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# Transmembrane signaling by an insulin receptor lacking a cytoplasmic β-subunit domain

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**ABSTRACT** **To assess the function of the cytoplasmic domain of the insulin receptor (IR) β subunit, we have studied a mutant IR truncated by 365 aa (HIRΔ978), thereby deleting >90% of the cytoplasmic domain. HIRΔ978 receptors were processed normally to homodimers that were expressed at the cell surface where they bind insulin with normal affinity. Although these truncated IRs were inactive with respect to ligand-induced internalization and autophosphorylation, insulin stimulated endogenous substrate (pp185) phosphorylation significantly more in HIRΔ978 cells than in untransfected Rat1 cells. Importantly, despite absence of the β-subunit cytoplasmic domain, fibroblasts expressing HIRΔ978 receptors displayed enhanced sensitivity to insulin for stimulation of glucose incorporation into glycogen, α-aminoisobutyric acid uptake, thymidine incorporation, and S6 kinase activity compared with parental fibroblasts. Insulin also induced the expression of the protooncogene c-*fos* and the early growth response gene *Egr-1* in HIRΔ978 cells far greater than in parental Rat1 fibroblasts. Furthermore, an agonistic monoclonal antibody specific for the human IR stimulated insulin action in fibroblasts expressing wild-type human IR but had no effect on HIRΔ978 cells. In conclusion, the HIRΔ978 truncated IRs appear to confer enhanced insulin sensitivity by augmenting the signaling properties of the endogenous rodent IRs.**

The insulin receptor (IR) is a heterotetrameric membrane protein possessing two extracellular α subunits and two transmembrane β subunits (1). Insulin binding to the extracellular α subunits activates the receptor tyrosine kinase properties of the cytoplasmic portion of the β subunit and a variety of studies have indicated that this activation is necessary for many, if not all, of insulin's biologic effects (2–7). For example, numerous studies have shown that cells expressing various tyrosine-kinase-defective IRs demonstrate impaired insulin signaling (2–7). On the other hand, not all reports agree with this concept, since a few studies have suggested that tyrosine-kinase-defective IRs can function normally with respect to biologic signaling (8–10). Kinase-defective IRs generally not only fail to mediate insulin's biologic actions but also impair the ability of coexpressed normal IRs to function properly. This dominant negative inhibitory effect has been noted *in vitro*, whereby transfected cells expressing kinase-defective mutant IRs display decreased insulin sensitivity compared with untransfected parental cells (2–4, 11). *In vivo*, certain patients who are heterozygous for IR mutations and express both normal and tyrosine-kinase-defective IRs can display severe insulin resistance (12–15). The degree of insulin resistance in these patients is far greater than would be predicted based on a loss of half of the functional IRs due to one defective allele.

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The mechanisms underlying this dominant negative effect are unclear and at least two possibilities exist (11, 16, 17). (*i*) Kinase-inactive IRs may form hybrid molecules with the normal endogenous receptors, and if these hybrid receptors are poorly functional, then this would provide a mechanism whereby a kinase-defective IR can inactivate a normal receptor (16). (*ii*) It is possible that the kinase-defective IRs compete with the normal endogenous receptor for cellular substrates or second messengers of insulin action (11). Therefore, this study had two goals: (*i*) to clarify the role of the β-subunit cytoplasmic domain in insulin signaling and (*ii*) to evaluate the mechanism of the dominant negative inhibitory effects of kinase-inactive IRs. Toward these ends, we have prepared and studied a truncated IR in which most of the intracellular region has been deleted. Since there is disagreement as to whether the tyrosine kinase or some other region of the IR is important for insulin action, deleting the cytoplasmic domain should shed some light on this issue. Then we reasoned that if substrate competition is a mechanism for dominant negative inhibition, then an IR lacking the cytoplasmic domain may not be inhibitory, since the intracellular region of the IR is the likely site for substrate–receptor interactions. In the current studies, we compared cells expressing normal human IRs with those expressing a C-terminal truncation mutant missing 365 aa, which is >90% of the β subunit cytoplasmic domain.

## MATERIALS AND METHODS

**Plasmid Construction and Transfection of Rat1 Cell Lines.** Construction of an expression plasmid containing the intact human IR cDNA and transfection of Rat1 cell lines have been described (2). The mutant IR was produced by constructing an expression plasmid (RLDN) (18), which was kindly provided by Mitchell E. Reff (SmithKline Beckman), containing the truncated IR cDNA generated by primer-directed *in vitro* mutagenesis (19) using an oligonucleotide in which the codon for amino acid residue 978 [according to the numbering system of Ullrich *et al.* (1)] was altered to a termination codon. The construction was verified by dideoxynucleotide sequence analysis (20). Rat1 cells were transfected with RLDN HIRΔ978 expression plasmids by the calcium phosphate coprecipitation methods as described (2). Geneticin (GIBCO)-resistant clonal cell lines expressing high levels of human IRs were selected by measurement of $^{125}$I-labeled insulin binding, and several clonal cell lines overexpressing the HIRΔ978 receptor were obtained.

**Insulin Binding.** Cells were exposed to 0.03 nM $^{125}$I-labeled insulin (Eli Lilly) and various concentrations of unlabeled

Abbreviations: IR, insulin receptor; IGF-I, insulin-like growth factor I; NAPA-insulin, B2(2-nitro-4-azidophenylacetyl)-des-Phe$^{B1}$-insulin; AIB, α-aminoisobutyric acid; IRS-I, IR substrate I.

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insulin for 3.5 hr at 15°C. Specific binding was determined by subtracting the counts bound in the presence of 350 nM unlabeled insulin (2).

**Ligand Internalization.** Cells were exposed to 0.07 nM $^{125}$I-labeled insulin or $^{125}$I-labeled insulin-like growth factor I (IGF-I; Eli Lilly) at 37°C. After 30 min, the cells were rinsed, surface-bound ligand was removed by acid treatment (pH 3.0), and residual internalized cell-associated radioactivity was determined (2).

**Cross-Linking and Cell Surface Labeling.** Cells were incubated with $^{125}$I-labeled B2(2-nitro-4-azidophenylacetyl)-des-Phe$^{B1}$-insulin (NAPA-insulin) for 2 hr at 12°C. UV-cross-linking of the insulin to its receptor was then carried out (21), or the cells were iodinated using Na$^{125}$I (Amersham), $H_2O_2$, and lactoperoxidase for 30 min at 4°C (22). Then, the cells were solubilized in the presence of 1% Triton X-100. After removal of insoluble material by centrifugation, the supernatants were immunoprecipitated with a monoclonal antibody specific for the human IR and analyzed by SDS/PAGE under reducing or nonreducing conditions.

**Autophosphorylation of Partially Purified IRs.** Autophosphorylation of lectin-purified IRs was measured *in vitro*. The IR preparations were incubated with various concentrations of insulin for 16 hr at 4°C. Autophosphorylation of the IR was measured after incubation with 50 μM [γ-$^{32}$P]ATP (6000 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) for 10 min at 4°C. Phosphorylated IRs were immunoprecipitated with a monoclonal antibody specific for the human IR and subjected to SDS/PAGE under reducing conditions (2).

**Endogenous Substrate Phosphorylation.** Cells were starved for 24 hr in Dulbecco's modified Eagle's medium (DMEM, GIBCO) containing 0.05% fetal calf serum. Immunoblot analysis of stimulated cell extracts was performed by exposing the cells to various concentrations of insulin for 1 min at 37°C. Cells were then solubilized directly in Laemmli buffer, and the phosphorylated proteins were detected by Western blot analysis using anti-phosphotyrosine antibody (11).

**Biological Action.** Measurements of glucose incorporation into glycogen (23), α-aminoisobutyric acid (AIB) uptake (24), thymidine incorporation (25), and S6 kinase activity (23, 26) were performed as published.

**c-Fos and Egr-1 Expression.** Prior to inducing c-*fos* and *Egr-1*, cells were grown to confluence and starved for 24 hr with serum-free DMEM. After stimulation with insulin for 30 min at 37°C, total cellular RNA was isolated from the cells by the modified method of single-step guanidinium thiocyanate/chloroform/phenol RNA extraction (27). cDNA from the total cellular RNA was synthesized by using a cDNA cycle kit and the manufacturer's instructions (Invitrogen, San Diego). Specific cDNA as defined by the c-*fos* and *Egr-1* primers was PCR-amplified with L30 primers as an internal standard in the presence of [$^{32}$P]dCTP (B. H. Jhun, J. L. Meinkoth, and J.M.O., unpublished data). PCR products were electrophoresed and visualized by autoradiography. The relative amounts of c-*fos* or *Egr-1* vs. L30 were quantitated by measuring the incorporated radioactivity on the gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

## RESULTS AND DISCUSSION

**Insulin Binding and Structure.** Insulin binding studies (Fig. 1*A*) showed that HIRΔ978 cells expressed 1.2 × 10$^5$ IRs per cell and cells expressing wild-type IRs displayed 2.7 × 10$^5$ IRs per cell (HIRc). Both HIRc and HIRΔ978 receptors have approximately the same affinity for insulin, with a half-maximal competition of tracer binding at 0.4–0.5 nM unlabeled insulin in both cell lines.

Next, cells were cross-linked with $^{125}$I-labeled NAPA-insulin, and SDS/PAGE of solubilized cell extracts revealed

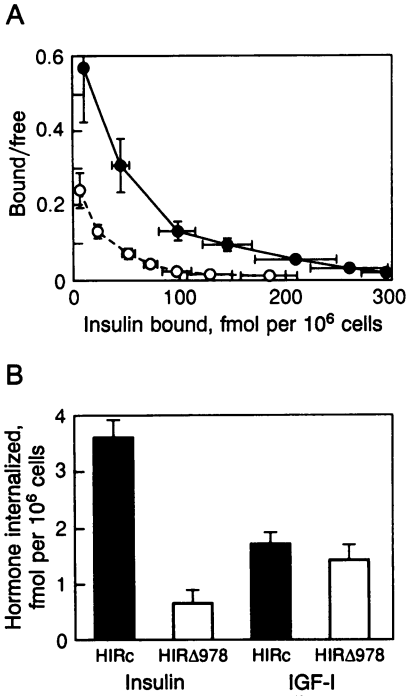


FIG. 1. Insulin binding and internalization in transfected cell lines. (*A*) Scatchard plots of binding data. The binding analysis was performed by a negative cooperative model. Results are the mean ± SEM of four experiments for HIRc (●) and HIRΔ978 (○) cells. (*B*) Ligand internalization by transfected cells. HIRc (solid bars) or HIRΔ978 (open bars) cells were exposed to 0.07 nM $^{125}$I-labeled insulin or $^{125}$I-labeled IGF-I at 37°C. After 30 min, the cells were rinsed with ice-cold phosphate-buffered saline. Surface-bound $^{125}$I-labeled insulin or $^{125}$I-labeled IGF-I was removed by treatment with buffer at pH 3.0, and residual internalized $^{125}$I-labeled insulin or $^{125}$I-labeled IGF-I was determined. Results are the mean ± SEM of three experiments.

intact α subunits (135 kDa) in both cell lines (since the ligand binds to the α subunit, β subunits are not visualized using this technique; Fig. 2*A*). However, when the $^{125}$I-labeled NAPA-insulin cross-linked IRs were analyzed by SDS/PAGE under nonreducing conditions, disulfide bonds linking the α and β subunits are not disrupted, and clear size differences between the wild-type and mutant IRs can be seen (Fig. 2*B*). Thus, the wild-type heterotetrameric IRs migrate to a position of ≈430 kDa, whereas the truncated IRs appear at 330 kDa. Cells were also surface labeled with $^{125}$I and the labeled proteins were analyzed by SDS/PAGE. As shown in Fig. 2*C*, bands corresponding to the α (135 kDa) and β (95 kDa) subunits are readily visualized with the HIRc cells, whereas only the α subunit (135 kDa) is seen with the HIRΔ978 cells, consistent with deletion of most of the β subunit from the HIRΔ978 receptor. A faintly visualized band migrating at ≈50 kDa was variably noted in the HIRΔ978 cells, which may represent the extracellular portion of the β subunit. Therefore, despite the severe truncation, mutant IRs can be processed to the heterotetrameric structure and transported to the plasma membrane where they bind insulin in an apparently normal fashion.

**Autophosphorylation and Kinase Activity.** The function of the mutant IR was analyzed by measuring its ability to autophosphorylate *in vitro*. To distinguish between endogenous and human receptors, a human-specific monoclonal antibody was used to immunoprecipitate the IRs prior to SDS/PAGE analysis. As expected, insulin stimulated autophosphorylation of the β subunit in a dose-dependent fashion by using HIRc receptors, whereas no autophosphorylation was detected with the HIRΔ978 and Rat1 receptors (Fig. 3*A*).

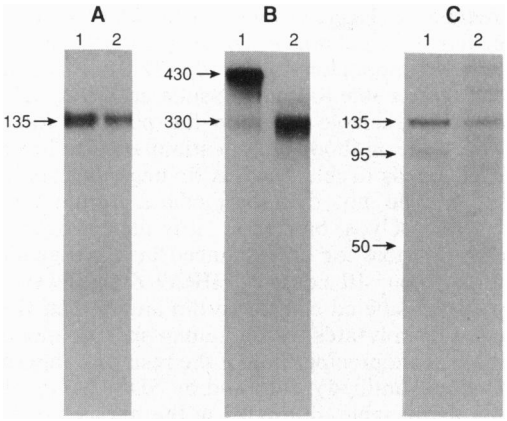


FIG. 2. Cross-linking and cell surface labeling of IRs in transfected cell lines. (*A* and *B*) Cross-linking of IRs. HIRc (lane 1) or HIRΔ978 (lane 2) cells were incubated with $^{125}$I-labeled NAPA-insulin at 12°C for 2 hr. After photolysis, cells were solubilized, immunoprecipitated with a monoclonal antibody specific for the human IR, and subjected to SDS/PAGE under reducing (*A*) or nonreducing (*B*) conditions. (*C*) Cell surface labeling of IRs. Surface proteins on HIRc (lane 1) or HIRΔ978 (lane 2) cells were iodinated. The cells were solubilized, and iodinated IRs were immunoprecipitated with a monoclonal antibody specific for the human IR and subjected to SDS/PAGE under reducing conditions. Molecular masses (kDa) are indicated.

To assess *in vivo* autophosphorylation and endogenous substrate phosphorylation, cells were incubated with various concentrations of insulin and cell lysates were subjected to Western blot analysis using anti-phosphotyrosine antibodies. As shown in Fig. 3*B*, two prominent insulin-stimulated phosphorylated bands can be visualized. The 95-kDa band corresponds to the β subunit of the IR, whereas the 185-kDa band represents an endogenous substrate. Insulin markedly stimulated β subunit autophosphorylation in HIRc cells, whereas the stimulation was small and comparable between the HIRΔ978 and Rat1 cells. Thus, in the HIRΔ978 cells, as expected, the truncated IR does not undergo autophosphorylation, but autophosphorylation of the endogenous rodent IRs is normal and comparable between HIRΔ978 and Rat1 cells. Insulin also stimulates phosphorylation of the endogenous pp185 substrate in all three cell lines. Most likely, pp185 represents the recently cloned IR substrate I (IRS-I) (28). The amount of insulin-stimulated pp185/IRS-I phosphorylation was similar in the HIRc and HIRΔ978 cells and far greater than that seen in Rat1 cells. This is in marked contrast to the decrease in β-subunit autophosphorylation in HIRΔ978 cells compared with HIRc cells. Thus, in the HIRΔ978 background, autophosphorylation of native Rat1 receptors without autophosphorylation of the HIRΔ978 receptors led to enhanced stimulation of pp185/IRS-I phosphorylation. This indicates that the coupling efficiency between phosphorylated β subunits and pp185/IRS-I was much more efficient in HIRΔ978 cells compared with parental Rat1 cells.

**Ligand Internalization.** Ligand-mediated endocytosis was assessed by measuring the ability of both cell lines to internalize $^{125}$I-labeled ligand (Fig. 1*B*). Wild-type IRs mediate insulin internalization in a normal manner. On the other hand, insulin internalization was markedly depressed in the HIRΔ978 cells, consistent with the known important role of tyrosine autophosphorylation of the β subunit in mediating endocytotic behavior (2, 15, 29). Interestingly, $^{125}$I-labeled IGF-I internalization was comparable between the two cell lines, demonstrating the specificity of the defect in insulin internalization.

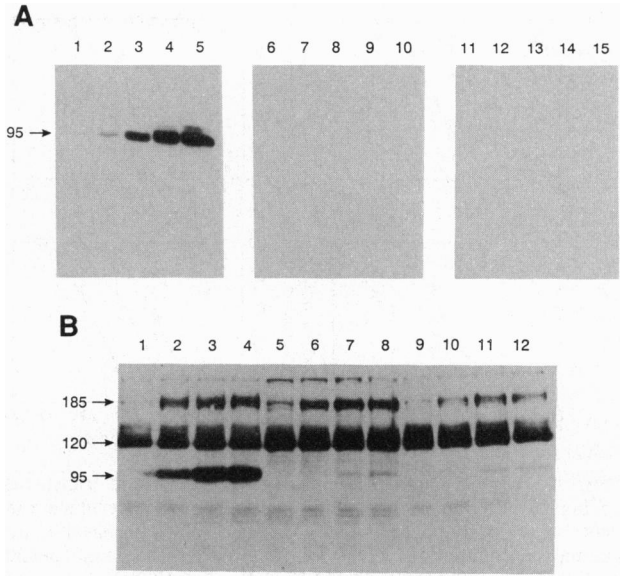


FIG. 3. Autophosphorylation and endogenous substrate phosphorylation in parental and transfected fibroblasts. (*A*) Autophosphorylation *in vitro*. The IR preparations from HIRc (lanes 1–5), HIRΔ978 (lanes 6–10), or Rat1 (lanes 11–15) cells were incubated with 0 (lanes 1, 6, and 11), 0.17 (lanes 2, 7, and 12), 1.7 (lanes 3, 8, and 13), 17 (lanes 4, 9, and 14), or 170 (lanes 5, 10, and 15) nM insulin for 16 hr at 4°C. The IRs were autophosphorylated, immunoprecipitated with a monoclonal antibody specific for the human IR, and analyzed by SDS/PAGE under reducing conditions. An autoradiogram representative of two experiments is shown. (*B*) Insulin-stimulated autophosphorylation and tyrosine phosphorylation of endogenous substrates. HIRc (lanes 1–4), HIRΔ978 (lanes 5–8), or Rat1 (lanes 9–12) cells were exposed to 0 (lanes 1, 5, and 9), 1.7 (lanes 2, 6, and 10), 17 (lanes 3, 7, and 11), or 83 (lanes 4, 8, and 12) nM insulin for 1 min at 37°C and then lysed in Laemmli buffer. An equivalent number of cells were processed for immunoblot analysis using an anti-phosphotyrosine antibody. An autoradiogram representative of four experiments is shown. Molecular masses (kDa) are shown.

**Biologic Effects.** To study the transmembrane signaling properties of the HIRΔ978 receptor, four biologic actions of insulin were evaluated. Fig. 4 presents dose–response curves for insulin stimulation of glucose incorporation into glycogen, AIB uptake, thymidine incorporation into DNA, and S6 kinase activity. With these analyses, the functional activity of the transfected IRs is manifested by their ability to shift the dose–response curve. That is, compared with the parental cells, a leftward shifted curve depicts enhanced sensitivity, presumably due to functional receptors, and a rightward shifted curve would be indicative of dominant inhibitory receptors; no change in the curve would indicate receptors that have neither intrinsic activity nor inhibitory effects. As can be seen, the wild-type IRs in the HIRc cells led to the predicted leftward shift (enhanced sensitivity) in all cases. In the HIRΔ978 cells, it is quite clear that insulin signaling is not inhibited for any of the biologic actions measured. On the other hand, and somewhat surprisingly, overexpression of the truncated IR led to strikingly enhanced insulin sensitivity in each instance. In general, the effect of the truncated IR to confer increased insulin sensitivity was comparable to that of the wild-type IR, even though ≈2-fold more IRs were expressed in HIRc compared with HIRΔ978 cells. These findings in the HIRΔ978 cells were not due to clonal variation, since identical results were obtained in two additional independent clonal lines.

Insulin is known to increase transcription of the protooncogene c-*fos* and the early growth response gene *Egr-1* (30, 31). This effect of insulin was measured in the three cell lines

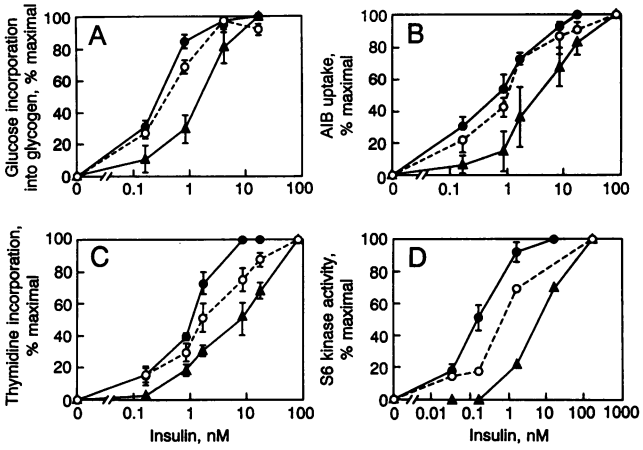


FIG. 4. Biologic actions of insulin in parental and transfected fibroblasts. (*A*) Glucose incorporation into glycogen. Results are the mean ± SEM of five experiments. Absolute values for basal (*b*) and maximal (*m*) stimulation were as follows: Rat1, $b$ = 15.7 nmol per 200 μg of protein per 2 hr and $m$ = 23.2; HIRc, $b$ = 12.2 and $m$ = 20.8; HIRΔ978, $b$ = 12.5 and $m$ = 17.2. (*B*) AIB uptake. Results are the mean ± SEM of three experiments. Absolute values for basal (*b*) and maximal (*m*) stimulations were as follows: Rat1, $b$ = 0.52 nmol per 200 μg of protein per 12 min and $m$ = 0.80; HIRc, $b$ = 0.70 and $m$ = 1.02; HIRΔ978, $b$ = 0.53 and $m$ = 0.68. (*C*) Thymidine incorporation. Results are the mean ± SEM of five experiments. Absolute counts of basal levels (*b*) and maximal stimulation (*m*) were as follows: Rat1, $b$ = 12,972 dpm and $m$ = 2.06-fold; HIRc, $b$ = 16,770 and $m$ = 1.67; HIRΔ978, $b$ = 14,692 and $m$ = 1.98. (*D*) S6 kinase activity. Results are the mean ± SEM of four experiments for HIRc cells and the mean of two experiments for Rat1 and HIRΔ978 cells. Absolute values of ATP incorporation into 40S protein for basal (*b*) and maximal (*m*) stimulations were as follows: Rat1, $b$ = 0.19 pmol per 5 μg of protein per 30 min and $m$ = 0.88; HIRc, $b$ = 0.40 and $m$ = 1.03; HIRΔ978, $b$ = 0.33 and $m$ = 1.16. All results are presented as the percent of maximal insulin stimulation of HIRc (●), HIRΔ978 (○), and Rat1 (▲) cells.

and the results are displayed in Table 1. As can be seen, insulin leads to a >2-fold effect on c-fos and Egr-1 mRNA levels in HIRc and HIRΔ978 cells, whereas only a minimal effect was seen in parental Rat1 fibroblasts.

All three cell types express 1.5 × $10^5$ IGF-I receptors, and these mediate IGF-I biologic effects. To assess IGF-I action, and in particular, to see if IGF-I signaling is enhanced in HIRΔ978 cells, dose–response studies of IGF-I-stimulated glucose incorporation into glycogen, AIB uptake, and thymidine incorporation were conducted. The dose–response curves were comparable across all three cell lines for each biologic action (data not shown). These results indicate the specificity of the increased insulin action in HIRΔ978 cells, since IGF-I signaling was unaffected.

**Endogenous Receptor Function.** In HIRΔ978 cells, insulin can bind to the overexpressed human IRs or the endogenous rodent receptors. To determine which set of receptors directly mediated the enhanced signaling, we used an agonistic human-specific monoclonal antibody (32–36). As expected, this antibody was able to mimic insulin action in HIRc cells but had no appreciable effect in the parental Rat1 cells. Interestingly, the antibody did not stimulate insulin action in the HIRΔ978 cells (Table 2). This finding suggests that the truncated IRs do not directly mediate insulin's biologic effects by themselves, but rather, it is the endogenous IRs that are responsible for the enhanced insulin signaling. To assess endogenous IR content, HIRΔ978 cells were cross-linked with $^{125}$I-labeled NAPA-insulin and human IRs were precipitated from lysates with a human-specific monoclonal antibody. Immunoprecipitation of the resulting supernatants with polyclonal antibody, followed by SDS/PAGE, demonstrated no appreciable difference in the number of endogenous rodent IRs among HIRc, Rat1, and HIRΔ978 cells (data not shown).

Either *in vitro* (2–4, 15) or *in vivo* (12–14), missence mutations that compromise kinase activity of the IR exert inhibitory effects on endogenous normal receptor function leading to decreased insulin sensitivity and severe insulin resistance. This dominant inhibitory effect may be due to formation of inactive kinase-defective–normal receptor hybrids (16) or competition for substrates between the kinase-defective and the normal receptors (11). Thus, the absence of inhibitory effects in HIRΔ978 cells could be explained if this IR was either incapable of forming hybrids or did not compete for substrates. Because the number of endogenous rodent IRs is so small (2–3000 IRs) in these cells, it is difficult to quantitate the existence or number of these hybrids. However, hybrids also form between insulin and IGF-I receptors in transfected cells (23, 37, 38) and using anti-IR antibodies to immunoprecipitate receptors from $^{125}$I-labeled IGF-I cross-linked cells, we observed that ≈40% of the endogenous IGF-I receptors had formed hybrids with the truncated HIRΔ978 receptors (data not shown). Thus, it seems likely that the HIRΔ978 receptor was capable of forming hybrids with the endogenous rodent IR. The lack of inhibition combined with the presence of hybrids raises the possibility that the truncated IR cannot effectively compete for downstream signaling molecules, obviating the dominant inhibitory effect. This hypothesis seems reasonable, since the sites of IR associations with signaling molecules are likely to reside in the cytoplasmic β-subunit domain, the region almost entirely missing from the HIRΔ978 receptor.

The most striking finding in this study was the enhanced insulin sensitivity conferred by the HIRΔ978 receptor. The mechanisms for this effect are not clear, but the fact that the agonistic human-specific monoclonal antibody did not mimic insulin action in HIRΔ978 cells indicates that the enhanced signaling is directly mediated by the existing endogenous receptors. It is unlikely that endogenous IGF-I receptors or IGF-I–HIRΔ978 receptor hybrids play a role because IGF-I action is normal in these cells (data not shown) and because

Table 1. Insulin induction of c-*fos* and *Egr-1* expression

| Cell line | Relative expression, % basal level | |
|---|---|---|
| | c-*fos* | *Egr-1* |
| HIRc | 240 ± 68 | 244 ± 66 |
| Rat1 | 138 ± 13 | 119 ± 4 |
| HIRΔ978 | 212 ± 25 | 205 ± 41 |

Cells were starved for 24 hr in serum-free DMEM and stimulated with 17 nM insulin for 30 min at 37°C. Total cellular RNA was isolated from the cells, and mRNA expression of c-*fos* and *Egr-1* was measured. Analysis was performed by measuring the relative amounts of c-fos and Egr-1 mRNA vs. L30 mRNA. Results are expressed as the percentage of the basal values (basal value = 100%). Each value is presented as the mean ± SEM of three experiments.

Table 2. Effect of monoclonal antibody (83-14) on thymidine incorporation into DNA and mRNA expression of c-*fos* and *Egr-1*

| Cell line | % above basal level | | |
|---|---|---|---|
| | Thymidine incorporation | c-*fos* expression | *Egr-1* expression |
| HIRc | 44.8 ± 3.6 | 42.8 ± 9.5 | 44.9 ± 9.6 |
| Rat1 | 10.2 ± 3.4 | 3.4 ± 9.2 | 6.4 ± 3.5 |
| HIRΔ978 | 9.7 ± 2.5 | 2.4 ± 1.9 | 4.2 ± 4.1 |

Cells were incubated with antibody 83-14 (1:1000 dilution) instead of insulin, and thymidine incorporation and c-fos and Egr-1 mRNA expression were measured. Results are expressed as the percentage above the basal values. Each value is presented as the mean ± SEM of three experiments.

the monoclonal antibody was not stimulatory. Therefore, we suggest that the truncated IRs enhance the ability of the endogenous rodent IRs and/or the rat insulin–HIRΔ978 receptor hybrids to mediate insulin's biologic effects.

Our current studies do not elucidate the manner in which this occurs. However, after insulin binding, IRs, which were evenly distributed as singletons over the cell surface, undergo microaggregation, clustering into clathrin-coated pits, and then endocytosis (39, 40). Using a morphometric analysis of gold-labeled IR complexes (40), we have found that the HIRΔ978 receptor does not undergo microaggregation or coated-pit clustering (41). Possibly, microaggregation plays some role in terminating or attenuating insulin's signal and, if it is impaired, then the net effect of insulin would be enhanced. Alternatively, the anti-phosphotyrosine Western blot data shown in Fig. 3*B* demonstrate that insulin-induced phosphorylation of the endogenous pp185/IRS-I substrate is enhanced in HIRΔ978 cells compared with Rat1 fibroblasts and is comparable to that seen in HIRc cells. This is despite the fact that β-subunit autophosphorylation is equal in Rat1 and HIRΔ978 cells and much lower than that observed in HIRc cells. This suggests that coupling between phosphorylated rodent insulin receptor β subunits and pp185/IRS-I is augmented in HIRΔ978 cells compared with Rat1 cells, consistent with the hypothesis that, in some way, the truncated IR confers increased efficiency to the interaction between activated endogenous IRs and pp185/IRS-I. Insofar as pp185/IRS-I serves as a signaling molecule upstream of the bioeffects presented in Fig. 4, then this could provide a mechanism for the augmented insulin action displayed by the HIRΔ978 cells.

Regardless of the precise mechanism(s), it is clear that overexpression of the truncated IR augments the signaling properties of the endogenous normal IR. In a sense, this is a dominant positive stimulatory effect. This has important implication for our understanding of insulin action and may also have some physiologic or clinical relevance. Perhaps there are patients with enhanced insulin sensitivity in whom this mechanism is operative. Furthermore, knowledge of the mechanism underlying this dominant positive effect may lead to a method to improve insulin action in insulin-resistant patients.

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