of experiments examined whether the IR itself altered conformation in the presence of glucose and cytochalasin B. UV spectrophotometry demonstrated that the IR undergoes easily observable conformational changes with changing glucose concentrations. At all wavelengths from 190 through 220 (selected data shown in Fig. 3), concentration-dependent shifts in the UV spectra show a binding constant for glucose that is around 1.5 mM, and some of the spectra suggest a second lower affinity binding constant around 70 mM. Similar shifts in the spectra occur upon binding to cytochalasin B indicating a binding constant of about 60 μM. These data are consistent with those found by studying isolated IR peptides (Table 1). The IR data were also amenable to the production of difference spectra showing that the IR spectrum as a whole alters in a concentration-dependent manner with increasing glucose or cytochalasin B (Fig. 3).

The fifth set of experiments examined the effect of glucose concentration on insulin binding to the IR. One set of experiments using insulin-HRP to directly determine binding demonstrated that increasing glucose concentration decreases insulin binding in an essentially linear fashion above 1 mM glucose (Fig. 4a). UV difference spectra yield a similar story. Adding insulin to the IR in the presence of glucose produces difference spectra significantly different from those caused by glucose or cytochalasin B binding to the IR. (Fig. 5c; compare with Fig. 4). These insulin-associated spectral shifts are dependent on glucose concentration (Fig. 5c), and the data permitted the calculation of the effect of glucose on insulin binding to the IR, which shows glucose produces a half-maximal effect at about 2 mM and a maximal effect at about 12 mM (Fig. 5b). The nature of these data do not reveal where the glucose-sensitive changes are occurring (in the IR itself, insulin, or both), so it is not possible to determine whether insulin binding increases or decreases as glucose concentration increases (Fig. 5b). The UV data confirm the insulin-HRP data, however, on the point that the insulin-IR interaction is most sensitive to glucose concentrations around normal physiological levels.

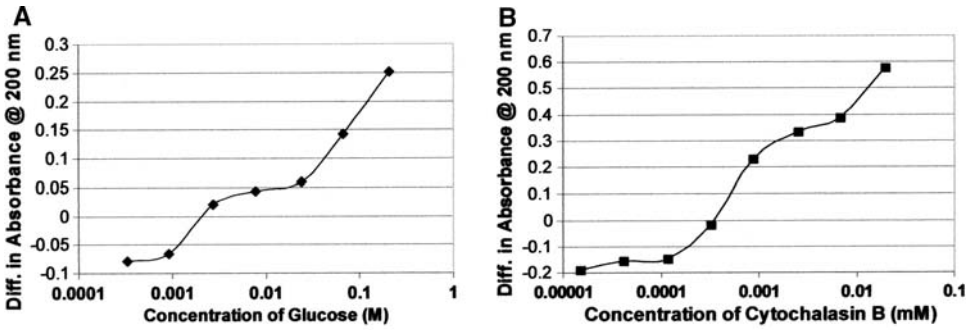

Fig. 3 Glucose and cytochalasin B bind to an IR preparation derived from rat liver. **a** UV spectroscopic data at 200 nm are suggestive of high-affinity (Kd ca. 1.5 mM) and low-affinity (Kd ca. 70 mM) binding, although at higher wavelengths, the binding appears to average out. **b** Cytochalasin B binding to the IR. The data at 200 nm are again suggestive of high-affinity (Kd ca. 0.6 μM) and low-affinity (Kd >1 mM) binding. These data are consistent with the binding constants found for individual peptides derived from the IR (see Table 1 and Fig. 2)

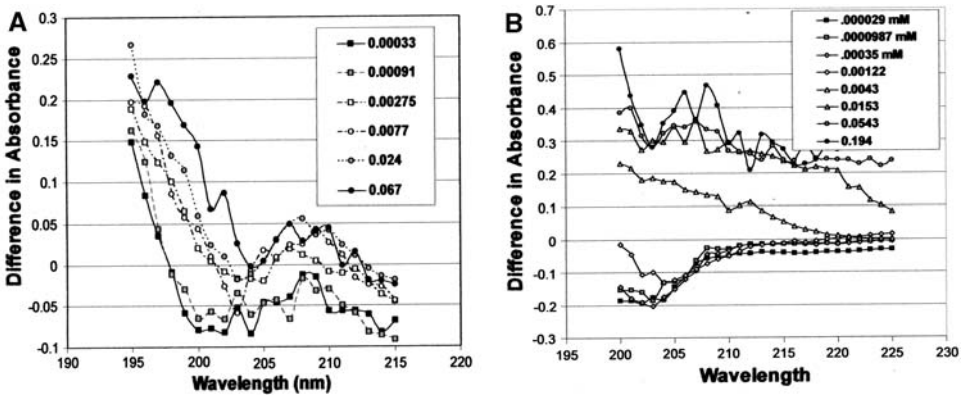

Fig. 4 Difference spectra show that the IR changes its conformation as a function of increasing glucose (**a**) or cytochalasin B (**b**) concentrations (molar, unless otherwise noted). Difference spectra were generated by obtaining spectra of an IR preparation as increasing concentrations of glucose or cytochalasin B were added, and spectra of an identical IR preparation to which buffer without glucose was added, and spectra of buffer with equivalent concentrations of glucose or cytochalasin B added. The spectra of the IR plus buffer were added to the spectra of the buffer plus glucose or cytochalasin B to generate "expected" spectra; the difference between the "expected" spectra and the experimentally observed spectra is what is plotted here. These difference spectra show that the IR alters conformation in a glucose concentration-dependent manner with the major shifts occurring within physiological ranges (0.9–25.0 mM)

Finally, since Eiesnberg et al. [17], Nevado et al. [8], and Gonzalez-Rodriguez et al. [18] report that IR binds directly to GLUT 1 and GLUT 2 but not GLUT 4, we tested our "purified" IR preparation (Sigma-Aldrich) for the presence of GLUT. As Fig. 6 shows, there was, as would be expected of a liver preparation, a very significant amount of GLUT 1, no GLUT 4 present, and a trace of GLUT 2. (We note in passing that all of our own attempts to produce a GLUT-free IR preparation from human muscle cells have so far failed, suggesting that IR-GLUT complexes are not only ubiquitous but very stable.) It is therefore likely that some of the modifications in the UV spectra of the IR in the presence of cytochalasin B are actually due to binding of cytochalasin B to GLUT 1. Some of the modifications in the UV spectra of the IR in the presence of glucose may also be attributable to binding to GLUT 1. While these results complicate the interpretation of each of the IR experiments described above, they are nonetheless consistent with the overall hypothesis being tested.

Discussion

To summarize, we set out to test the hypothesis that the IR may act as a glucose-binding protein that interacts allosterically with GLUT. Various possibilities were proposed. Glucose may alter insulin binding to its receptor, or glucose may alter IR affinity for insulin, or both; IR may interact allosterically with GLUT so that

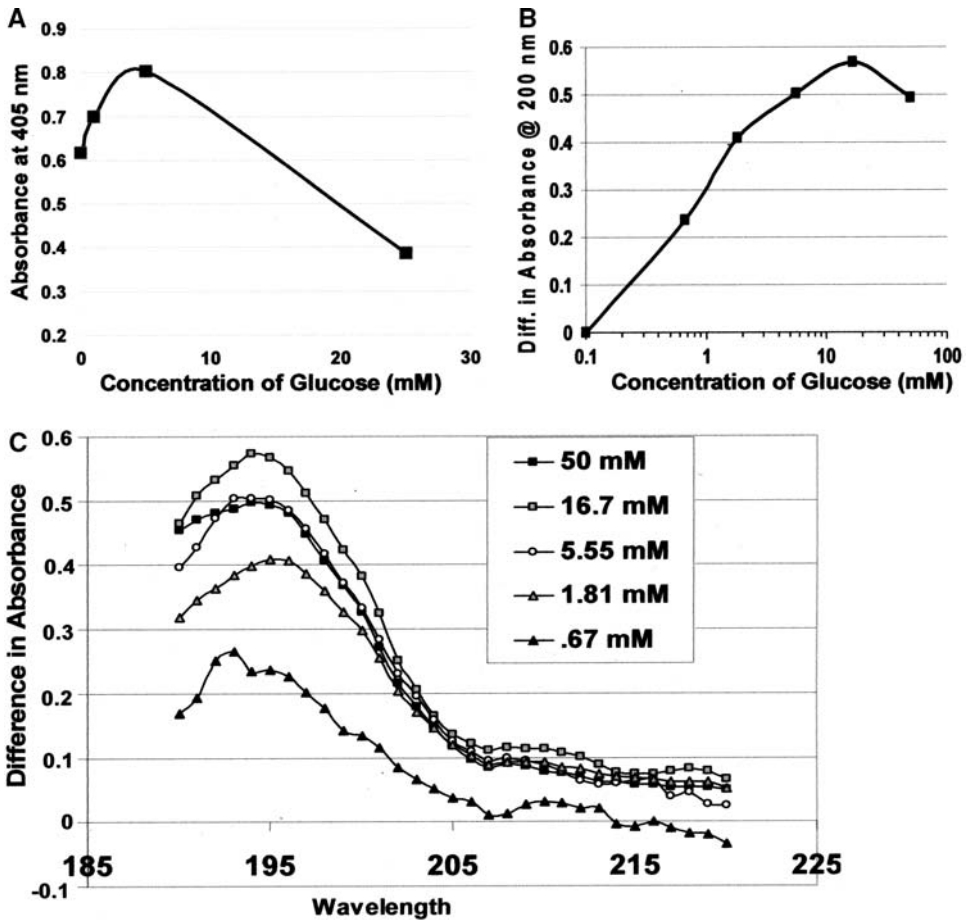

Fig. 5 Two different types of experiments demonstrate that glucose concentration alters insulin binding to its receptor. **a** In the first experiment, we measured insulin-HRP (1 nM) binding to a rat liver IR preparation as glucose increased from 0 to 25 mM. Insulin binding increased to 5 mM glucose and decreased thereafter, which is consistent with previous reports [21, 24]. **b** and **c** In the second experiment, a constant amount of insulin was added to a rat liver IR extract and the concentration of glucose was varied. UV spectra of the combination were obtained and compared with the sum of the UV spectra of insulin plus buffer, the IR plus buffer, and buffer plus glucose each measured separately (see description for Fig. 4). The difference spectrum obtained clearly differs from that of the IR in the presence of glucose, indicating that insulin alters the receptor conformation in a different manner than does glucose. Insulin binding to the receptor is clearly glucose concentration-dependent (**c**). The differences are such that it is possible to obtain binding curves from the spectra (**b**). The effect of glucose on insulin-dependent changes in receptor conformation is half-maximal at about 1 mM, maximal at about 15 mM, and decreases insulin binding above 15 mM. Both sets of experiments (**a–c**) demonstrate glucose modulation of insulin binding to its receptor

either GLUT alters IR function or IR alters GLUT function, or both; glucose should therefore modify insulin binding to IR, and structural changes in IR should be observable on glucose binding (whether that binding is directly to IR or involves allosteric modification due to glucose binding to GLUT).

Our results demonstrate that glucose and cytochalasin B bind to insulin and to peptides isolated from the IR that mimic insulin; insulin binds to several of these same IR peptides; glucose and cytochalasin B modify the binding of insulin to these peptides; the IR conformation is sensitive to glucose and cytochalasin B; and this conformational sensitivity translates into glucose-sensitive changes in insulin affinity for its receptor. Two phenomena complicate the interpretation of these experiments: glucose binds to insulin as well as the IR, so that it is not possible to determine the extent to which changes in insulin binding to IR and to IR-derived peptides are due to glucose binding to insulin, glucose binding to IR (or IR peptides), or glucose binding to both. What can be said for certain is that glucose binds both to insulin and to the IR and some IR-derived peptides altering their conformation, and that the presence of glucose modifies insulin binding to the IR and IR-derived peptides. It is reasonable to assume that glucose affects the mutual affinity of insulin for the IR and its peptides.

As expected [7, 17, 18], our IR preparation contained GLUT 1. GLUT 1 binds glucose and cytochalasin B. Thus,

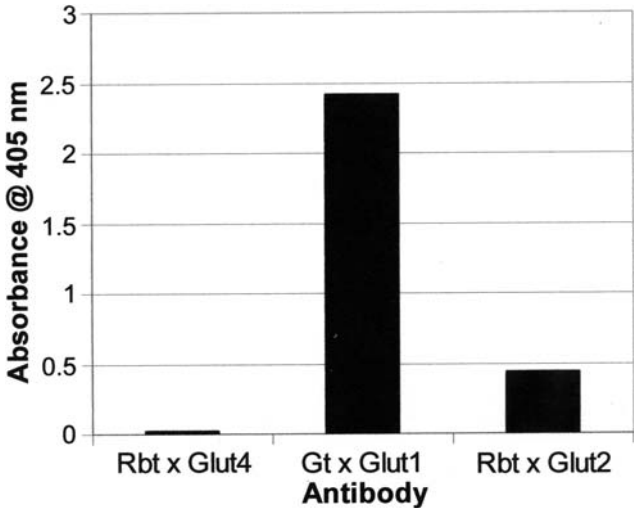

Fig. 6 Enzyme-linked immunoadsorption assay (ELISA) demonstrating the presence of glucose transporter 1 (GLUT 1) but very little GLUT 2 and no GLUT 4 in our rat liver-derived IR preparation. The co-purification of GLUT with IR has been reported by other laboratories as well [7, 17, 18] suggesting that the IR may interact directly with GLUT. *Rbt* Antibody raised in rabbit, *Gt* antibody raised in goat

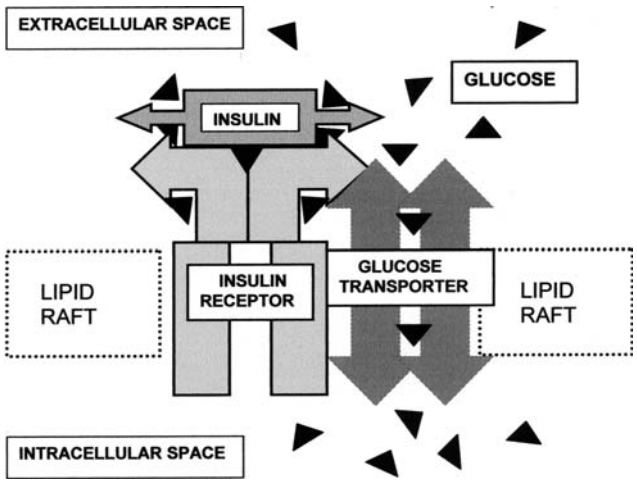

Fig. 7 A schematic model summarizing the results obtained in the previous table and figures. Glucose binds to insulin; insulin binds to the insulin receptor (IR); glucose also binds to the IR; glucose is transported by glucose transporters (GLUT); the IR binds to GLUT to produce a complex that co-purifies within lipid rafts. Glucose binding to insulin and to the IR alters the affinity of insulin for its receptor, increasing binding under hypoglycemic conditions and decreasing binding under hyperglycemic conditions. Binding is associated with observable changes in receptor conformation. These changes in IR conformation may allosterically modify glucose transport by GLUT. Conversely, GLUT may play a role as allosteric regulators of the IR in a glucose-dependent manner. The result of these interactions is a highly integrated insulin-dependent system that is very sensitive to changes in glucose concentration. Interference with this system, as might occur if the IR became glycosylated at glucose-binding sites under hyperglycemic conditions could result in the insulin resistance that typifies diabetes

some of the spectral changes attributed to IR in the presence of glucose and cytochalasin B may be due to changes in GLUT structure. Since glucose and cytochalasin B bind to several isolated IR-derived peptides, to which insulin also binds, it is not possible to attribute all of the glucose- and cytochalasin-related spectral changes in our IR-GLUT preparations to GLUT alone. Since glucose and cytochalasin B bind directly to peptides of the IR at appropriate Kd, the IR must be playing a role in the observed glucose-dependent spectral shifts. Moreover, we also demonstrate, using two methods, that insulin binding to the IR is glucose sensitive (Figs. 4, 5). Glucose can antagonize insulin binding to the IR only if that antagonism is mediated by glucose binding directly to the IR, allostery between GLUT and the IR (Fig. 6), or both. Thus, despite the ambiguities present in the interpretations of the mechanisms involved in some of the individual experiments, the set of experiments clearly demonstrates that insulin binding to the IR is glucose-sensitive. The data also clearly demonstrate that insulin-glucose, IR-peptide-glucose, and IR-glucose are all involved in this sensitivity. The data further suggest that GLUT-IR allostery may be involved. Indeed, the data as a whole suggest that it is the system of interactions among glucose, insulin, the IR, and GLUT that makes this glucose sensor system so homeostatically sensitive (Fig. 7).

Our data concerning an effect of glucose on insulin binding are consistent with previous physiological studies. Jarrett and Smith [5] reported that the IR often exists in aggregates of two to six receptors within lipid rafts and that cytochalasin B caused these IR aggregates to disaggregate. Such disaggregation would be consistent with IRs binding either to each other (via glucose-binding regions) or to glucose transporters by means of glucose-sensitive interactions. Jarrett and Smith [5] also reported that cytochalasin B, but not cytochalasin D, increased I 125-insulin binding to adipocytes, which is consistent with our observation that cytochalasin B increased insulin binding to insulin-like peptides derived from the IR (e.g., Fig. 2). They reported in addition that pre-incubating cells with insulin prevented the increase in binding caused by cytochalasin B, as would be expected if some of the cytochalasin B bound directly to insulin. Raizada, Fellows, and Wu [20] similarly reported that cytochalasin B increased insulin binding to its receptor, though interpretation of these data are complicated by the same factors (e.g., probable complexation with GLUT) as are our experiments. Martinnen [21] found that 25 mM glucose increased high-affinity and decreased low-affinity insulin binding to intact erythrocytes, which is consistent again with our data (e.g., Fig. 3). Garvey and his collaborators [4, 6, 22, 23] have shown that glucose alone or insulin alone at any reasonable physiological concentration had very little or no effect on rates of glucose transport, but that insulin

and glucose at almost any physiological concentrations control glucose transport synergistically. The synergy is such that increasing glucose concentrations impair insulin activity, as we have demonstrated here, and have the physiological effect of impairing translocation of GLUT to the cell surface in adipocytes. Their data are consistent with a model that involves the IR directly as a glucose sensor and also the IR forming a regulatory complex with GLUT.

Notably, glucose may be required for full insulin activity: Yu and Erikkson [24] found that insulin binding to rat adipocytes was increased up to three-fold by medium containing 5.6 mM D-glucose as compared with glucose-free medium, again pointing to glucose-dependent insulin binding to the IR. Other sugars, such as fructose and pyruvate, could partly substitute for D-glucose, but they increased insulin binding only about 1.5-fold. They also found that cytochalasin B completely blocked enhanced insulin binding. Our data also suggest such a glucose-enhancing effect under hypoglycemic conditions (Fig. 5). And we have found, as have several previous studies [7, 17, 18], that the IR forms a complex with GLUT (Fig. 6) so that all studies of glucose and cytochalasin B effects on insulin binding and activity must be re-interpreted in light of possible GLUT participation in the experimental effects.

It is important to stress that the affinities that we have documented between the glucose and IR peptides and between insulin and IR peptides are appropriate to explaining physiological functions. Normal blood glucose is about 5.5 mM. Two of the IR peptides (95–113 and 897–916) had binding constants between 11 and 14 mM, which would mean that at normal blood glucose, they are each about 10% saturated. Under hyperglycemic conditions (15–30 mM glucose), these binding sites would be 50–90% saturated, resulting in resistance to insulin binding (Figs. 3, 4, 5). It is possible that the affinities of the various peptides studied are somewhat lower than would occur if they were in their tertiary conformations within an intact receptor. Tertiary conformation plays an important role in insulin binding, as multiple regions of the IR are known to participate in hormone binding [25–31], and it is important to bear in mind that the method used here to measure insulin affinity to IR peptides utilized insulin conjugated to horseradish peroxidase, which may interfere with binding. Nonetheless, the highest affinities observed in this study for insulin-HRP binding to IR peptides were only between 7- and 30-fold higher [1.5–8.5 nM (Table 1)] than those reported for insulin binding to its native receptor (ca. 250 pM for binding to adipocytes [32]). Moreover, the binding constants for glucose binding to the IR itself (1.5 and 70 mM, Figs. 4a, 5a) are very close to the kDs measured for the isolated IR peptides and would result in two-stage binding under normoglycemic and hyperglycemic conditions. As demonstrated in Figs. 4b, 5b and c, a range of glucose concentrations that spans hypoglycemic, normoglycemic, and hyperglycemic conditions causes significant differences in insulin-HRP binding to the intact IR, again in harmony with the data derived from isolated IR peptides. Thus, some of the peptides described here are likely to be components of more complex binding sites within the tertiary structure of the intact IR, and the experiments reported here may be relevant to elucidating how various regions of the IR contribute to insulin binding. Moreover, the results obtained with the IR peptides concerning the glucose sensitivity of insulin binding were borne out with direct experiments on the IR itself using 1 nM insulin-HRP, which is within normal variations for physiological glucose concentrations.

Our IR-derived peptide data are also consistent with previous studies of where insulin binds to the IR. Regions thought to be involved in insulin binding (Table 1) include its N-terminus (SwissProt numbers) 34–84 [25, 26]; 107–112 [26, 27]; 238–393 [25, 28]) and more specifically 264–272 and 344–352 [27]; 496–619 [29]; 677–785 [30] and more specifically 728–737 [26, 27]; and 792–797 [29]. Additionally, known mutations to the IR [summarized on the SwissProt website (http://www.expasy.ch) for human IR P06213] show that the 232–387, 496–619, and 677–797 regions as well as two additional regions, 113–119 and 925–937, are associated with loss of insulin binding resulting in leprechaunism. Longo, Langley, and Still [31] and Whitaker et al. [26], for example, demonstrated that the Arg 86 (SwissProt, 113; Table 1) is critical to activation of glucose transport. All of the glucose- and insulin-binding sequences listed in Table 1 are either within one of the regions defined by previous investigators as insulin-binding sites or they are immediately adjacent to one of them, as would be required for glucose concentration-dependent modification of insulin binding.

Our data suggest that insulin-binding regions of the IR evolved from insulin-like modules that may represent low-affinity insulin binding sites on their own, or participate in tandem through tertiary and quaternary structures to form parts of high affinity sites [15, 16]. We note, however, that our data suggest that a novel region not previously identified as an insulin binding site—SwissProt 897–916—may be involved in insulin binding. Since this sequence sits at the intersection of the alpha and beta chains of the IR and is glucose sensitive as well, it might play a very important role in any allosteric mechanisms that may be at work in the insulin-glucose-IR-GLUT system. The other important addition to our current knowledge of the IR that our study makes is the demonstration that insulin-like regions of the IR bind glucose as well.

Taken together, our data suggest a new model of glucose sensing that augments previous research demonstrating

IR-mediated GLUT transport and a variety of second-messenger-related alterations in insulin activity due to hyperglycemic conditions. The new model incorporated into this larger system glucose binding to insulin and the IR, combined with complexation of IR with GLUT (Figs. 6, 7). IR and GLUT bind to each other through glucose-sensitive and cytochalasin B-sensitive interactions to form complexes that, as Jarrett and Smith [5] demonstrated, may involve anywhere from two to six IR and (we predict) as many GLUT. These complexes may exert allosteric control within themselves. The binding of insulin to the IR thereby may regulate not only GLUT translocation to the cell surface, but also regulation of glucose transport more directly by altering GLUT structure. This allosteric mechanism may be sensitive to glucose concentration by one or more of three mechanisms: glucose binding to insulin itself, glucose binding to the IR, and/or glucose transport through GLUT producing allosteric changes in insulin activity on the IR. The effect of glucose binding to insulin, the IR and/or GLUT is to decrease insulin binding and activity (glucose-mediated insulin densensitization). Because glucose causes multiple effects on this highly integrated system, it is likely to be extremely sensitive to glucose concentration. A decrease in available insulin (as occurs in type 1 diabetes), or exposure of the system to persistent hyperglycemia (as in type 2 diabetes) would also make this system extremely susceptible to unrecoverable loss of control, since hyperglycemia will impair insulin-dependent responses in a vicious cycle.

The experimental exploration of the hypothesis of a potential allosterically regulated insulin-receptor-GLUT glucose sensor unit would appear to be particularly important in light of the demonstration that GLUT 1 or GLUT 2 (but not GLUT 4) are necessary components of whatever glucose sensor exists in the portal vein (and perhaps other glucose sensors in other parts of the body) [33–35]. Moreover, the hypothesis is completely consistent with previous studies on alterations in phospholipid/atypical PKC post-receptor second messenger signaling for insulin-stimulated glucose transport that occur in obesity and type II diabetes [36, 37]. Clearly, glucose-mediated modification of insulin signaling at the receptor-transporter unit would produce the well-documented cascade of second-messenger consequences involving AMPK, PPARgamma, PKCs, PI-3K, etc., that are associated with insulin-resistance [33–38].

The “Achilles’ heel” of such a system of glucose regulation may follow from the very fact that glucose binds to the IR. Insulin is very rapidly (days to weeks) glycosylated in hyperglycemic conditions [39–41], resulting in significantly reduced insulin binding and activity [42–47]. Since insulin itself turns over rapidly in the body, however, most investigators believe that glycosylation of insulin has little physiological or pathological effect. Similar glucose-mediated glycosylation of the IR (and/or GLUT, as we have proposed elsewhere [48]) might result in more serious insulin resistance since IRs turn over very slowly. Our data demonstrate that glucose binds to regions of the IR that also bind insulin (Table 1, Figs. 1, 2) and that insulin binding is glucose-sensitive (Figs. 2–5). The IR peptides that bind both glucose and insulin are similar to insulin in their sequences [15, 16]. Thus, it is possible that the IR itself is rapidly glycosylated under hyperglycemic conditions, and we are currently testing this possibility. Evidence for such an effect may already have been presented by Watanabe et al. [49], who report that exposing IM-9 cells to 25 mM glucose resulted in a decrease in insulin binding beginning at 6 days and very significantly decreased binding after 14 days, despite no change in the number of available insulin-binding sites. The greater the hyperglycemia and the longer it lasts or repeats, the greater the glycosylation of insulin-binding sites on the IR would be expected to be, and the greater the resulting insulin resistance. Such a scenario would result in ever more serious vicious cycles of hyperglycemia and insulin resistance that might help to explain the unusually high, yet ineffective, levels of insulin in type 2 diabetes and the need for many type 1 diabetics to increase their insulin doses over time.

Glycosylation of the IR under hyperglycemic conditions may also help to explain the difference between short-term, reversible glucose desensitization of beta cells and long-term, irreversible glucose toxicity proposed by Robertson, Olson, and Zhang [50]: glucose desensitization would result (at least in part) from glucose-mediated decrease in insulin activity, and glucose toxicity would result (at least in part) from glycosylation of the IR (and perhaps GLUT as well). Glucose toxicity resulting in increased glycosylation of additional proteins, such as those in nerves, may lead to long-term negative effects on sensation, movement, and cognition that are associated with chronic diabetes and dementias (e.g., [51, 52]).

Conclusions

It is worth noting that a number of investigators (e.g., [5, 6, 33, 53, 54]) have produced tantalizing evidence that there may be a “glucoreceptor” or glucose sensor on beta cells, neurons, the portal vein, and other cell types, but have been unable to identify it. The discovery that the IR itself binds glucose, and that insulin binding to the IR is glucose-sensitive, suggests new ways to view glucose sensing and leads to a novel model making unique, testable predictions about how glucose regulation is lost in types 1 and 2 diabetes through direct binding of glucose to insulin and the IR and through GLUT-IR allostery. It also suggests how

chronic hyperglycemia can lead to long-term glucose toxicity by glycating the IR.

Conflict of interest statement Neither author has any commercial interest or any other conflict of interest related to the contents of this study.

References

1. Cushman SW, Wardzala LJ (1980) Potential mechanism of insulin action on glucose transport in isolated rat adipose cell: apparent translocation of intracellular transport systems to the plasma membrane. J Biol Chem 255:4758–4762
2. Suzuki K, Kono T (1980) Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. Proc Natl Acad Sci USA 77:2542–2545
3. Field JB (1964) Factors concerned with insulin synthesis and release. Metabolism 13:407–421
4. Marshall S, Garvey WT, Traxinger RR (1991) New insights into the metabolic regulation of insulin action and insulin resistance: role of glucose and amino acids. FASEB J 5:3031–3036
5. Jarett L, Smith RM (1979) Effect of cytochalasin B and D on groups of IRs and on insulin action in rat adipocytes. Possible evidence for a structural relationship of the IR to the glucose transport system. J Clin Invest 63:571–579
6. Garvey WT, Olefsky JM, Matthaei S, Marshall S (1987) Glucose and insulin co-regulate the glucose transport system in primary cultured adipocytes. A new mechanism of insulin resistance. J Biol Chem 262:189–197
7. Minokoshi Y, Kahn RC, Kahn BB (2003) Tissue-specific ablation of GLUT4 glucose transporter or the IR challenges assumptions about insulin action and glucose homeostasis (minireview). JBC 278:33609–33612
8. Nevado C, Valverde AM, Benito M (2006) Role of the IR in glucose uptake in neonatal hepatocytes. Endocrinology 147: 3709–3718
9. Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR (1999) Tissue specific knockout of the IR in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 96:329–339
10. Anzenbacher P, Kalous V (1975) Binding of D-glucose to insulin. Biochim Biophys Acta 386:603–607
11. Kalous V, Anzenbacher P (1979) Mechanism of the insulin–glucose interactions. Acta Diabetol Lat 16:169–174
12. Root-Bernstein RS, Dillon PF (1997) Molecular complementarity I. The complementarity theory of life and its origins. J Theor Biol 188:447–479
13. Yu B, Caspar DL (1998) Structure of cubic insulin crystals in glucose solutions. Biophys J 74:616–622
14. Zoete V, Meuwly M, Karplus M (2004) Investigation of glucose binding sites on insulin. Proteins 55:568–581
15. Root-Bernstein RS (2002) Molecular complementarity III. Peptide complementarity as a basis for peptide receptor evolution: a bioinformatic case study of insulin, glucagon and gastrin. J Theor Biol 218:71–84
16. Root-Bernstein RS (2005) Peptide self-aggregation and peptide complementarity as bases for the evolution of peptide receptors: a review. J Mol Recognit 18:40–49
17. Eisenberg ML, Maker AV, Slezak LA, Nathan JD, Sritharan KC, Jena BP, Geibel JP, Andersen DK (2005) IR (IR) and glucose transporter 2 (GLUT2) proteins form a complex on the rat hepatocyte membrane. Cell Physiol Biochem 15:51–58
18. González-Rodriguez A, Nevado C, Escrivá F, Sesti G, Rondinone CM, Benito M, Valverde AM (2008) PTP1B deficiency increases glucose uptake in neonatal hepatocytes: involvement of IRA/GLUT2 complexes. Am J Physiol Gastrointest Liver Physiol 295:G338–G347
19. Dillon PF, Root-Bernstein RS, Lieder CM (2007) Ascorbate enhancement of H1 histamine receptor coincides with ascorbate oxidation inhibition by histamine receptors. Am J Physiol Cell Physiol 60:C977–C984
20. Raizada MK, Fellows RE, Wu B (1981) Cytochalasin B-induced alterations of insulin binding and microfilament organization in cultured fibroblasts. Exp Cell Res 136:335–341
21. Martinnen A (1989) Insulin degradation by intact erythrocytes is associated with low-affinity insulin binding sites. J Endocrinol Invest 12:455–459
22. Lima FB, Thies RS, Garvey WT (1991) Glucose and insulin regulate insulin sensitivity in primary cultured adipocytes without affecting IR kinase activity. Endocrinology 128:2415–2426
23. Lima FB, Bao S, Garvey WT (1994) Biological actions of insulin are differentially regulated by glucose and insulin in primary cultured adipocytes. Chronic ability to increase glycogen synthase activity. Diabetes 43:53–62
24. Yu ZW, Eriksson JW (2000) The upregulating effect of insulin and vanadate on cell surface IRs in rat adipocytes is modulated by glucose and energy availability. Horm Metab Res 32:310–315
25. Yip CC, Grunfeld C, Goldfine ID (1991) Identification and characterization of the ligand-binding domain of IR by the use of an anti-peptide antiserum against amino acid sequence 241–251 of the alpha subunit. Biochemistry 30:695–701
26. Whittaker J, Sørensen H, Gadsbøll VL, Hinrichsen J (2002) Comparison of the functional insulin binding epitopes of the A and B isoforms of the IR. JBC 277:47380–47384
27. Ottensmeyer FP, Beniac DR, Luo RZ-T, Yip CC (2000) Mechanisms of transmembrane signaling: insulin binding and the IR. Biochemistry 39:12103–12112
28. Yip CC (1992) The insulin-binding domain of IR is encoded by exon 2 and 3. J Cell Biochem 48:19–25
29. Prigent SA, Stanley KK, Siddle K (1990) Identification of epitopes on the human IR reacting with rabbit polyclonal sera and mouse monoclonal antibodies. JBC 265:9970–9977
30. Fabry M, Schaefer E, Ellis L, Kojro E, Fahrenholz F, Brandenburg D (1992) Detection of a new hormone contact site within the IR ectodomain by the use of a novel photoreactive insulin. J Biol Chem 267:8950–8956
31. Longo N, Langley MD, Still SJ (1998) Role of arginine 86 of the IR in insulin binding and activation of glucose transport. Biochim Biophys Acta 1402:86–94
32. Pedersen O, Hjøllund E, Beck-Nielsen H, Lindskov HO, Sonne O, Gliemann J (1981) IR binding and receptor-mediated insulin degradation in human adipocytes. Diabetologia 20(6):636–641
33. Burcelin R, Dolci W, Thorens B (2000) Glucose sensing by the hepatoportal sensor is GLUT2-dependent. In vivo analysis in GLUT2-null mice. Diabetes 49:1643–1648
34. Thorens B, Guillam M-T, Beermann F, Burcelin R, Jaquet M (2000) Transgenic reexpression of GLUT1 or GLUT2 in pancreatic β cells rescues Glut2-null mice from early death and restores normal glucose-stimulated insulin secretion. J Biol Chem 275:23751–23758
35. Burcelin R, Crivelli V, Perrin C, Da Costa A, Mu J, Kahn BB, Birnbaum MJ, Kahn CR, Vollenweider P, Thorens B (2003) GLUT4, AMP kinase, but not the IR, are required for hepatoportal glucose sensor-stimulated muscle glucose utilization. J Clin Invest 111(10):1555–1562
36. Farese RV (2001) Insulin-sensitive phospholipid signaling systems and glucose transport. Update II. Exp Biol Med (Maywood) 226(4):283–295

37. Farese RV, Sajan MP, Standaert ML (2005) Insulin-sensitive protein kinases (atypical protein kinase C and protein kinase B/Akt): actions and defects in obesity and type II diabetes. Exp Biol Med (Maywood) 230(9):593–605
38. McGarry J (2002) Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes 51(1):7–18
39. O'Harte FPM, Hojrup P, Barnett CR, Flatt PR (1996) Identification of the site of glycation of human insulin. Peptides 17:1323–1330
40. McKillop AM, Meade A, Flatt PR, O'Harte FPM (2003) Evaluation of the site(s) of glycation in human proinsulin by ion-trap LCQ-electrospray ionization mass spectrometry. Regul Pept 113:1–8
41. Farah MA, Bose S, Lee JH, Jung HC, Kim Y (2005) Analysis of glycated insulin by MALDI-TOF mass spectrometry. Biochim Biophys Acta 1725:269–282
42. Dollhofer R, Wieland OH (1979) Preparation and biological properties of glycosylated insulin. FEBS Lett 100:133–136
43. Abdel-Wahab YHA, O'Harte FPM, Barnett CR, Flatt PR (1997) Characterization of insulin glycation in insulin-secreting cells maintained in tissue culture. J Endocrinol 152:59–67
44. Abdel-Wahab YHA, O'Harte FPM, Boyd AC, Barnett CR, Flatt PR (1997) Glycation of insulin results in reduced biological activity in mice. Acta Diabetol 34:265–270
45. Abdel-Wahab YHA, O'Harte FPM, Mooney MH, Barnett CR, Flatt PR (2002) Vitamin C supplementation decreases insulin glycation and improves glucose homeostasis in obese hyperglycemic (ob/ob) mice. Metabolism 51:514–517
46. Boyd AC, Abdel-Wahab YHA, McKillop A, McNulty H, Barnett CR, O'Harte FPM, Flatt PR (2000) Impaired ability of glycated insulin to regulate plasma glucose and stimulate glucose transport and metabolism in mouse abdominal muscle. Biochim Biophys Acta 1523:128–134
47. O'Harte FPM, Boyd AC, McKillp AM, Abdel-Wahab YHA, McNulty H, Barnett CR, Conlon JM, Hojrup P, Flatt PR (2000) Structure, antihyperglycemic activity and cellular actions of a novel diglycated human insulin. Peptides 21:1519–1526
48. Root-Bernstein RS (2007) An insulin-like modular basis for the evolution of glucose transporters with implications for diabetes. Evol Bioinform 3:317–331
49. Watanabe N, Kobayashi M, Iwasaki M, Ishibashi O, Takata Y, Shigeta Y (1987) The effect of high-glucose condition on insulin binding to IM-9 lymphocytes. Horm Metab Res 19:316–318
50. Robertson RP, Olson LK, Zhang HJ (1994) Differentiating glucose toxicity from glucose desensitization: a new message from the insulin gene. Diabetes 43:1085–1089
51. Baynes JW (2001) The role of AGEs in aging: causation or correlation. Exp Gerontol 36:1527–1537
52. Vlassara H, Palace MR (2002) Diabetes and advanced glycation endproducts. J Intern Med 251:87–101
53. Kobayashi K, Yoshida K, Tsumura Y, Kagawa S, Matsuoka A (1980) Studies on glucoreceptor. I. Specific binding of D-glucose to partially purified membrane of rat islets of Langerhans. J Pharmacobiodyn 3:33–40
54. Niki A, Niki H, Hashioka T (1989) Receptors of paraneurons, with special reference to glucoreceptors. Arch Histol Cytol 52:33–38