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Transdominant inhibition of tyrosine kinase activity in mutant insulin/insulin-like growth factor I hybrid receptors

(*in vitro* assembly/heterologous receptors/substrate phosphorylation/autophosphorylation)

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ABSTRACT **Classical insulin and insulin-like growth factor I (IGF-I) receptors exist as well defined $\alpha_2\beta_2$ heterotetrameric complexes that are assembled from two identical $\alpha\beta$ heterodimeric half-receptor precursors. Recent evidence suggests that insulin and IGF-I half-receptors can heterologously assemble to form $\alpha_2\beta_2$ insulin/IGF-I hybrid receptor complexes *in vivo* and *in vitro*. We have utilized hybrid receptor complexes to examine ligand-stimulated transmembrane signaling of wild-type insulin ($\alpha\beta_{INS.WT}$) or IGF-I ($\alpha\beta_{IGF.WT}$) half-receptors assembled with a kinase-defective insulin half-receptor mutant ($\alpha\beta_{INS.A/K}$). *In vitro* assembly of either $(\alpha\beta)_{IGF.WT}/(\alpha\beta)_{INS.A/K}$ or $(\alpha\beta)_{INS.WT}/(\alpha\beta)_{INS.A/K}$ hybrid receptors resulted in decreased substrate protein kinase activity. The degree of protein kinase inactivation directly correlated with the amount of immunologically cross-reactive hybrid receptors formed. In contrast to substrate kinase activity, insulin-stimulated autophosphorylation of the $(\alpha\beta)_{INS.WT}/(\alpha\beta)_{INS.A/K}$ hybrid receptor complex was completely unaffected in comparison to the wild-type $(\alpha\beta)_{INS.WT}/(\alpha\beta)_{INS.WT}$ receptor. To assess a molecular basis for this difference, autophosphorylation of a hybrid receptor composed of a truncated β-subunit insulin half-receptor with the kinase-defective half-receptor, $(\alpha\beta)_{INS.\Delta CT}/(\alpha\beta)_{INS.A/K}$, demonstrated the exclusive autophosphorylation of the $(\alpha\beta)_{INS.A/K}$ half-receptor β subunit. These results demonstrate that ligand-dependent substrate phosphorylation by insulin and IGF-I holoreceptors requires interactions between two functional β subunits within the $\alpha_2\beta_2$ heterotetrameric complex and occurs through an intramolecular trans-phosphorylation reaction.**

Insulin and insulin-like growth factor I (IGF-I) holoreceptors share a large degree of structural and functional similarity (1, 2). The mature $\alpha_2\beta_2$ holoreceptors are synthesized from $\alpha\beta$ fusion proreceptor precursors that are cotranslationally acylated and glycosylated and then transported to the Golgi apparatus where additional glycosylation, proteolytic cleavage, and assembly into an $\alpha_2\beta_2$ complex occurs (3–9). The mature $\alpha_2\beta_2$ receptor state is required for ligand-stimulated transmembrane signaling, since lower oligomeric forms such as isolated $\alpha\beta$ heterodimers (10–14) and truncated $\alpha_2\beta\beta'$ receptors (15) are kinase-inactive species. Immunologically cross-reactive $\alpha_2\beta_2$ insulin/IGF-I hybrid receptors have been described that appear to result from the heterologous assembly of individual $\alpha\beta$ insulin and IGF-I receptor precursor proteins *in vivo* (16, 17). In parallel, we have observed that insulin/IGF-I hybrid receptor complexes can be assembled from purified insulin and IGF-I $\alpha\beta$ half-receptors *in vitro* (18).

The identification of hybrid receptor complexes has raised the question whether functional alterations in transmembrane signaling could result from the assembly of wild-type receptor precursors with dysfunctional receptor subtypes. For example, it has been observed that heterozygote individuals that express both wild-type and mutant tyrosine kinase-defective insulin receptor precursors have severe insulin resistance and diabetes (19–22). In these patients, the degree of insulin resistance is significantly greater than would be predicted from a simple loss of half the normal complement of insulin receptors. Similarly, cell lines that coexpress both endogenous wild-type and transfected kinase-defective insulin receptors display a marked decrease in insulin sensitivity and/or responsiveness (23–26). To determine whether defective insulin signaling through hybrid receptors could contribute to the effects observed, we have examined the kinase activity of $\alpha_2\beta_2$ heterotetrameric hybrid receptors composed of kinase-defective A/K_{1018} mutant insulin half-receptors (24) assembled *in vitro* with a wild-type insulin or IGF-I half-receptor.

MATERIALS AND METHODS

Isolation of $\alpha\beta$ Heterodimeric Insulin and IGF-I Receptor Complexes. Human placenta membranes (20 mg/ml) were treated with 2 mM dithiothreitol for 5 min at pH 8.5 to reduce and dissociate the $\alpha_2\beta_2$ heterotetrameric receptors, followed by detergent solubilization and partial purification by Bio-Gel A-1.5m gel filtration or wheat germ agglutinin-Sepharose chromatography (27). The $(\alpha\beta)_{IGF.WT}$ half-receptors were isolated by immunoaffinity purification by using the anti-IGF-I receptor monoclonal antibody αIR-3 or by immunodepletion of $(\alpha\beta)_{INS.WT}$ half-receptors by using the anti-insulin receptor monoclonal antibody 83-7 (28–31). The $(\alpha\beta)_{INS.WT}$, $(\alpha\beta)_{INS.A/K}$, and $(\alpha\beta)_{INS.\Delta CT}$ half-receptors were obtained from cDNA-transfected cell lines expressing the wild-type human insulin receptor (32), the A/K_{1018} mutant insulin receptor (24), and a deletion-mutated insulin receptor lacking 43 COOH-terminal amino acid residues (33), respectively. Cell membranes (3 mg/ml) were prepared (27) and treated with alkaline pH plus dithiothreitol as described above for the human placenta membranes followed by partial purification by Bio-Gel A-1.5m gel filtration chromatography or polylysine-Sepharose affinity chromatography (34).

Bio-Gel A-1.5m Column Gel Filtration Chromatography. Isolated $(\alpha\beta)_{IGF.WT}$ and $(\alpha\beta)_{INS.A/K}$ half-receptors (2 pmol/ml) were mixed and incubated in 50 mM Hepes (pH 7.8) containing 100 nM insulin, 100 nM IGF-I, or 100 nM insulin/100 nM IGF-I for 1 hr at 23°C. Samples were then applied to

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Abbreviation: IGF-I, insulin-like growth factor I.

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Bio-Gel A-1.5m gel filtration columns (1.6 × 50 cm) equilibrated in 50 mM Tris·HCl, pH 7.6/0.1% Triton X-100/150 mM NaCl/0.02% NaN_3 (Bio-Gel buffer) at 4°C. Fractions containing the $\alpha_2\beta_2$ heterotetrameric and $\alpha\beta$ heterodimeric insulin and/or IGF-I receptors were identified by ^{125}I-labeled IGF-I and ^{125}I-labeled insulin binding.

Immunoabsorption of Insulin and IGF-I Receptor Complexes. Bio-Gel A-1.5m gel filtration column fractions containing the $\alpha_2\beta_2$ heterotetrameric or $\alpha\beta$ heterodimeric insulin and IGF-I receptors were incubated (1:500 dilution) with Sepharose-coupled anti-insulin receptor monoclonal antibody 83-7 and anti-IGF-I receptor monoclonal antibody αIR-3 for 16 hr at 4°C. The antibody–receptor complexes were collected by centrifugation and the supernatants were assayed for ^{125}I-labeled insulin and ^{125}I-labeled IGF-I binding.

Kinase Assays. Isolated $(\alpha\beta)_{INS.WT}$, $(\alpha\beta)_{INS.\Delta CT}$, and $(\alpha\beta)_{INS.A/K}$ half-receptors (0.1–5 pmol/ml) were mixed and incubated in 50 mM Hepes, pH 7.8/100 nM insulin for 1 hr at 22°C. The samples were immunoabsorbed with Sepharose-coupled monoclonal antibody 83-7 and resuspended in 50 mM Hepes, pH 7.8/100 nM insulin/10 mM $MnCl_2$/10 mM $MgCl_2$. Substrate phosphorylation was initiated by the addition of poly(Glu-Tyr) (2 mg/ml) and [γ-^{32}P]ATP (100 μM, 3 μCi/nmol; 1 Ci = 37 GBq), and the reaction was terminated (20 min) by precipitation onto Whatman 3MM filter paper with 10% (wt/vol) trichloroacetic acid. Autophosphorylation was initiated by the addition of [γ-^{32}P]ATP (100 μM, 3 μCi/nmol) and terminated (5 min) by addition of 5 mM ATP/5 mM EDTA/100 mM sodium fluoride/10 mM sodium pyrophosphate. Samples were centrifuged at 12,000 × *g*, resuspended in Laemmli sample buffer (35) containing 300 mM dithiothreitol, heated for 5 min at 100°C, and resolved on 7.5% polyacrylamide gels containing SDS as described (33).

The isolated $(\alpha\beta)_{IGF.WT}$ and $(\alpha\beta)_{INS.A/K}$ half-receptors (0.3–3 pmol/ml) were mixed and incubated for 1 hr at 22°C with 100 nM insulin or 100 nM insulin/100 nM IGF-I, followed by the addition of 10 mM $MnCl_2$/10 mM $MgCl_2$/poly(Glu-Tyr) (2 mg/ml). Substrate phosphorylation was then assayed directly in solution by the addition of [γ-^{32}P]ATP (100 μM, 3 μCi/nmol) and terminated as described above.

RESULTS AND DISCUSSION

Heterologous Assembly of $(\alpha\beta)_{INS.A/K}$ and $(\alpha\beta)_{IGF.WT}$ Half-Receptors. To determine whether mutant/wild-type hybrid receptors could be formed *in vitro*, the highly related but immunologically distinct $(\alpha\beta)_{IGF.WT}$ and $(\alpha\beta)_{INS.A/K}$ half-receptor species were examined for heterologous assembly into $(\alpha\beta)_{IGF.WT}/(\alpha\beta)_{INS.A/K}$ complexes (Fig. 1). Equal amounts of $(\alpha\beta)_{IGF.WT}$ and $(\alpha\beta)_{INS.A/K}$ were incubated with insulin alone, IGF-I alone, or insulin/IGF-I and then subjected to Bio-Gel A-1.5m gel filtration chromatography to determine the receptor association state. Insulin treatment of the mixed $\alpha\beta$ half-receptors resulted in a characteristic mobility shift of insulin binding activity (Fig. 1*A*), consistent with an insulin-induced *in vitro* assembly of $\alpha_2\beta_2$ heterotetrameric complexes (12). The formation of an $(\alpha\beta)_{INS.A/K}/(\alpha\beta)_{INS.A/K}$ heterotetrameric receptor was specific for insulin, since IGF-I treatment had no effect on the association state of the $(\alpha\beta)_{INS.A/K}$ half-receptor. Similarly, IGF-I incubation of the mixed half-receptors resulted in a specific mobility shift of IGF-I binding activity, consistent with the formation of an $(\alpha\beta)_{IGF.WT}/(\alpha\beta)_{IGF.WT}$ heterotetrameric IGF-I receptor complex, whereas insulin was without effect (Fig. 1*B*). The simultaneous treatment of the mixed half-receptors with a combination of insulin plus IGF-I resulted in a mobility shift of both insulin and IGF-I binding activity to the expected position of an $\alpha_2\beta_2$ heterotetrameric receptor (Fig. 1).

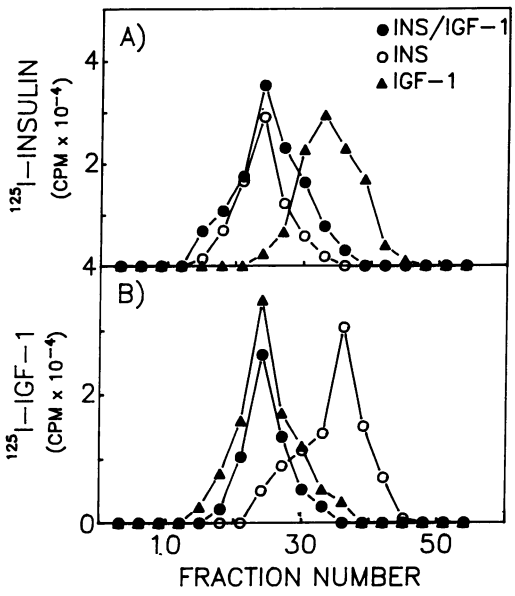

FIG. 1. Ligand-dependent association of $(\alpha\beta)_{IGF.WT}$ and $(\alpha\beta)_{INS.A/K}$ heterodimeric half-receptors into an $\alpha_2\beta_2$ heterotetrameric state. $(\alpha\beta)_{IGF.WT}$ half-receptor and the $(\alpha\beta)_{INS.A/K}$ half-receptor were obtained from human placenta membranes and transfected Rat-1 fibroblast membranes. Equal amounts of $(\alpha\beta)_{IGF.WT}$ and $(\alpha\beta)_{INS.A/K}$ half-receptors (2 pmol/ml) were mixed and incubated with 100 nM insulin (○), 100 nM IGF-I (▲), or 100 nM insulin/100 nM IGF-I (●) for 1 hr at 23°C. The samples were then applied to Bio-Gel A-1.5m gel filtration columns (1.6 × 50 cm) equilibrated in 50 mM Tris·HCl, pH 7.6/150 mM NaCl/0.1% Triton X-100. Fractions (0.45 ml) were collected after voiding 20 ml and assayed (200 μl) for ^{125}I-labeled insulin (^{125}I-insulin) (*A*) and ^{125}I-labeled IGF-I (^{125}I-IGF-I) binding (*B*). In the absence of ligand treatment, the mixed $\alpha\beta$ heterodimeric half-receptors displayed peak insulin and IGF-I binding at fractions 33–36 (data not shown).

Although these data demonstrate that both the $(\alpha\beta)_{INS.A/K}$ and $(\alpha\beta)_{IGF.WT}$ half-receptors associate, in a ligand-specific manner, to a heterotetrameric state, such association could have occurred in either a homologous or heterologous fashion. To determine the degree of insulin/IGF-I hybrid receptor formation, the peak fractions in Fig. 1 were immunoabsorbed with the anti-insulin receptor specific monoclonal antibody 83-7 and the anti-IGF-I receptor monoclonal antibody αIR-3. In the absence of ligand, the mixed $\alpha\beta$ heterodimeric half-receptors displayed absolute antibody specificity without any detectable cross-reactivity (e.g., 98–100% of binding precipitated only by the cognizant monoclonal antibody) (Table 1). Antibody specificity (90–100%) was also maintained subse-

Table 1. Insulin and IGF-I induced heterologous assembly of $(\alpha\beta)_{IGF.WT}$ and $(\alpha\beta)_{INS.A/K}$ half-receptors into an immunological cross-reactive $\alpha_2\beta_2$ heterotetrameric hybrid receptor complex

Treatment	Receptor species	^{125}I-labeled insulin precipitated, %		^{125}I-labeled IGF-I precipitated, %	
		83-7	αIR-3	83-7	αIR-3
Untreated	$\alpha\beta$	98	0	1	100
Insulin	$\alpha_2\beta_2/\alpha\beta$	95	3	10	100
IGF-I	$\alpha_2\beta_2/\alpha\beta$	93	0	9	100
Insulin/IGF-I	$\alpha_2\beta_2$	95	55	54	100

Bio-Gel A-1.5m gel filtration column fractions containing the $\alpha_2\beta_2$ heterotetrameric or $\alpha\beta$ heterodimeric insulin and IGF-I receptors (Fig. 1) were immunoabsorbed with 83-7 or αIR-3 followed by ^{125}I-labeled insulin and ^{125}I-labeled IGF-I binding.

quent to separate insulin or IGF-I treatment of the mixed $\alpha\beta$ heterodimeric half-receptors. However, incubation of the mixed $\alpha\beta$ heterodimeric half-receptors with the combination of insulin plus IGF-I resulted in $\alpha_2\beta_2$ heterotetrameric receptors that displayed partial (54–55%) cross-reactivity of ligand binding precipitation by both 83-7 and αIR-3 (Table 1). Thus, incubation of approximately equal amounts of $(\alpha\beta)_{IGF.WT}$ and $(\alpha\beta)_{INS.A/K}$ half-receptors with a combination of insulin plus IGF-I resulted in the random formation of $\alpha_2\beta_2$ heterotetrameric hybrid receptors. This is in accord with our previous observations of random heterologous association between the wild-type insulin and IGF-I half-receptors (18).

Inhibition of $(\alpha\beta)_{IGF.WT}$ Kinase Activity by $(\alpha\beta)_{INS.A/K}$ Half-Receptor. We next examined the effect of hybrid formation between the $(\alpha\beta)_{INS.A/K}$ and $(\alpha\beta)_{IGF.WT}$ half-receptors on IGF-I-stimulated substrate protein kinase activity (Fig. 2). A fixed concentration of $(\alpha\beta)_{IGF.WT}$ was incubated with various amounts of $(\alpha\beta)_{INS.A/K}$ prior to the simultaneous addition of insulin plus IGF-I. The addition of increasing amounts of $(\alpha\beta)_{INS.A/K}$ resulted in a progressive decrease in IGF-I-stimulated substrate kinase activity (Fig. 2*A*), which directly correlated ($r^2 = 0.91$) with an increased *in vitro* assembly of $(\alpha\beta)_{IGF.WT}/(\alpha\beta)_{INS.A/K}$ heterotetrameric hybrid receptors. Kinase inactivation did not simply result from heterologous hybrid formation between the insulin and IGF-I $\alpha\beta$ half-receptors *per se*, since $(\alpha\beta)_{INS.WT}/(\alpha\beta)_{IGF.WT}$ hybrid receptor complexes displayed ligand-stimulated kinase activity in response to both insulin and IGF-I (data not shown). In addition, the $(\alpha\beta)_{IGF.WT}$ displayed a linear 4.5-fold increase in substrate protein kinase activity as the concentration of $(\alpha\beta)_{IGF.WT}$ was increased 6-fold in the presence of IGF-I alone (data not shown) or insulin plus IGF-I (Fig. 2*B*).

In these experiments it was necessary to drive the formation of the insulin/IGF-I hybrid receptors by a combination of insulin plus IGF-I (Fig. 1). To confirm the ligand specificity of substrate kinase activation of the $(\alpha\beta)_{IGF.WT}$ half-receptor, a comparison between insulin and IGF-I stimulation was determined (Fig. 2*B*). As observed in Fig. 1, insulin did not induce the self-association of the $(\alpha\beta)_{IGF.WT}$ into an $\alpha_2\beta_2$ heterotetrameric state and was relatively ineffective in activating the IGF-I receptor kinase (Fig. 2*B*). In the presence of IGF-I alone or insulin plus IGF-I, the linear relationship between $\alpha\beta$ half-receptor concentration and kinase activity reflected the complete formation of $\alpha_2\beta_2$ IGF-I heterotetrameric receptor complexes under these conditions (27, 29).

Inhibition of $(\alpha\beta)_{INS.WT}$ Kinase Activity by the $(\alpha\beta)_{INS.A/K}$ Half-Receptor. In a similar paradigm, we examined the effect of the $(\alpha\beta)_{INS.A/K}$ half-receptor on insulin-stimulated substrate kinase activity by $(\alpha\beta)_{INS.WT}$. As observed for $(\alpha\beta)_{IGF.WT}$ (Fig. 2), the addition of increasing amounts of $(\alpha\beta)_{INS.A/K}$ to a fixed concentration of $(\alpha\beta)_{INS.WT}$ resulted in a dose-dependent inhibition of $(\alpha\beta)_{INS.WT}$ substrate protein tyrosine kinase activity (Fig. 3*A*). Half-maximal inhibition of the $(\alpha\beta)_{INS.WT}$ kinase activity occurred at approximately equal molar amounts of $(\alpha\beta)_{INS.A/K}$ whereas maximal inhibition required a 10-fold excess. As previously reported (23–25), the kinase-defective $(\alpha\beta)_{INS.A/K}$ was substrate-kinase-inactive (Fig. 3*B*). In contrast, the isolated $(\alpha\beta)_{INS.WT}$ displayed a linear 6-fold increase in insulin-stimulated substrate phosphorylation over an 8-fold $(\alpha\beta)_{INS.WT}$ concentration range (Fig. 3*B*). In addition, we have observed that $(\alpha\beta)_{INS.A/K}$ inhibition of substrate kinase activity is specific for the $(\alpha\beta)_{INS.WT}$ half-receptor, since addition of $(\alpha\beta)_{INS.A/K}$ to $\alpha_2\beta_2$ wild-type insulin receptors had no effect on substrate kinase activity (data not shown). Furthermore, *in vitro* assembly of the $(\alpha\beta)_{INS.A/K}$ half-receptor with prephosphorylated and autoactivated $(\alpha\beta)_{INS.WT}$ half-receptors (10) did not result in diminished substrate kinase activity (data not shown). These data demonstrate that the transdominant inhibition of $(\alpha\beta)_{INS.WT}$ substrate kinase activity by

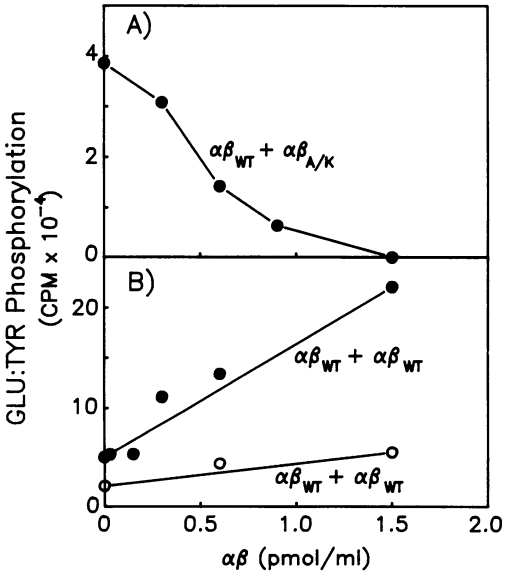

FIG. 2. Transdominant inhibition of $(\alpha\beta)_{IGF.WT}$ half-receptor substrate kinase activity by *in vitro* assembly with the $(\alpha\beta)_{INS.A/K}$ half-receptor. (*A*) A fixed amount of $(\alpha\beta)_{IGF.WT}$ half-receptor (0.3 pmol/ml) was mixed with increasing relative amounts of $(\alpha\beta)_{INS.A/K}$ half-receptor and incubated with 100 nM insulin/100 nM IGF-I for 1 hr at 22°C and substrate phosphorylation was determined. (*B*) The $(\alpha\beta)_{IGF.WT}$ half-receptor (0.3 pmol/ml) was mixed with increasing relative amounts of homologous $(\alpha\beta)_{IGF.WT}$ half-receptors in the presence of 100 nM insulin (○) or 100 nM insulin/100 nM IGF-I (●) for 1 hr at 22°C. Poly(Glu-Tyr) substrate phosphorylation was then determined as described in *A*.

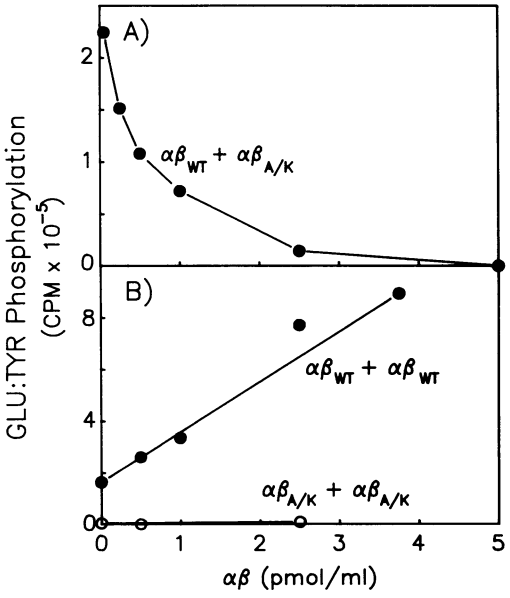

FIG. 3. $(\alpha\beta)_{INS.A/K}$ inhibition of insulin-stimulated protein kinase activity of the $(\alpha\beta)_{INS.WT}$ half-receptor. (*A*) A fixed amount of $(\alpha\beta)_{INS.WT}$ half-receptor (0.5 pmol/ml) was mixed with increasing amounts of $(\alpha\beta)_{INS.A/K}$ half-receptor prior to the addition of 100 nM insulin for 1 hr at 22°C. The samples were immunoabsorbed to anti-insulin receptor monoclonal antibody 83-7 and substrate phosphorylation was initiated. (*B*) $(\alpha\beta)_{INS.WT}$ (●) and $(\alpha\beta)_{INS.A/K}$ (○) insulin half-receptors (0.5 pmol/ml) were mixed with increasing amounts of homologous half-receptor in the presence of 100 nM insulin for 1 hr at 22°C. Samples were then immunoabsorbed to monoclonal antibody 83-7 and assayed for poly(Glu-Tyr) substrate phosphorylation as described in *A*.

$(\alpha\beta)_{INS.A/K}$ results from impaired insulin-signaling within a wild-type/mutant hybrid receptor complex.

Autophosphorylation of Wild-Type/Mutant Hybrid Receptor Complexes. To further investigate the insulin-signaling defect in the wild-type/mutant hybrid receptor, insulin-stimulated β-subunit autophosphorylation was examined (Fig. 4). In contrast to the complete inhibition of substrate kinase activity (Fig. 3), the $(\alpha\beta)_{INS.WT}/(\alpha\beta)_{INS.A/K}$ hybrid receptor (Fig. 4*A*, lane 2) displayed β-subunit autophosphorylation that was essentially identical compared to the $(\alpha\beta)_{INS.WT}/(\alpha\beta)_{INS.WT}$ holoreceptor complex (Fig. 4*A*, lane 1). As expected, the *in vitro*-assembled $(\alpha\beta)_{INS.A/K}/(\alpha\beta)_{INS.A/K}$ holoreceptors alone were completely devoid of insulin-stimulated β-subunit autophosphorylation (Fig. 4*A*, lane 3).

One possible explanation for apparently normal β-subunit autophosphorylation but defective substrate kinase activity in the $(\alpha\beta)_{INS.WT}/(\alpha\beta)_{INS.A/K}$ hybrid receptor would be an altered intramolecular autophosphorylation cascade. To address this issue, autophosphorylation was reexamined in hybrid receptors formed from the heterologous assembly of the $(\alpha\beta)_{INS.A/K}$ half-receptor with the β-subunit C-terminal-truncated αβ half-receptor, $(\alpha\beta)_{INS.\Delta CT}$. Insulin-stimulated autophosphorylation of the $(\alpha\beta)_{INS.\Delta CT}/(\alpha\beta)_{INS.A/K}$ hybrid receptor complex demonstrated the specific labeling of the M_r 95,000 $(\alpha\beta)_{INS.A/K}$ β subunit, without significant autophosphorylation of the truncated M_r 90,000 $(\alpha\beta)_{INS.\Delta CT}$ β subunit (Fig. 4*B*, lane 2). As reported (33), autophosphorylation of the $(\alpha\beta)_{INS.\Delta CT}/(\alpha\beta)_{INS.\Delta CT}$ receptors exclusively identified the truncated M_r 90,000 β subunit species (Fig. 4*B*, lane 1). These data directly demonstrate an intramolecular trans-phosphorylation of the $(\alpha\beta)_{INS.A/K}$ β subunit by the kinase-active $(\alpha\beta)_{INS.\Delta CT}$ β subunit within the $\alpha_2\beta_2$ heterotetrameric $(\alpha\beta)_{INS.\Delta CT}/(\alpha\beta)_{INS.A/K}$ hybrid receptor complex.

In summary, we have identified a transdominant inhibition of ligand-stimulated substrate kinase activity in hybrid insulin and IGF-I holoreceptors composed of αβ wild-type and αβ kinase-defective half-receptors. However, these hybrid receptors display essentially normal β-subunit autophosphorylation activity that occurs by an intramolecular transphosphorylation mechanism. Thus, insulin binding must necessarily stimulate the kinase activity of one of the αβ half-receptors that subsequently utilizes the other αβ half-receptor as a phosphotyrosine acceptor substrate. Since autophosphorylation has been established (36–39) to result in the activation of substrate kinase activity, we hypothesize that the presence of a kinase-defective half-receptor within an $\alpha_2\beta_2$ hybrid receptor complex results in premature termination of the activating signal by preventing a secondary back phosphorylation of the wild-type half-receptor species.

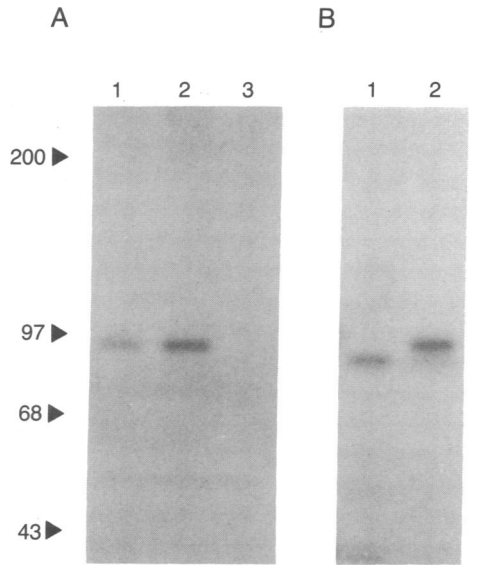

FIG. 4. Insulin-stimulated autophosphorylation of wild-type/mutant hybrid receptor complexes. (*A*) The $(\alpha\beta)_{INS.WT}$ (0.2 pmol/ml; lane 1), $(\alpha\beta)_{INS.WT}$ plus $(\alpha\beta)_{INS.A/K}$ (0.2 pmol/ml + 1.0 pmol/ml; lane 2), and $(\alpha\beta)_{INS.A/K}$ (1.0 pmol/ml; lane 3) half-receptors were incubated with 100 nM insulin for 1 hr at 22°C, then immunoabsorbed with the anti-insulin receptor monoclonal antibody 83-7, and autophosphorylated. (*B*) The $(\alpha\beta)_{INS.\Delta CT}$ (0.1 pmol/ml; lane 1) and $(\alpha\beta)_{INS.\Delta CT}$ plus $(\alpha\beta)_{INS.A/K}$ (0.1 pmol/ml + 0.6 pmol/ml; lane 2) half-receptors were incubated with 100 nM insulin for 1 hr at 22°C, then immunoabsorbed with the monoclonal antibody 83-7, and autophosphorylated as described in *A*. Positions of molecular weight markers are indicated ($\times 10^{-3}$).

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