Hybrid Receptors Formed by Insulin Receptor (IR) and Insulin-like Growth Factor I Receptor (IGF-IR) Have Low Insulin and High IGF-1 Affinity Irrespective of the IR Splice Variant*

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Insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) are both from the same subgroup of receptor tyrosine kinases that exist as covalently bound receptor dimers at the cell surface. For both IR and IGF-IR, the most described forms are homodimer receptors. However, hybrid receptors consisting of one-half IR and one-half IGF-IR are also present at the cell surface. Two splice variants of IR are expressed that enable formation of two isoforms of the IGF-IR/IR hybrid receptor. In this study, these two splice variants of hybrid receptors were studied with respect to binding affinities of insulin, insulin-like growth factor I (IGF-I), and insulin-like growth factor II (IGF-II). Unlike previously published data, in which semipurified receptors have been studied, we found that the two hybrid receptor splice variants had similar binding characteristics with respect to insulin, IGF-I, and IGF-II binding. We studied both semipurified and purified hybrid receptors. In all cases we found that IGF-I had at least 50-fold higher affinity than insulin, irrespective of the splice variant. The binding characteristics of insulin and IGF-I to both splice variants of the hybrid receptors were similar to classical homodimer IGF-IR.

Most mammalian cells express both insulin receptor (IR) and IGF-I receptor (IGF-IR) but at different expression levels. The two receptors are in the same tyrosine kinase family and have a high degree of sequence similarity (1–5). Two splice variants of IR are expressed in mammalian cells. They differ by the presence of exon 11 encoding 12 amino acids at the C-terminal of the α-subunit (IR+/ex11 or IR-B) or by the absence of exon 11 (IR−/ex11 or IR-A) (6). Hence, the two splice-variant IGF-IR/IR+/ex11 and IGF-IR/IR−/ex11 hybrid receptors can be formed. They have been detected in all tissues that express both IGF-IR and IR (7, 8) and appear to be present in amounts that suggest that the monomers of IR and IGF-IR assemble into homo- and heterodimers in a stochastic fashion (9–12).

Insulin predominantly induces metabolic effects, whereas IGFs are far more potent as growth factors and anabolic agents (13). The IR and IGF-IR have high affinity for their cognate ligand, whereas the binding affinities of insulin to IGF-IR and IGF-I to IR are ~100-fold lower (14, 15). The functional role of hybrid receptors in vivo remains unclear (16, 17). However, the composition of IR, IGF-IR, and hybrid receptors can change during differentiation. This was observed in 3T3-L1 adipocytes, where virtually all IGF-IR monomers were present as hybrid receptors in fully matured cells (18). The expression levels of hybrid receptors have been found to be elevated in some types of cancer cells (19, 20). Furthermore, an increased level of, specifically, IGF-IR/IR+/ex11 has been reported in breast cancer cells (21, 22). It is therefore important to determine whether there is a specificity difference between the two splice variants of the hybrid receptors with respect to insulin, IGF-I, and IGF-II binding.

Placenta expresses hybrid receptors, and insulin was shown to bind with low affinity to them (23). The hybrid receptors formed in placenta were also studied by Kasuya et al. (24), who found that the hybrid receptors have binding properties similar to IGF-IR. However, in these studies, the hybrid receptors examined were a mix of the two splice variants, as placenta expresses equal levels of the two IR isoforms (25). Pandini et al. (26) studied the two splice variants of the hybrid receptors separately and reported that the IGF-IR/IR+/ex11 hybrid receptor had high affinity for insulin in contrast to IGF-IR/IR−/ex11. There are several other reports in which IGF-I induces tyrosine phosphorylation of the hybrid receptors but no insulin response could be detected (27–29). Because the literature contains some conflicting data on the binding properties of hybrid receptors, we decided to investigate this question in more detail using both methods similar to those previously described as well as other methods.

**EXPERIMENTAL PROCEDURES**

Cells and Culture Conditions—The human IR−/ex11, IR+/ex11, and IGF-IR (14) were inserted in pZem vector and stably expressed alone or in combination in baby hamster kidney (BHK) cells to achieve the desired receptor expressions. In brief, 1 × 10⁶ cells were transfected with 10 μg of plasmid by
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liposome transfer using Lipofectamine (Invitrogen) and allowed to recover 3 days before selection pressure, in the form of 1 μM methotrexate, was applied. After ~3 weeks, single cell clones from each transfection appeared, and 12 clones from each transfection were picked and subjected to analysis for the expression of IGF-IR and IR by Western blot analysis. Based on the highest expression level, one clone for each transfection was selected and used in the subsequent experiments. The cells were cultured in Dulbecco’s modified Eagle’s medium containing high glucose, 10% fetal calf serum, 2 mM l-glutamine, 10 mg/ml streptomycin, and 100 units/ml penicillin (Invitrogen) at 37 °C in a 5% CO₂-enriched, humidified atmosphere.

Chemicals and Antibodies—125I-(Tyr³⁴)-IGF-I, human IGF-I, and insulin were from Novo Nordisk A/S ( Bagsvaerd, Denmark). IGF-II was from GroPep (Adelaide, Australia). Human serum albumin (HSA) A-1887 (batch 094K7640) was from Sigma-Aldrich. The IR-specific antibodies, 83-7 (30) and IR-CT1 (31), and the IGF-IR-specific antibody and IGFR 1–2 (23) were licensed from Dr. K. Siddle (University of Cambridge, Cambridge, UK).

Wheat Germ Agglutinin (WGA)–Agarose Purification of Solubilized Receptors—Cells were lysed in 50 mM Hepes, pH 8.0, 150 mM NaCl, 1% Triton X-100, 2 mM EDTA, and 10% glycerol. The cleared cell lysate was batch-absorbed with WGA-agarose (lectin from Triticum vulgaris-agarose, L1394, Sigma-Aldrich) for 90 min. After 20 volumes of wash with 50 mM Hepes, pH 8.0, 150 mM NaCl, and 0.1% Triton X-100, the receptors were eluted with 50 mM Hepes, pH 8.0, 150 mM NaCl, 0.1% Triton X-100, 0.5 M N-acetyl glucosamine, and 10% glycerol. All buffers contained Complete protease inhibitor mixture (Roche Diagnostics).

Immunopurification of Solubilized Hybrid Receptors—Hybrid receptors were isolated by two consecutive immunofinity purifications using monoclonal antibodies specifically recognizing the C termini of the β-subunit of IGF-IR (IGFR 1–2) and IR (IR CT-1). The antibodies were coupled to CNBr-activated Sepharose™ 4B beads according to the manufacturer’s instructions (Amersham Biosciences AB, Uppsala, Sweden). Cell lysates from BHK cells co-expressing IGF-IR and one of the two IR isoforms were cleared by centrifugation at 15,000 × g for 10 min, mixed with two volumes of binding buffer (BB) (100 mM Hepes, pH 8.0, 100 mM NaCl, 10 mg of MgCl₂, 0.025% Triton X-100), and added to the IGFR 1–2-Sepharose beads for batch absorption. After 18 h of incubation at 4 °C, unbound receptors (i.e., IR homodimer) were removed by washing the beads 10 times with one volume of BB. Hybrid receptors and classical homodimer IGF-IR were eluted by incubation with 10 volumes of BB containing 1 mg/ml peptide with the amino acid sequence KKKKKNGRILTLPRSNPS overnight. The amino acid sequence corresponded to the IR CT-1 antibody epitope with three additional lysines at the N terminus, which increased the solubility of the peptide. All buffers contained Complete protease inhibitor mixture (Roche Diagnostics).

Plate Antibody Capture Binding Assays—WGA-purified, solubilized receptors were analyzed by plate antibody capture assay done essentially as described by Pandini et al. (26). Microtiter plates (Nunc; Lockwell C8, Maxisorb catalog number 446469; Roskilde, Denmark) were coated with IR-specific antibody by adding 100 μl of 17 μg/ml 83–7 IR antibody in phosphate-buffered saline (PBS) (Amersham Biosciences catalog number RPNQ0017) to each well. Plates were incubated 1 h at room temperature before three washes with 0.05% Tween 20 in PBS and blocking with 250 μl/well of 0.2% Tween 20 in PBS and incubated for 1 h at room temperature. The plates were then washed three times with 0.05% Tween 20 in PBS. To each well a 10 μl of solubilized receptors at a suitable concentration, adjusted to give 20% bound tracer when no competing ligand was added. After 2 h of incubation at room temperature, unbound receptors were removed by three washes with 0.05% Tween 20 in PBS. Displacements of tracer was done with increasing concentrations of the competitor insulin or IGF-I in 150 μl/well 20 mM Hepes, pH 8.0, 10 mM MgSO₄, 137.5 mM NaCl, 0.05% Triton X-100, and 0.5% (w/v) HSA with 5000 cpm/well of 125I-IGF-I and incubated overnight at room temperature. The receptor-bound tracer was counted on a γ counter after three washes with 250 μl of 20 mM Hepes, pH 8.0, 1 mM EDTA, 150 mM NaCl, and 0.025% Triton X-100. EC₅₀ values were determined using the non-linear regression algorithm in GraphPad Prism, version 4.02 (GraphPad Software, Inc., San Diego, CA).

Scintillation Proximity Assay (SPA)—WGA-purified hybrid receptors of both isoforms of IR were used. SPA PVTr antisemouse beads (Amersham Biosciences) were incubated with IR antibody 83–7 and hybrid receptors for 5 h at room temperature. The SPA beads were washed twice with buffer to remove IGF-IR and any other receptors not bound to the SPA beads, and 125I-IGF-I was added. A 2-fold dilution series of human insulin (starting from 100 nm), IGF-I (from 10 nm), or IGF-II (from 10 nm) was prepared in an Optiplate 96-well plate (PerkinElmer Life Sciences) and the SPA beads added. The final concentration of 125I-IGF-I was 5000 cpm/200 μl, and the buffer composition was 100 mM Hepes, pH 7.8, 100 mM NaCl, 10 μM MgCl₂, and 0.025% Tween 20. The plate was rocked gently for 18 h at room temperature, centrifuged, and counted in a TopCounter. Kᵣ values were calculated by non-linear regression analysis.

Polyethylene Glycol (PEG) Precipitation Binding Assay—For the PEG assay, a suitable dilution of solubilized, immunopurified hybrid receptor isoform samples or WGA-purified, solubilized IGF-IR (giving ~20% bound tracer when no competing ligand was added) was incubated for 18 h at 4 °C in a total volume of 200 μl with 2500 cpm of 125I-IGF-I and various concentrations of competing ligands in BB + 0.05% (w/v) HSA. Subsequently bound tracers were recovered by precipitation with 0.04% gammaglobulin and 24% (w/v) PEG 8000. Bound 125I-labeled IGF-I was counted on a γ counter. EC₅₀ values
were calculated using the non-linear regression algorithm in GraphPad Prism, version 4.02 (GraphPad Software, Inc.).

PCR IR Isoform Test—Isolation of total RNA from BHK cell lines was done using the RNeasy minikit (Qiagen GmbH, Hilden, Germany). cDNA was prepared from the RNA using the Omiscript kit (Qiagen GmbH, Hilden, Germany). One common human IR-specific antisense primer annealing in exon 12 with the sequence 5'-CCACCGTGAGCAGGCACC-3' was used and two IR isoform-specific sense primers. For IR⁺ex11, the primer sequence was 5'-GCACGTTGAGCAGGACC-3' annealing in exon 11, and for IR⁻ex11, the specific primer was annealing in the overlapping region of exon 10 and exon 12 with the sequence 5'TGGTTTTCGTCCCCAGGCAGGACCC-3'. PCR was done with the Expand High Fidelity kit (Roche Applied Science). The PCR products were analyzed by agarose gel electrophoresis.

RESULTS

To study IR/IGF-IR hybrid receptors, five stable BHK cell lines were generated, which expressed the human IGF-IR, IR⁻ex11, and IR⁺ex11 alone or co-expressed IGF-IR with IR⁻ex11 or IGF-IR with IR⁺ex11. The receptor expressions were evaluated by Western blot analysis (data not shown) and by PCR analysis of cDNA prepared from individual cell lines (Fig. 1). The primers for the PCR analysis were designed to distinguish between the two splice variants of IR. One common human IR-specific antisense primer annealing in exon 12 and two IR isoform-specific sense primers were used. For testing the presence of IR⁺ex11 mRNA, the specific primer annealed inside exon 11, and for IR⁻ex11 mRNA, the specific primer annealed the overlapping region of exon 10 and exon 12. For each cell line, cDNA was prepared and analyzed by two PCR reactions with the specific primers for either IR⁻ex11 or IR⁺ex11. Only in the two cell lines expected to express IR⁺ex11 did the PCR reaction with the IR⁺ex11-specific primer give the specific 81-bp PCR product (i.e. IR⁺ex11 and IGF-IR/IR⁺ex11). The IR⁻ex11-specific primer produced the specific 87-bp PCR product in the two cell lines expected to express IR⁻ex11 (i.e. IR⁻ex11 and IGF-IR/IR⁻ex11). Hence, the BHK cell lines expressed the expected human IR isoforms.

IGF-IR/IR⁺ex11 and IGF-IR/IR⁻ex11 hybrid receptors were studied using plate antibody capture binding assay essentially as described by Pandini et al. (26). The receptors were solubilized and semipurified by WGA purification from two BHK cell lines overexpressing IGF-IR and IR⁻ex11 or IGF-IR and IR⁺ex11. In the plate antibody capture binding assay, the IR-specific antibody 83-7 was used. Both the homodimer IR and the hybrid receptors were retained in the plates, but the homodimer IGF-IR was washed off. Binding of 125I-IGF-I to IR⁻ex11 or IR⁺ex11 was insignificant in this assay and could be ignored. Therefore, the EC₅₀ values for displacement of 125I-IGF-I from IGF-I/IR⁻ex11 hybrid (A) and IGF-I/IR⁺ex11 hybrid (B) with IGF-I (■) or insulin (▲) in plate antibody capture binding assay. The graphs are representatives of at least three experiments. Each point in the graph is the mean (±S. D.) of two measurements.
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**TABLE 1**

|                         | Insulin competitor | IGF-I competitor | IGF-I competitor |
|-------------------------|--------------------|------------------|------------------|
| **Plate antibody capture binding assay (EC\(_{50}\)** |                     |                  |                  |
| IGF-IR/IR\(^{-\text{ex11}}\) | 2.6 ± 1.3          | 0.017 ± 0.011    |                  |
| IGF-IR/IR\(^{+\text{ex11}}\) | 2.8 ± 1.4          | 0.012 ± 0.010    |                  |

**Scintillation proximity assay (K\(_d\))**

|                          |                     |                  |                  |
|--------------------------|---------------------|------------------|------------------|
| IGF-IR/IR\(^{-\text{ex11}}\) | 1.1 ± 0.2           | 0.022 ± 0.006    | 0.18 ± 0.04      |
| IGF-IR/IR\(^{+\text{ex11}}\) | 1.1 ± 0.3           | 0.023 ± 0.007    | 0.19 ± 0.04      |

Fig. 2. The EC\(_{50}\) values for insulin displacement were determined to be 2.6 ± 1.3 nM for IGF-IR/IR\(^{-\text{ex11}}\) hybrid receptors and 2.8 ± 1.4 nM for IGF-IR/IR\(^{+\text{ex11}}\) hybrid receptors, and the EC\(_{50}\) values for displacement with IGF-I were 0.017 ± 0.011 and 0.012 ± 0.010 nM, respectively (Table 1). Thus, we did not find a significant difference between the two splice variants of hybrid receptors in any of the experiments. Furthermore, the EC\(_{50}\) value for displacement of \(^{125}\)I-IGF-I from the receptors with insulin was >100-fold higher than with IGF-I, irrespective of the isoforms.

We studied the binding characteristics of hybrid receptors further by another binding assay. In the scintillation proximity assay, the IR-specific antibody 83-7 was used, and unlike in the plate antibody capture assay, no HSA or bovine serum albumin was present. Instead, nonspecific binding of peptides was prevented by the presence of 0.025% Tween 20. We determined the K\(_d\) values for insulin displacement of \(^{125}\)I-IGF-I to be 1.1 ± 0.2 nM for IGF-IR/IR\(^{-\text{ex11}}\) hybrid receptors and 1.1 ± 0.3 nM for IGF-IR/IR\(^{+\text{ex11}}\) hybrid receptors and for displacement with IGF-I to be 0.022 ± 0.006 and 0.023 ± 0.007 nM, respectively. Thus, all binding affinities were comparable with the EC\(_{50}\) values obtained with the plate antibody capture binding assay (Table 1). The SPA was further used to determine the hybrid receptor binding affinity for IGF-II. We found the K\(_d\) values for IGF-II displacement of \(^{125}\)I-IGF-I to be 0.18 ± 0.04 nM for IGF-IR/IR\(^{-\text{ex11}}\) hybrid receptors and 0.19 ± 0.04 nM for IGF-IR/IR\(^{+\text{ex11}}\) hybrid receptors (Table 1). These values were similar to those found for the displacement of \(^{125}\)I-IGF-I from IGF-IR by IGF-II (data not shown).

To compare the hybrid receptors directly with IGF-IR, we purified the hybrid receptors using two consecutive antibody purifications. This enabled a direct comparison using PEG binding assay. First, IGF-IR and hybrid receptors were immunopurified with the specific IGF-IR antibody IGFR 1–2. The homodimer IGF-IR and hybrid receptors were eluted with the specific peptide for the antibody IGFR 1–2 epitope. Second, the eluted receptors were purified with an IR-specific antibody IR CT-1 retaining only the hybrid receptors. The hybrid receptors were eluted with the specific IR CT-1 antibody epitope peptide. The purified hybrid receptors allowed us to determine the EC\(_{50}\) values in a PEG precipitation binding assay for \(^{125}\)I-IGF-I displacement from IGF-IR/IR\(^{-\text{ex11}}\) and IGF-IR/IR\(^{+\text{ex11}}\) and directly compare the binding characteristics to solubilized IGF-IR. In Fig. 3, the binding curves for IR\(^{-\text{ex11}}\)/IGF-IR hybrid receptors and IGF-IR are shown. The EC\(_{50}\) values for insulin displacement of \(^{125}\)I-IGF-I were 4.6 ± 1.9 nM for IGF-IR/IR\(^{-\text{ex11}}\) hybrid receptors, 5.1 ± 2.3 nM for IGF-IR/IR\(^{+\text{ex11}}\) hybrid receptors, and 3.8 ± 0.5 nM for IGF-IR. The EC\(_{50}\) values for displacement of \(^{125}\)I-IGF-I with IGF-I were 0.018 ± 0.001 nM for IGF-IR/IR\(^{-\text{ex11}}\) hybrid receptors, 0.017 ± 0.001 nM for IGF-IR/IR\(^{+\text{ex11}}\) hybrid receptors, and 0.019 ± 0.003 nM for IGF-IR (Table 2).

**DISCUSSION**

There are some contradicting reports as to whether insulin is a biologically relevant ligand for IGF-IR/IR hybrid receptors. Recently, it was published by Pandini et al. (26) that there is a significant difference between the two splice variants of the hybrid receptors with respect to insulin binding. They report that IGF-IR/IR\(^{-\text{ex11}}\) bound insulin with high affinity, whereas IGF-IR/IR\(^{+\text{ex11}}\) did not. They have determined the EC\(_{50}\) value for \(^{125}\)I-IGF-I displacement with insulin to be only 10-fold higher than the EC\(_{50}\) value for IGF-I for IGF-IR/IR\(^{-\text{ex11}}\) hybrid receptors, 5.1 ± 2.3 nM for IGF-IR/IR\(^{+\text{ex11}}\) hybrid receptors, and 3.8 ± 0.5 nM for IGF-IR. Because these results are somewhat different from most other data in the literature, we decided to study the hybrid receptors by a variety of methods to clarify this issue. The binding characteristics of both receptors were first studied in a plate antibody capture assay, essentially as described by Pandini et al. (26). In this assay, the specific IR antibody 83-7 was used, capturing both hybrid receptors and homodimer IR but not homodimer IGF-IR. Displacement of \(^{125}\)I-IGF-I from hybrid receptors could be studied, as the \(^{125}\)I-IGF-I binding to the
homodimer IR was insignificant and could be ignored in this assay. In contrast to Pandini et al. (26), we found a >100-fold difference between the EC\textsubscript{50} values for insulin and IGF-I displacement of \textsuperscript{125}I-IGF-I from both isoforms of the hybrid receptors.

We had noticed that some commercially available purified radioimmunoassay grade bovine serum albumin preparations could have an effect on the IGF-I binding properties determined. We therefore studied the binding in an assay without bovine serum albumin or HSA, as was used in the plate antibody capture assay. Nonspecific peptide binding was avoided by the presence of 0.025% Tween 20. This was done in an SPA, which was based on the specific IR antibody 83-7 as in the plate antibody capture assay. In the SPA, the solubilized WGA-purified hybrid receptors as well as IR homodimers were retained on the beads, and the IGF-IR was washed off. As in the plate antibody capture assay, displacement of \textsuperscript{125}I-IGF-I from hybrid receptors could be studied, and in this assay, we found an ~50-fold difference for both isoforms. In this assay, we also measured the affinity of IGF-II for the hybrid receptors and found an affinity intermediate between that of insulin and IGF-I. The affinity was similar for both isoforms, which is interesting in view of the fact that IGF-II has been shown to bind better to IR\textsuperscript{+ex11} than to IR\textsuperscript{-ex11} (32). One explanation for this result could be that, although insulin requires a dimeric receptor to achieve high affinity binding (15), IGF-I (and IGF-II) may only require a receptor monomer. Thus, the IGFs may bind to the IGF-IR half of either isoform of the hybrid receptor irrespective of what the other half-receptor is, whereas insulin has lower affinity for the hybrid receptors, because the IGF-IR half does not contain binding epitopes that are able to contribute to a high affinity binding site for insulin.

To ensure that effects of homodimer receptors and ratios between homodimers and hybrid receptors did not influence the binding assays, we purified both hybrid receptors by sequential immunopurification. This allowed us to study the binding properties in a PEG precipitation binding assay, and again we found that both hybrid receptor isoforms had binding characteristics similar to classical IGF-1R. These results are in agreement with Soos et al. (23) and Kasuya et al. (24). The EC\textsubscript{50} values determined for IGF-I binding to solubilized hybrid receptors was in the picomolar range, as is solubilized homodimer IGF-IR/IR for their respective ligands (14, 15). IGF-I and not insulin is therefore likely to be the biologically relevant ligand of the two hormones. We did not find a significant difference in the binding characteristics of the two splice variants of the hybrid receptors as has been reported previously (26). We do not have an explanation for this discrepancy. However, we have found that the determined EC\textsubscript{50} values for displacement of \textsuperscript{125}I-IGF-I can be greatly affected by contaminations of IGF-binding proteins in some commercial radioimmunoassay grade bovine serum albumin preparations.\textsuperscript{3}

There are several reports indicating that insulin does not appear to activate hybrid receptors, whereas IGF-I does. Brown adipocytes and human and bovine endothelial cells have been reported to form IR/IGF-IR hybrid receptors that could be activated by IGF-I but not with physiological concentrations of insulin (8, 11, 27). Furthermore, in rat skeletal muscle, insulin could not displace IGF-I from the IR/IGF-IR hybrid receptor (12). Moreover, in human coronary artery smooth muscle cells, IGF-IR was not phosphorylated by low concentration of insulin but rather IR was tyrosine-phosphorylated with low amounts of IGF-I, indicating that the IR was present in hybrid receptors and that they could not be activated by low levels of insulin. In that study (28), insulin could only activate its own homodimer IR. It is not always clear whether one or both IR isoforms were present, but the studies indicate that IGF-I is the biologically relevant ligand for IGF-IR/IR hybrid receptors. Because formation of hybrid receptors appear to be stochastic and is therefore receptor concentration-dependent, the formation of hybrid receptors in cells could have an effect on their insulin responsiveness. It could be speculated that, in a situation where the level of IGF-IR is higher than the level of IR, the insulin-responsive receptor, namely the homodimer IR, could be depleted. Subsequently, IR monomers would be present mainly as IR/IGF-IR hybrid receptors and have high affinity for IGF-I, thereby increasing the number of receptors with high IGF-I affinity and decreasing the number of receptors with high insulin affinity. The functional role of hybrid receptors is still unclear. Further studies on the signaling properties of hybrid receptors may provide more information on this issue.

In conclusion, we have studied the hybrid receptors in three different binding assays, and in all cases, IGF-I had at least 50-fold higher affinity than insulin. Results were similar for both isoforms of the hybrid receptors and indicated that the binding properties of the hybrid receptors were very similar to those of native IGF-IR. These data suggest that signaling of insulin through hybrid receptors is unlikely to be physiologically relevant.

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REFERENCES

1. Ullrich, A., Gray, A., Tam, A. W., Yang-Feng, T., Tsubokawa, M., Collins, C., Henzel, W., Le Bon, T., Kathuria, S., Chen, E., Jacobs, S., Francke, U., Ramachandran, J., and Fujita-Yamaguchi, Y. (1986) EMBO J. 5, 2503–2512
2. Nakae, J., Kido, Y., and Accili, D. (2001) Endocr. Rev. 22, 818–835
3. De Meyts, P., and Whittaker, J. (2002) Nat. Rev. Drug Discov. 1, 769–783
4. Adams, T. E., Epa, V. C., Garrett, T. P., and Ward, C. W. (2000) Cell. Mol. Life Sci. 57, 1050–1093
5. Siddle, K., and Soos, M. A. (1999) Contemporary Endocrinology, pp. 199–225, Humana Press, Totowa, NJ
6. Mostchaf, L., Grako, K., Dull, T. J., Coutens, L., Ullrich, A., and McClain, D. A. (1990) EMBO J. 9, 2409–2413
7. Moxham, C. P., Duronio, V., and Jacobs, S. (1989) J. Biol. Chem. 264, 13238–13244
8. Entingh-Pearsall, A., and Kahn, C. R. (2004) J. Biol. Chem. 279, 38016–38024

\textsuperscript{3} R. Slaby, A. S. Andersen, and J. Brandt, manuscript in preparation.
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9. Valensise, H., Liu, Y. Y., Federici, M., Lauro, D., Dellanna, D., Romanini, C., and Sesti, G. (1996) *Diabetologia* **39**, 952–960
10. Baileyes, E. M., Nave, B. T., Soos, M. A., Orr, S. R., Hayward, A. C., and Siddle, K. (1997) *Biochem. J.* **327**, 209–215
11. Dekker Nitert, M., Chisalita, S., Olsson, K., Bornfeldt, K. E., and Arnqvist, H. J. (2005) *Mol. Cell. Endocrinol.* **229**, 31–37
12. Federici, M., Giaccari, A., Hriba, M. L., Giovannone, B., Lauro, D., Morviducci, L., Pastore, L., Tamburrano, G., Lauro, R., and Sesti, G. (1999) *Diabetes* **48**, 2277–2285
13. Heald, A., Stephens, R., and Gibson, J. M. (2006) *Diabet. Med.* **23**, 19–24
14. Andersen, A. S., Kjeldsen, T., Wiberg, F. C., Vising, H., Schäffer, L., Rasmussen, J. S., De Meyts, P., and Möller, N. P. H. (1992) *J. Biol. Chem.* **267**, 13681–13686
15. Schäffer, L. (1994) *Eur. J. Biochem.* **221**, 1127–1132
16. White, M. F. (2003) *Science* **302**, 1710–1711
17. Denley, A., Cosgrove, L. J., Booker, G. W., Wallance, J. C., and Forbes, B. E. (2005) *Cytokine Growth Factor Rev.* **16**, 421–439
18. Modan-Moses, D., Janicot, M., McLernihan, J. C., Lane, M. D., and Casella, S. J. (1998) *Biochem. J.* **333**, 825–831
19. Pandini, G., Vigneri, R., Costantino, A., Frasca, F., Ippolito, A., Fujita-Yamaguchi, Y., Siddle, K., Goldfine, I. D., and Belfiore, A. (1999) *Clin. Cancer Res.* **5**, 1935–1944
20. LeRoith, D., and Roberts, C. T. (2003) *Cancer Lett.* **195**, 127–137
21. Sachdev, D., Singh, R., Fujita-Yamaguchi, Y., and Yee, D. (2006) *Cancer Res.* **66**, 2391–2402
22. Sachdev, D., and Yee, D. (2001) *Endocr. Relat. Cancer* **8**, 197–209
23. Soos, M. A., Field, C. E., and Siddle, K. (1993) *Biochem. J.* **290**, 419–426
24. Kasuya, I., Paz, I. B., Maddux, B. A., Goldfine, I. D., Hefta, S. A., and Fujita-Yamaguchi, Y. (1993) *Biochemistry* **32**, 13531–13536
25. Möller, D. E., Yokota, A., Caro, J. F., and Flier, J. S. (1989) *Mol. Endocrinol.* **3**, 1263–1269
26. Pandini, G., Frasca, F., Mineo, R., Sciaccia, L., Vigneri, R., and Belfiore, A. (2002) *J. Biol. Chem.* **277**, 39684–39695
27. Li, G., Barrett, E. J., Wang, H., Chai, W., and Liu, Z. (2005) *Endocrinology* **146**, 4690–4696
28. Chisalita, S. I., and Arnqvist, H. J. (2005) *Diabetologia* **48**, 2155–2161
29. Seely, B. L., Reichart, D. R., Takata, Y., Yip, C., and Olefsky, J. M. (1995) *Endocrinology* **136**, 1635–1641
30. Soos, M. A., Siddle, K., Baron, M. D., Heward, J. M., Luzio, J. P., Bellatin, J., and Lennox, E. S. (1986) *Biochem. J.* **235**, 199–208
31. Ganderton, R. H., Stanley, K. K., Field, C. E., Coghlan, M. P., Soos, M. A., and Siddle, K. (1992) *Biochem. J.* **288**, 195–205
32. Frasca, F., Pandini, G., Scalia, P., Sciaccia, L., Mineo, R., Costantino, A., Goldfine, I. D., Belfiore, A., and Vigneri, R. (1999) *Mol. Cell. Biol.* **19**, 3278–3288