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Insulin/IGF-I Receptor Hybrids: A Mechanism for Increasing Receptor Diversity

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Abstract Insulin and IGF-I receptors are homologous disulfide linked $\alpha_2\beta_2$ tetramers. These tetramers are formed biosynthetically when proreceptors containing α and β subunits in a single uninterrupted linear peptide form disulfide linked homodimers and are subsequently proteolytically cleaved at the α-β junctions. Cells expressing both receptors also express hybrid receptors that contain one insulin receptor α and β subunit, and one IGF-I receptor α and β subunit. These presumably form by the association of mixed proreceptors. Hybrid receptors greatly expand the possible repertoire of cellular responses to hormonal stimulation. Although not yet examined in detail, both the hormone binding and the signaling properties of the hybrid receptor appear to be different from that of either insulin or IGF-I receptor. Regulatory mechanisms that involve either insulin or IGF-I receptor, at the level of expression or subsequently, could alter the expression or function of the hybrid receptor or the other receptor. Similarly, pathology in one receptor could affect both the hybrid and other receptor, or perhaps be partially compensated for by a hybrid receptor. The magnitude of these effects could vary greatly in different tissues depending upon the relative level of expression of the different receptor forms. These postulated responses might explain some of the complex heterogeneity and linkage of these receptors that have been observed previously.

Key words: insulin resistance, insulin receptor, receptor antibodies, peptide maps, transdominant negative mutations, insulin-like growth factor receptor, tyrosine kinase

IMMUNOLOGIC CROSS-REACTIVITY AND HYBRID RECEPTORS

The existence of hybrid receptors was first suggested by the apparent immunologic cross-reactivity of insulin and IGF-I receptors [1]. A battery of anti-insulin receptor antibodies, which reacted with different epitopes from widely different regions of the insulin receptor, was found to immunoprecipitate high affinity IGF-I binding activity from solubilized placenta membranes. Based upon the known amino acid sequences of the two receptors, it was difficult to explain this apparent cross-reactivity. The existence of hybrid receptors provided an alternative explanation for these observations.

That hybrid receptors were in fact the explanation for this cross-reactivity was convincingly demonstrated by investigating the immunoreactivity of rodent insulin and IGF-I receptors in cell lines in which recombinant human receptors had been expressed [2]. A species specific anti-human insulin receptor antibody failed to immunoprecipitate either insulin receptor or IGF-I receptor extracted from mouse or hamster cell lines. However, after human insulin receptors were expressed at high levels in these cell lines, this antibody acquired the ability to immunoprecipitate high affinity IGF-I binding activity. Conversely, a human specific anti–IGF-I receptor antibody acquired the ability to immunoprecipitate high affinity insulin binding activity after human IGF-I receptors were expressed in a mouse cell line.

β-SUBUNIT HETEROGENEITY AND HYBRID RECEPTORS

In most cells and tissues in which it has been examined, the IGF-I receptor β subunit migrates as a doublet when examined on SDS-polyacrylamide gels [3,4,11]. This β subunit heterogeneity results from the formation of hybrid receptors [5,6]. There is a degree of uncertainty about the nature of the components comprising these hybrids. In many respects, they appear to be insulin receptor–IGF-I receptor hybrids [5]. However, some investigators have suggested that a third, not yet well-characterized receptor might be a component of a hybrid receptor [6,7]. The evidence that forms the basis for these views will reviewed below.

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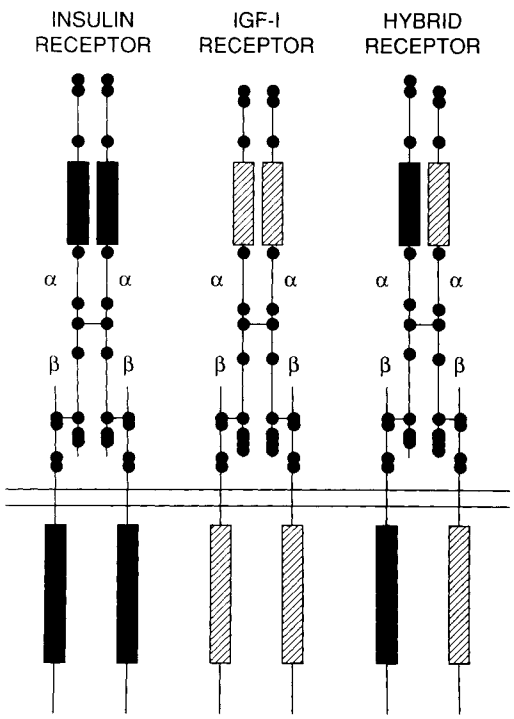

Fig. 1. Hybrid receptor formation by heterologous subunit assembly.

In NIH 3T3 cells, HepG2 cells, and brain membranes, low concentrations of insulin stimulate the tyrosyl phosphorylation of a single receptor β subunit, while low concentrations of IGF-I stimulate the tyrosyl phosphorylation of two distinct receptor β subunits with slightly different molecular weights, the lower having the same molecular weight as the insulin stimulated β subunit [5,6,7].

Stimulation of phosphorylation of this lower molecular weight β subunit by IGF-I is not simply due to cross-reactivity with ordinary insulin receptors. In a cell line expressing few insulin receptors, IGF-I is more effective than insulin at stimulating its phosphorylation [5]. Furthermore, the phosphorylation of both higher and lower molecular weight β subunits is stimulated by IGF-I with a similar concentration response which agrees with the expected potency of IGF-I to produce biological effects [5,6,7]. Although with respect to stimulation of phosphorylation, this subunit does not behave like the insulin receptor β subunit, structurally and immunologically it is very similar. Phosphopeptide maps of the lower molecular weight β subunit stimulated by IGF-I are clearly different from the higher molecular weight subunit but similar to the insulin stimulated β subunit. Furthermore, anti-insulin receptor carboxy-terminal peptide antibodies react with the lower molecular weight IGF-I stimulated β subunit as well as the insulin receptor β subunit, but not the higher molecular weight IGF-I stimulated β subunit [5,6].

Both subunits that are phoshorylated in response to IGF-I exist in a hybrid tetramer [5,6]. An antibody specific to the carboxy-terminal region of the insulin receptor β subunit immunoprecipitates both IGF-I stimulated β subunits from solubilized, intact tetrameric receptors; however, after the receptors are dissociated into α-β heterodimer halves with low concentrations of DTT, only the lower molecular weight β subunit is immunoprecipitated. Furthermore, only the lower molecular weight β subunit is recognized by this antibody on Western blots. Thus, the higher molecular weight β subunit is immunoprecipitated not because it is directly recognized by this antibody, but because it is associated with the lower molecular weight β subunit in a hybrid complex. Similarly, αIR-3, an antibody specific for the α subunit of the IGF-I receptor, precipitates both subunits if the tetramers are intact, but only the higher molecular weight one if the receptors are first dissociated into α-β heterodimeric halves [5]. Thus the higher molecular weight β subunit is associated in an α-β heterodimer with the classical IGF-I receptor α subunit recognized by αIR-3, while the lower molecular weight β subunit is not.

What is the identity of the lower molecular weight β subunit component of the hybrid receptor? Is it the insulin receptor β subunit? The following evidence suggests that it is [5]. It is indistinguishable from the insulin receptor β subunit on the basis of mobility on SDS-polyacrylamide gel electrophoresis and reactivity with the anti-insulin receptor-peptide antibody, P5. In NIH 3T3 cells and HepG2 cells, it is also indistinguishable on the basis of phosphopeptide maps. In particular, a phosphothreonine containing peptide is present in both the lower molecular weight β subunit and the insulin receptor β subunit but not the higher molecular weight β subunit. This threonine corresponds to threonine 1336 in the insulin receptor sequence. There is no corresponding threonine in the IGF-I receptor sequence. Furthermore, when the IGF-I receptor is purified from human placenta with an αIR-3 affinity column, and the amino-terminal α and β subunit sequences determined, components with sequences corresponding to both

the IGF-I receptor and the insulin receptor are present in approximately equal amounts [Y. Fujita-Yamaguchi, personal communication]. Since αIR-3 does not react with insulin receptor, these data indicate that in placenta, hybrid receptors are present which are composed of IGF-I receptor and insulin receptor halves.

However, in brain, evidence suggests that the lower molecular weight hybrid β subunit is different from either insulin or IGF-I receptor β subunits [6,7]. Phosphopeptide maps of the lower molecular weight hybrid receptor β subunit are different from maps of the insulin receptor β subunit, although they are more similar to it than to the higher molecular weight IGF-I receptor β subunit. Furthermore, although some antibodies that specifically recognize the insulin receptor β subunit also recognize the lower molecular weight hybrid receptor β subunit, others do not. Whether these subtle differences are intrinsic to the β subunits themselves or are a functional consequence of their being components of a hybrid receptor is unclear.

IN VITRO FORMATION OF HYBRID RECEPTORS

Treatment of insulin or IGF-I receptor tetramers with low concentrations of DTT at alkaline pH causes them to dissociate into disulfide linked α-β heterodimer halves [8,9]. Purified insulin or IGF-I receptor heterodimers spontaneously reassociate in the presence of Mg-ATP or hormone to form disulfide linked tetramers. When insulin and IGF-I receptor heterodimer halves are mixed, hybrid receptors form [10]. The distribution of different species suggests that mixed heterodimer halves are as likely to associate as identical halves. Thus formation of hybrid receptors can occur as a spontaneous process requiring no special cellular machinery.

RELATIVE PROPORTION OF HYBRID SPECIES

An assessment of the amount of hybrid receptors present in a cell or tissue requires quantitative assumptions about their hormone binding properties, immunoreactivity, and labeling efficiency. These have not yet been validated. Although there is little direct data, in cells that express both insulin receptors and IGF-I receptors, the proportion of hybrid receptors appears to be high.

In solubilized human placenta, insulin receptor monoclonal antibodies immunoprecipitate up to 70% of the high affinity IGF-I binding activity [1]. Assuming that this is due to hybrid receptors, and that on a molar basis they bind half as much IGF-I as ordinary IGF-I receptors, 85% of the IGF-I receptors in human placenta are actually hybrids. This is consistent with amino-terminal sequence data indicating that approximately one-half of the IGF-I receptor sequence in placenta is actually insulin receptor [Y. Fujita-Yamaguchi, personal communication].

In HepG2 cells, the high and low molecular weight β subunits immunoprecipitated by αIR-3 are labeled to a similar extent by either [35S]methionine or 32P suggesting that the hybrid receptor is a major species [5,11]. Similarly in brain, low concentrations of IGF-I stimulate the phosphorylation of both high and low molecular weight β subunits to a similar extent, suggesting that the hybrid is a major species [6]. In an NIH 3T3 cell line that had sixfold more IGF-I binding than insulin binding, phosphorylation of the lower molecular weight β subunit was stimulated more by IGF-I than insulin, indicating that most of the insulin receptor β subunit was involved in hybrid receptor than in ordinary insulin receptor [5].

FUNCTIONAL PROPERTIES OF THE HYBRID RECEPTOR

The ligand binding properties of insulin receptors depend upon a cooperative interaction between heterodimers [12]. Dissociated heterodimers have a tenfold lower affinity for binding insulin than do intact tetramers [8,9]; however, insulin binding to one site on the tetramer decreases the affinity of binding to the other site. There are, therefore, two ways in which unlabeled insulin can inhibit the binding of labeled insulin to its receptor: by directly competing for the same site, and by inducing a negatively cooperative interaction at the adjacent site. Evidence for a cooperative interaction between IGF-I receptor subunits is less clear. Most but not all investigators find that Scatchard plots of IGF-I binding are linear, suggesting that there is no cooperativity. Consistent with this, Feltz et al. [13] found that IGF-I binds to dissociated heterodimers with the same affinity as to the intact receptor. However, Tollefsen and Thompson [14] found that the dissociated receptor bound IGF-I with lower affinity than the intact receptor and that there was half site reactivity in the intact receptor, which would also result in a linear Scatchard plot. Given the, at least, quan-

titative differences in inter-subunit interactions for insulin and IGF-I receptors, it would be surprising if the hormone binding properties of the hybrid receptor were simply a linear combination of those of the homologous receptors.

Because of the difficulty in obtaining hybrid receptors free of contaminating insulin or IGF-I receptors, determining the binding and signaling properties of hybrid receptors has been difficult. Under conditions in which most of the labeled ligand binding could be assumed to be to solubilized hybrid receptors, ^{125}I-insulin binding was inhibited by cold insulin with an IC_{50} of 1 nM and by IGF-I with an IC_{50} of .2 nM, while ^{125}I-IGF-I binding was inhibited by cold insulin with an IC_{50} of 40 nM and by cold IGF-I with an IC_{50} of .2 nM [2]. Thus IGF-I appears to have a higher affinity for its high affinity binding site on the unoccupied hybrid than insulin does for its high affinity site on the unoccupied hybrid. Also, IGF-I binding to its site is more effective in inducing a negatively cooperative change in the conformation of the insulin receptor site than vice versa [2]. The higher affinity of IGF-I than insulin for the hybrid receptor and its greater ability to induce a conformational change perhaps also account for its greater potency in stimulating autophosphorylation of both β subunits of the hybrid receptor [5]. Based on the greater potency of IGF-I than insulin to activate autophosphorylation of the hybrid receptor and presumably its tyrosine kinase activity, it would be anticipated that hybrid receptors function physiologically more as IGF-I receptors than as insulin receptors.

CONSEQUENCES OF HYBRID RECEPTORS: TRANSDOMINANT MUTATIONS

An important consequence of the formation of hybrid receptors is that pathology in one receptor type can affect the function of the other. Such a phenomenon could result from interaction between subunit halves in hybrid receptors inhibiting hormone binding or tyrosine kinase activation, from defective post-translational processing or transport to the cell surface, or from increased turnover. The magnitude of this interaction would be expected to vary depending upon the relative level of expression of the different receptors. Recent studies have provided experimental support for this.

Hybrid receptors formed in vitro from tyrosine kinase defective insulin receptors and normal IGF-I receptors are defective in their ability to phosphorylate exogenous substrates in response to either hormone [15]. In such constructs, autophosphorylation of the inactive β subunit occurs normally, while autophosphorylation of the normal β subunit is blocked, suggesting that autophosphorylation is an intersubunit reaction.

Furthermore, expression of a tyrosine kinase defective insulin receptor mutant in rat fibroblasts at levels comparable to that of endogenous rat IGF-I receptors blocked responsiveness to IGF-I as well as insulin [16]. These results were interpreted as being due to competition for a shared substrate between IGF-I receptors and defective insulin receptors. However, an alternate explanation is the formation of functionally defective hybrids [20].

Some forms of severe insulin resistance that are due to coding region mutations in the insulin receptor gene have been associated with abnormalities in IGF-I binding and action. Fibroblasts cultured from a patient with Leprechaunism and insulin resistance had markedly lowered affinity for IGF-I and diminished IGF-I stimulation of glucose transport [17]. Fibroblasts from a patient with severe insulin resistance and diabetes had a parallel decrease in the number of receptors for both insulin and IGF-I [18]. The simplest mechanism for explaining how a single genetic abnormality could affect both receptors which are products of separate genes is through the formation of hybrid receptors.

CONCLUSIONS

Hybrid receptors composed of insulin and IGF-I receptor halves exist and appear to be major components of the species of these receptors that are found in various tissues. These hybrids appear to have unique properties and modes of regulation that may partially account for the atypical and heterogeneous characteristics previously observed for insulin and IGF-I receptors. The complexity resulting from the existence of hybrid receptors could greatly increase the range of cellular responses to hormonal stimulation. If, as suggested [6,7,19], new receptor isotypes exist that are the result of RNA splicing variants or the products of newly discovered or yet to be discovered genes, the resulting diversity will likely be multiplied by their involvement in receptor hybrid formation.

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