**Insulin and Insulin-like Growth Factor-I Stimulate a Common Endogenous Phosphoprotein Substrate (pp185) in Intact Neuroblastoma Cells**

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Joshua Shemer, Martin Adamo, Gaye Lynn Wilson, Dafna Heffez, Yehiel Zick, and Derek LeRoith

From the Diabetes Branch, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892 and the Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, 76100 Israel

*Mouse neuroblastoma N18 cells contain specific high affinity insulin and insulin-like growth factor-I (IGF-I) receptors. Insulin and IGF-I induce phosphorylation, in intact cells, of their respective receptor β subunits. The insulin receptor β subunit is represented by a 95-kDa phosphoprotein that is recognized by a specific antiserum (B10). The IGF-I receptor β subunit is represented by two phosphoproteins of molecular mass 95 and 105 kDa. The hormone-induced phosphorylation was rapid and dose-dependent occurring on both phosphoserine and phosphotyrosine residues. In addition, both insulin and IGF-I induced phosphorylation of an endogenous protein of molecular mass 185 kDa (pp185). The rapidity and dose dependency of the phosphorylation of pp185 suggested that it may represent a common endogenous substrate for the insulin and IGF-I receptors in these neural-derived cells. Phosphorylation was primarily on phosphoserine and phosphotyrosine residues. pp185 did not adsorb to wheat germ agglutinin and was not stimulated by either epidermal growth factor or platelet-derived growth factor. The finding of pp185 in these neural-related cells as well as in non-neuronal tissues suggests that it may represent a ubiquitous endogenous substrate for both the insulin and IGF-I receptor kinases.*

Insulin and insulin-like growth factor-I (IGF-I) 1 both induce autophosphorylation of their respective receptor β subunits (1–8). This effect is predominantly on tyrosine residues in detergent extracts of cells (9–12), whereas in intact cells exposure to these ligands results in receptor β subunit phosphorylation on both tyrosine and serine residues (7, 8, 13–16). Autophosphorylation of the β subunit as well as tyrosine phosphorylation of other proteins may be the initial signals for the intracellular effects of both insulin and IGF-I (17–19). Typical insulin and IGF-I receptors are widespread throughout the central nervous system and have also been characterized on primary cultures of neuronal and glial cells as well as established neural-derived cell lines (20–23, 25, 38). In detergent extracts of these cells, insulin and IGF-I stimulate receptor autophosphorylation and tyrosine kinase activity. In addition, in these cells insulin is capable of inducing glucose uptake, and IGF-I stimulates thymidine incorporation (21, 22, 25).

In this study, we demonstrate that insulin and IGF-I induce phosphorylation of their respective receptor β subunits in intact mouse neuroblastoma cells (N18). Furthermore, they stimulate the phosphorylation of a common endogenous substrate of M, 185,000 (pp185). Since a phosphoprotein with a similar molecular weight has been demonstrated previously in non-neural cells including rat hepatoma (15), rat and canine kidney cells (7), and human epidermoid carcinoma cells (6), our findings suggest that pp185 may represent a ubiquitous endogenous substrate for insulin and IGF-I receptor kinases.

**MATERIALS AND METHODS**

**Cell Culture**—Mouse neuroblastoma cell line N18 was a gift of Dr. Marshall Nirenberg, Bethesda, MD. The cells were grown in Dulbecco’s modified Eagle’s medium with 5% fetal bovine serum at 37 °C in a humidified atmosphere composed of 90% air, 10% CO₂. Cells were grown to confluency in 175-mm² dishes. Twelve hours prior to the phosphorylation experiment, the medium was changed to serum-free medium (with 2 mM glutamine).

**Phosphorylation Reaction**—The cells were labeled using 8 ml of phosphate-free, serum-free minimum Eagle’s medium containing carrier-free ²⁵³⁸Porthophosphate (0.5 mCi/ml, Du Pont-New England Nuclear) for 2 h at 37 °C and 5% CO₂. Stimulation of ²⁵³⁸P incorporation into phosphoproteins was achieved using insulin (Elanco Products Co., Indianapolis, IN) and IGF-I (AMGEN Biologicals, Thousand Oaks, CA) at various concentrations. For time course experiments, the reaction was stopped at various time intervals by rapidly removing the media and freezing the cells using liquid nitrogen. The cells were solubilized by the addition of a "solubilization solution" containing 50 mM Hepes, pH 7.4, 1% Triton X-100, 10 mM sodium pyrophosphate, 10 mM sodium fluoride, 4 mM EDTA, 2 mM sodium orthovanadate, and 2 mM phenylmethylsulfonyl fluoride (15). The cells were removed from the dishes during thawing.

**Whole Cell Extract Preparations**—In studies with whole cell extracts, solubilization of the cells was performed using 1.4 ml of the solubilization solution (see above) per dish. The suspensions were vortexed and centrifuged at 12,000 x g for 60 min at 4 °C. Each resultant supernatant anti-phosphotyrosine antibody (anti-P-Tyr) was added at a serum dilution of 1:50 and incubated for 2 h at 4 °C. The anti-phosphotyrosine antibodies were raised by injecting rabbits with phosphotyrosine coupled to KLH. The detailed procedure of the production of the antigen and characterization of these antibodies will be described elsewhere.² The immune complexes were immobilized on Pansorbin, and the precipitates were washed five times with

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² To whom correspondence should be addressed: Diabetes Branch, NIDDK, NIH, Bldg. 10, Rm. AS-243, 9000 Rockville Pike, Bethesda, MD 20892.

³ The abbreviations used are: IGF-I, insulin-like growth factor-I; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; Hepes, 4-(2-hydroxyethyl) 1-piperazineethanesulfonic acid; WGA, wheat germ agglutinin; anti-P-Tyr Ab, anti-phosphotyrosine antibody.

² D. Heffez and Y. Zick, manuscript in preparation.
a solution containing 50 mM Hepes, pH 7.4, 1% Triton X-100, 0.1% SDS, 150 mM NaCl, 10 mM sodium fluoride, 4 mM EDTA, and 2 mM sodium orthovanadate.

The phosphoproteins were eluted from the anti-P-Tyr-Pansorbin complex following a 1-h incubation at 4°C with 10 mM p-nitrophenyl phosphate (8). Half of the volume of the eluted phosphoproteins was added to sample buffer (22) and run on 7.5% SDS-PAGE under reducing conditions. The second half was reimmunoprecipitated using anti-insulin receptor antibodies B10 and B2 (which also recognized IGF-I receptors (26)), as well as anti-EGF receptor antiserum (a kind gift of Dr. Joseph Schlessinger, Rorer Biotechnology Incorporation, Rockville MD). The incubation period was 18 h at 4°C, and the receptor-antibody complex was recovered by using Pansorbin and sample buffer as described before (15) and was run on 7.5% SDS-PAGE under reducing conditions.

WGA-purified Preparations—For WGA purification, cells were solubilized as described for whole-cell preparations using 6 ml of solubilization solution per plate. The solubilized suspension was centrifuged at 40,000 × g for 50 min at 4°C. The supernatant was applied to 0.5-ml WGA-agarose columns (Miles-Yeda, Rehovot, Israel). The flow through was recycled twice. The agarose columns were washed with 90 ml of washing solution containing 50 mM Hepes, pH 7.4, 0.1% Triton X-100, 0.1% SDS, 150 mM NaCl, 10 mM sodium fluoride, 4 mM EDTA, 2 mM sodium orthovanadate, and 2 mM phenylmethylsulfonyl fluoride. The adsorbed glycoproteins were eluted using 1-ml fractions of the washing solution containing 0.3 M GlcNAc. Fractions 1 and 2 were found to contain the highest radioactivity and were used for immunoprecipitation of the phosphorylated glycoproteins using anti-P-Tyr Ab or antireceptor Ab (B10) in overnight incubations at 4°C. The phosphoproteins were eluted from the Pansorbin-anti-P-Tyr Ab complex using p-nitrophenyl phosphate as described above and were applied to 7.5% SDS-PAGE. When using B10 antiserum, the immunoprecipitated proteins were recovered from the Pansorbin-antibody complex using sample buffer prior to SDS-PAGE. The samples were then applied to 7.5% SDS-PAGE.

Phosphoamino Acid Analysis—Phosphoamino acid content of the phosphoproteins was determined by the method of Cooper et al. (27) with minor modifications (22). The whole-cell extract was immunoprecipitated using anti-P-Tyr Ab as described above. Following SDS-PAGE, the phosphoproteins of interest were located by autoradiography of the dried gel and excised. The phosphoproteins were eluted and hydrolyzed using 2 ml of 6 N HCl at 110°C for 90 min. The samples were then chilled on ice, diluted by the addition of 3 ml of water, and centrifuged at 2,000 rpm in a bench-top centrifuge for 5 min at 4°C. The supernatant was dried in vacuo at room temperature, and the yellowish brown residue was resuspended in 7.5 ml of water. Phosphoamino acid standards (5.5 μg each of phosphoserine, phosphothreonine, and phosphotyrosine purchased from Sigma) plus 1.0 ml of a 50% slurry of Bio-Rad AG 1-X8 (100-200 mesh, chloride form) resin were added. The pH was adjusted to 7.5 using 200 mM NH₄OH. The samples were shaken overnight at room temperature and filtered into disposable minicolumns. The resin was washed three times using 3 ml of distilled water, and the phosphoamino acids were eluted from the resin with two applications of 1 ml each of 0.1 M HCl. The eluates were dried in vacuo and the samples were resuspended in 10 μl of water. Thin-layer electrophoresis in one dimension in pyridine-acetate buffer, pH 3.1, for 1 h at 850 V was used to separate the phosphoamino acids. The phosphoamino acids were then localized by autoradiography. Unlabeled phosphoamino acid standards were added to the samples and located by staining with 6% ninhydrin in acetone.

RESULTS

Kinetic Studies of Insulin and IGF-I-stimulated Phosphoproteins—Insulin and IGF-I at a concentration of 10⁻⁷ M stimulated the phosphorylation of tyrosine-containing phosphoproteins in a time-dependent manner (Fig. 1). Insulin-induced phosphorylation of a 95-kDa (presumed β subunit) protein as well as a 185-kDa (presumed endogenous substrate) protein. The phosphorylation of both bands was extremely rapid, occurring at least as early as 20 s and reaching a maximum at 1 min. The dephosphorylation reaction was more rapid with the 185-kDa protein compared with the 95-kDa protein following insulin stimulation. Thus, after 10 min, the radioactivity in the 185-kDa protein was less than 50% of the 1-min level (Fig. 1). IGF-I-stimulated phosphorylation of three phosphoproteins of apparent molecular mass 95, 105, and 185 kDa. Similar to the insulin response, all three proteins were phosphorylated very rapidly (within 20 s) with a maximal effect at 1 min. The 185-kDa phosphoprotein was dephosphorylated more rapidly than the 95- and 105-kDa phosphoproteins following stimulation with IGF-I (Fig. 1). In addition to the 95- and 185-kDa phosphoproteins, insulin and IGF-I stimulated △P incorporation into at least three additional proteins (~45-, 60-, and 70-kDa bands). This phosphorylation did not appear time-dependent, and further characterization of these phosphoproteins was not undertaken.

Dose Response of Insulin- and IGF-I-stimulated Phosphoproteins—Insulin stimulated the phosphorylation of a 95- and a 185-kDa protein in a dose-dependent manner (Fig. 2). IGF-
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Fig. 2. Dose response of insulin- and IGF-I-induced phosphorylation in whole cells. N18 cells were labeled with $^{32}$P orthophosphate for 2 h prior to the addition of varying concentrations of insulin and IGF-I (10$^{-9}$–10$^{-7}$ M). The cells were incubated in the absence or presence of the hormones for 1 min, and the reaction was stopped as described under “Materials and Methods.” The phosphoproteins that were immunoprecipitated with anti-P-Tyr Ab were run on SDS-PAGE (upper panel). The tyrosine-containing phosphoprotein bands (95, 105, and 185 kDa) were then excised and the radioactivity was quantitated (lower panel). The degree of $^{32}$P incorporation into each band is expressed as a percentage of maximal incorporation at 10$^{-7}$ M hormone.

I similarly stimulated the phosphorylation of a 95- and a 185-kDa protein but, in addition, stimulated the phosphorylation of a 105-kDa protein (Fig. 2). The 185-kDa phosphoprotein following insulin and IGF-I stimulation appeared to be a doublet, the nature of which requires further study. The extent of $^{32}$P incorporation into the 95- and 185-kDa proteins, stimulated by insulin, was similar at each dose (when expressed as a percentage of maximal $^{32}$P incorporation). Likewise, $^{32}$P incorporation into the 95-, 105-, and 185-kDa proteins was enhanced to a similar extent at each concentration of IGF-I (Fig. 2). The phosphorylation of the 70-kDa protein by insulin and IGF-I was also dose-dependent.

The ED$_{50}$ of 10$^{-7}$ M for insulin and IGF-I is higher than the typical ED$_{50}$ for biological activities of these hormones. This difference may be due to an amplification system at the postreceptor level (28).

Identification of the Receptor β Subunits—To determine which phosphoproteins represented the β subunits of the insulin and IGF-I receptors, specific antisera were used. The specific anti-insulin receptor antiserum (B10) immunoprecipitated a 95-kDa phosphoprotein following insulin stimulation but not following IGF-I stimulation, suggesting that this protein represents the insulin receptor β subunit (Fig. 3). The anti-insulin receptor antiserum B2, like B10, immunoprecipitated an insulin-stimulated 95-kDa protein but, in addition, this antibody (which also binds to the IGF-I receptor) immunoprecipitated a 95-kDa as well as a 105-kDa protein whose phosphorylation was stimulated by IGF-I. This suggests that the 105-kDa protein is related to the already known 95-kDa β subunit of the IGF-I receptor (Fig. 3).

To further characterize the insulin and IGF-I receptor β subunits (known to be glycoproteins), $^{32}$P-labeled extracts were further purified on WGA-agarose. When such preparations were precipitated with anti-phosphotyrosine antibodies, it was found that IGF-I (added to $^{32}$P-labeled cells) stimulated the phosphorylation of WGA-agarose-purified 95- and 105-kDa proteins in a dose- and time-dependent manner (Figs. 4 and 5). By contrast, insulin only stimulated phosphorylation of its own 95-kDa receptor β subunit which could be precipitated by the anti-insulin receptor antibody B10 (Fig. 5, lanes F–H). The phosphorylation of this 95-kDa protein was not

Fig. 3. Identification of the β subunits of the insulin and IGF-I receptors. Insulin- and IGF-I-induced phosphorylation in intact N18 cells was performed for 1 min as described in the legends to Figs. 1 and 2. To identify the β subunits of the insulin and IGF-I receptors, whole-cell preparations were first immunoprecipitated with preparations using anti-P-Tyr antibodies. The tyrosine-containing phosphoproteins were recovered from the immune complexes using p-nitrophenyl phosphate and were then reimmunoprecipitated using the specific anti-insulin receptor antibodies B10 or B2 (that, in addition to the insulin receptor, also recognizes the IGF-I receptor). The immunoprecipitated phosphoproteins were then run on SDS-PAGE.
enhanced when the cells were treated with IGF-I at $10^{-9} - 10^{-7}$ M (Fig. 5, lanes J and K). This further emphasizes that the 95-kDa phosphoprotein stimulated by insulin probably represents the $\beta$ subunit of the insulin receptor. Because anti-P-Tyr antibody failed to immunoprecipitate this 95-kDa protein in $^{32}$P-labeled and untreated cells, it suggests that the $\beta$ subunit of the insulin receptor is phosphorylated on serine or threonine residues in the basal state.

To further determine whether the 105-kDa protein was a subtype of the IGF-I receptor $\beta$ subunit, the phosphorylated proteins that were stimulated by IGF-I (for 0–30 min as described in Figs. 4 and 5), WGA-purified, and immunoprecipitated by anti-P-Tyr Ab were run on 5% SDS-PAGE under nonreducing conditions (Fig. 6). Two major protein bands were identified: band I (molecular mass > 300 kDa) and band II (molecular mass < 300 kDa). Both bands bind represent heterotrimeric or oligomeric forms of the IGF-I receptor. When the bands were excised, reduced, and run on 7.5% SDS-PAGE, band I revealed two protein spots of molecular mass 105 and 95 kDa, whereas band II surprisingly revealed only a 105-kDa protein (data not shown). These results, in addition to other experiments, e.g. phosphopeptide mapping,\(^7\) suggest that the 105-kDa protein is an IGF-I receptor-related protein and probably represents a subtype of the $\beta$ subunit.

Phosphoamino Acid Analysis of the $\beta$ Subunit—Phosphoserine was the predominant amino acid seen in the basal phosphorylation state of the 95-kDa protein. Insulin induced an increase in phosphoserine and phosphotyrosine content of this specific $\beta$ subunit (95 kDa) after 1 min. The increase in phosphoserine was three times that seen for phosphotyrosine. Minimal incorporation of phosphate into threonine was also seen (Fig. 7). IGF-I similarly increased the phosphoserine and phosphotyrosine content of the 95-kDa IGF-I receptor $\beta$ subunit. After 1-min treatment of cells with IGF-I, the increase in phosphoserine was twice that of phosphotyrosine (Fig. 7). Phosphoserine was the predominant phosphoamino acid in the 105-kDa protein in the basal state. After 1-min stimulation of the cells with IGF-I, the phosphoserine and phosphotyrosine content of pp110 equally increased, whereas phosphothreonine content was only minimally altered (data not shown).

Characterization of the 185-kDa Phosphoprotein—The 185-kDa phosphoprotein (pp185) demonstrated rapid phosphorylation in response to insulin and IGF-I at concentrations of $10^{-9}$ M (Fig. 1). The dephosphorylation of pp185 was, however, more rapid than the $\beta$ subunits of both the insulin and IGF-I receptors (Fig. 1). Phosphorylation of pp185 in response to insulin and IGF-I was dose-dependent, which was similar to the dose dependency for insulin and IGF-I receptor $\beta$ subunit phosphorylation (Fig. 2). Antisera B10 and B2 failed to recognize pp185 (Fig. 3), strongly suggesting that pp185 represents an endogenous substrate for both insulin and IGF-I receptor kinase and is not a precursor form of these receptors. Moreover, pp185 failed to adsorb to WGA-agarose, unlike the insulin and IGF-I preceptor. To exclude the possibility that pp185 may represent the EGF or PDGF receptors that have a Mr, close to 185,000, experiments were performed where EGF or PDGF were added to $^{32}$P-labeled cells. Neither EGF nor PDGF stimulated phosphorylation of pp185 (Fig. 8). Furthermore, an anti-EGF receptor antibody failed to immunoprecipitate the 185-kDa phosphoprotein whose phosphorylation was stimulated by insulin and IGF-I. By contrast, this antibody immunoprecipitated a 175-kDa protein (presumed EGF receptor) whose phosphorylation was enhanced in response to EGF (Fig. 8). Phosphoamino acid analysis of pp185 demonstrated that treatment of N18 cells with insulin or IGF-I increased the phosphoserine, phosphotyrosine, as well as phosphothreonine content of this protein, with a maximal increase in phosphoserine content following stimulation by IGF-I (Fig. 9).

**DISCUSSION**

Specific insulin and IGF-I receptors are present on mouse neuroblastoma N18 cells (38). In cell-free extracts, both insulin and IGF-I induced autophosphorylation of their respective receptor $\beta$ subunits in a time- and dose-dependent manner. Furthermore, the phosphorylation occurred almost entirely on tyrosine residues. Both ligands also stimulated the

\(^3\) A. Ota, G. L. Wilson, R. Pruss, and D. LeRoith, submitted for publication.
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**Fig. 6. Characterization of the β subunit of the IGF-I receptor; non-reducing and reducing conditions.** N18 cells were treated as described in the legend to Fig. 5 using IGF-I (10^{-7} M) for varying time intervals. The samples were run on SDS-PAGE (5%) under nonreducing conditions and revealed two high molecular weight bands (left panel). Band I, with molecular mass greater than 300 kDa was excised and run on SDS-PAGE (7.5%) under reducing conditions (right panel).

**Fig. 7. Phosphoamino acid analysis of the β subunits for insulin and IGF-I receptors.** To demonstrate the phosphoamino acid composition of the β subunits of the insulin and IGF-I receptor of N18 cells, we utilized the method described in the legend to Fig. 5. The 95- and 105-kDa bands following IGF-I stimulation as well as the 95-kDa band following insulin stimulation (after antiphosphotyrosine antiseraum immunoprecipitation), and the 95- and 105-kDa bands in the basal state were excised and analyzed as under “Materials and Methods.” P-THR, phosphothreonine; P-SER, phosphoserine.

tyrosine kinase activity associated with the β subunits in these preparations (38).

In the present work, we have studied in intact N18 cells the effects of insulin and IGF-I on phosphorylation of their receptor β subunits as well as endogenous substrates. We could demonstrate the presence of an endogenous substrate (pp185) common to both the insulin and IGF-I receptors. We could also demonstrate that insulin and IGF-I very rapidly stimulate phosphorylation of their own receptor β subunits in a time- and dose-dependent manner.

The Endogenous Substrate pp185—No direct evidence is available from our studies that pp185 is a substrate of either the insulin or IGF-I receptor kinases; however, suggestive evidence for this includes the following. 1) Ligand-induced phosphorylation of pp185 is extremely rapid, reaching a maximum within 1 min, similar to that for the autophosphorylation of both the insulin and IGF-I receptors. Dephosphorylation of pp185 was, however, more rapid than that seen with the receptor β subunits. 2) Dose response of the phosphorylation of pp185 using insulin and IGF-I is similar to that for the receptor autophosphorylation, with similar effects at the same concentrations of the hormone. 3) pp185 does not adsorb to WGA-agarose nor is it recognized by antibodies that recognize the insulin and IGF-I receptors. 4) The phosphorylation of pp185 was not stimulated by EGF and PDGF, further supporting the hypothesis that pp185 represents an endogenous substrate specific to the insulin and IGF-I receptor kinases, as has been suggested by previous studies in non-
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Insulin and IGF-I-induced phosphorylation of a 95-kDa protein. Because IGF-I at 10^{-8} M probably does not bind in significant amounts to the insulin receptor, we propose that this 95-kDa protein may also represent a subtype of the IGF-I receptor β subunit. Further indirect evidence that the β subunit of the IGF-I receptor may be comprised of two types of subunits comes from the studies in which the IGF-I-stimulated phosphoproteins were gel electrophoresed under nonreducing conditions. This resulted in the formation of two ~300-kDa bands that upon reduction were shown to contain the 105-kDa protein, whereas the larger molecular mass band (>300 kDa) also contains a 95-kDa protein. The exact structure of these two oligomeric forms remains undefined. The presence of two β subunits of the IGF-I receptor has been previously reported in human epidermoid carcinoma KB cells (8). In that study, a specific monoclonal antibody directed towards the human IGF-I receptor (aIR3) identified two IGF-I-sensitive phosphoproteins of 92 and 98 kDa. Final confirmation that the IGF-I-sensitive 95- and 105-kDa phosphoproteins in N18 cells represent subtypes of the IGF-I receptor β subunits will require specific antibodies towards mouse IGF-I receptors, peptide mapping, and sequence analysis. In cell-free extracts of N18 cells, peptide mapping and enzymatic digestion using exo- and endoglycosidases have demonstrated that the 95- and 105-kDa proteins differ primarily in their carbohydrate composition.

An interesting difference was noted when comparing insulin- and IGF-I-induced autophosphorylation in intact N18 cells and cell-free extracts. In cell-free extracts, insulin at a concentration of 10^{-8} M induced the phosphorylation of the 105-kDa β subunit of the IGF-I receptor and the 95-kDa protein, whereas in intact cells, insulin failed to stimulate phosphorylation of the 105-kDa protein. This suggests that in cell-free systems, insulin may cause phosphorylation of the IGF-I receptor β subunit by binding to IGF-I receptor, or, alternatively, the IGF-I receptor β subunit may be an in vitro substrate for the insulin receptor kinase. Because this phenomenon does not occur in intact cells, it could be of non-physiological significance. The lack of in vivo cross-reactivity between insulin and IGF-I in phosphorylating each others receptors is further suggestive evidence that, unlike the receptor β subunits, pp185 indeed serves as a common in vivo substrate for both the insulin receptor and the IGF-I receptor kinases.

Another difference between insulin and IGF-I receptor phosphorylation in cell-free systems and in intact cells is the amino acid residues that incorporate the radioactive phosphate. In cell-free systems, insulin and IGF-I induce autophosphorylation of their respective receptor β subunits almost entirely on tyrosine residues. In contrast, in intact cells, serine residues are phosphorylated quantitatively at least as much as tyrosine residues. This phenomenon has been described previously in cells of non-neural origin (7-15). The in vivo phosphorylation of pp185 has similar characteristics, with phosphoserine being the predominant phosphoamino acid after hormonal stimulation. The significance of the differences between phosphorylation in intact cells and cell-free systems remains undefined.

Our results in neural-derived cells suggest that pp185 may be a ubiquitous endogenous substrate for both the insulin and IGF-I receptor kinases in neural and non-neural cells. Furthermore, pp185 may be involved in the early transmission of the events following the autophosphorylation of the insulin and IGF-I receptors. Because the insulin and IGF-I receptors are very similar in structure (24, 36, 37), certain functions or mechanisms of signal transduction may be common to both,
and pp185 may represent an example of this commonality.

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