Distinct Biologically Active Receptors for Insulin, Insulin-like Growth Factor I, and Insulin-like Growth Factor II in Cultured Skeletal Muscle Cells*

(Received for publication, June 28, 1985)

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The expression of insulin-like growth factor (IGF) receptors at the cell surface and the changes in IGF responsiveness during differentiation were studied in the L6 skeletal muscle cell line. Throughout the entire developmental sequence, distinct receptors for IGF I and IGF II that differed in structure and peptide specificity could be demonstrated. During differentiation, both 125I-IGF I and 125I-IGF II binding to the L6 cells decreased as a result of a 3–4-fold reduction in receptor number, whereas 125I-insulin binding increased. Under nonreducing conditions, disuccinimidyl suberate cross-linked 125I-IGF I and 125I-IGF II to two receptor complexes with apparent $M_r > 300,000$ (type I) and 220,000 (type II). Under reducing conditions, the apparent molecular weight of the type I receptor changed to $M_r > 150,000$ (distinct from the 120,000 insulin receptor) and the type II receptor changed to 250,000. IGF I and IGF II both stimulated 2-deoxy-D-glucose and $\alpha$-aminoisobutyric acid uptake in the L6 cells with a potency close to that of insulin, apparently through interaction with their own receptors. The stimulatory effects of IGF II correlated with its affinity for the type II but not the type I IGF receptor, as measured by inhibition of affinity labeling, whereas the effects of IGF I correlated with its ability to inhibit labeling of the type I receptor. In spite of the decrease in type I and type II receptor number, stimulation of 2-deoxy-D-glucose and $\alpha$-aminoisobutyric acid uptake by the two IGFs increased during differentiation.

The insulin-like growth factors (IGFs) are a family of polypeptide hormones that have close structural and functional homologies with insulin. The two IGF types found in human plasma (IGF I and IGF II) have approximately 70% amino acid sequence identity with each other and almost 50% identity with human insulin (1, 2). Insulin and IGFs produce similar biological effects in most cells, including stimulation of hexose and amino acid uptake and DNA synthesis (3, 4). Separate receptors for insulin and the two IGFs have been demonstrated in several tissues (5, 6) and, depending on the particular tissue, each peptide exhibits a weaker affinity for the specific receptors of the other hormones than for its own receptor (6). The precise biological roles of the insulin and IGF receptors in different tissues have not been established.

Using anti-insulin receptor antibodies, King et al. (7) demonstrated in rat adipocytes that both insulin and rat IGF II stimulated glucose oxidation via the insulin receptor. Since IGF II exhibited weaker affinity for the insulin receptor than insulin itself, this finding explained its lower potency in stimulating metabolic responses in these cells. Czech and coworkers (8, 9) also concluded that IGF II exerts its effects through either IGF I or insulin receptors, based on correlations of dose-response curves and the failure of anti-IGF II receptor antibodies to block IGF II action in soleus muscle and cultured hepatoma cells. However, since other studies in muscle have shown that the relative potency of IGFs and insulin in stimulating glucose metabolism is higher than in adipose tissue (10), different functional roles might be expected for insulin and IGF receptors in muscle as compared to adipose tissue.

In the present study, we have addressed this question using the L6 skeletal muscle cell line. Our previous studies, as well as those of Merrill et al. (12) have suggested that the L6 skeletal muscle cells not only maintain in culture the ability to differentiate into mature skeletal muscle cells with many properties of adult skeletal muscle, but also respond to insulin and IGFs and exhibit a progressive appearance of insulin receptors during the differentiation process. In the current study, we demonstrate that the L6 cells also possess distinct receptors for IGF I and IGF II and that these receptors are separately regulated during differentiation. All three receptors appear to initiate stimulatory effects on hexose and amino acid uptake. We suggest that the coordinated modulation of insulin and IGF receptors may represent an important event in skeletal muscle development.

EXPERIMENTAL PROCEDURES

Materials—Insulin and IGF I were purchased from Eli Lilly & Co. (Indianapolis, IN) and AMGen Biologicals (Thousand Oaks, CA), respectively. Rat IGF II (or MSA) was purified by a modification of the procedure of Moses et al. (13). MSA III-2 ($M_r = 7100$) was a kind gift of S. Peter Nissley (National Institutes of Health) and was used to prepare $^{125}$I-labeled rat IGF II. Unlabeled IGF II had an $M_r$ value of 8700 (peak II MSA, see Ref. 13) and is known to be approximately

\[ 2^{125}I-\text{IGF II} \]

\[ \alpha\text{-aminoisobutyric acid} \]

\[ \text{DNA synthesis} \]

\[ \text{Adipose tissue} \]

\[ \text{Skeletal muscle} \]

\[ \text{Stimulation} \]

\[ \text{Hexose and amino acid uptake} \]

\[ \text{IGF I and IGF II} \]

\[ \text{Insulin receptor} \]

\[ \text{Different functional roles} \]

\[ \text{IGF I and IGF II} \]

\[ \text{Skeletal muscle development} \]

\[ \text{Distinct Biologically Active Receptors for Insulin, Insulin-like Growth Factor I, and Insulin-like Growth Factor II in Cultured Skeletal Muscle Cells} \]
Acid (AIS) Uptake—The effect of insulin, IGF I, and IGF II presence of the indicated concentrations of insulin, IGF I, or IGF II was studied as previously described. Briefly, cells were plated on 60-mm tissue culture dishes and cultured for 4 days (undifferentiated myoblasts) or 18 days (differentiated myotubes) as previously reported. Specific [125I]-IGF I, [125I]-IGF II, and [125I]-insulin binding of cell protein was determined at both stages of differentiation as described under “Experimental Procedures.” Each bar represents the mean ± S.D. of duplicate plates from at least ten separate experiments. IGFI II, rat IGF II.

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Determination of 2-Deoxy-D-glucose (2-DG) and a-Aminoisobutyric Acid (AIB) Uptake—The effect of insulin, IGF I, and IGF II on 2-DG uptake was studied as previously described. Briefly, the cells were grown to confluence and allowed to differentiate into multinucleated myotubes. After 8–10 h, the incubation medium was replaced with 2.0 ml of Kreb’s solution (122 mM NaCl, 5 mM KCl, 2.5 mM MgSO4, 1 mM CaCl2, 23 mM Tris, pH 7.5) containing the same hormone concentrations and appropriate amounts of labeled and unlabeled 2-DG or AIB. The plates were then rapidly rinsed with cold phosphate-buffered saline, and the cells lysed with 1 N NaOH and counted for 14C.

RESULTS

Reciprocal Changes in Insulin and IGF Binding during Differentiation—Undifferentiated myoblasts were grown to confluence and allowed to differentiate into multinucleated myotubes over a period of 18 days. [125I]-IGF I, [125I]-IGF II, and [125I]-insulin binding were measured on day 4, when the myoblasts had nearly reached confluence, and on day 18, when the differentiation process was complete as assessed by cell morphology, maximal creatine kinase content, and the appearance of spontaneous contractions. At both the myoblast and the myotube stages, L6 cells bound IGFI, IGFI I, and insulin (Fig. 1). IGFI binding was about 100-fold greater than insulin binding at the myoblast stage. As the cells differentiated into myotubes, IGFI I binding fell by 70% and IGFI II binding decreased 55% (Fig. 1, left). This is the reverse of the situation observed for [125I]-insulin binding, where myoblasts have 2-fold lower binding than myotubes (Fig. 1, right).

To investigate the nature of the decrease in IGFI binding during L6 cell differentiation, steady-state [125I]-IGFI and [125I]-IGFI II binding to myoblasts and myotubes was studied over the concentration range of 10^{-12}–10^{-6} M (Fig. 2). Scatchard analysis in both cases was linear and revealed a decrease in IGFI II binding capacity from 43 pmol mg^{-1} (myoblasts) to 9 pmol mg^{-1} (myotubes) and a decrease in IGFI I binding capacity from 2.7 pmol mg^{-1} (myoblasts) to 1.0 pmol mg^{-1} (myotubes). The K_I values remained similar at approximately 3 × 10^{-8} M and 5 × 10^{-9} M, respectively. Thus, the decreased IGFI I binding after L6 cell differentiation was accounted for by a diminished receptor number at the cell surface with no changes in receptor affinity.

Characterization of Two Types of IGF Receptors in the L6 Cells—To characterize the receptors for IGFI I and II, binding competition studies were performed. [125I]-IGFI I binding to the L6 myotubes was inhibited in a dose-dependent fashion by IGFI I (Fig. 3, left). In six experiments the mean half-maximal
Inhibition occurred at $5 \times 10^{-6}$ M. IGF II and insulin also competed with tracer $^{125}$I-IGF I but their potencies were 30- to 50-fold and 100-fold lower than that of IGF I, respectively. Similar results were obtained in undifferentiated myoblasts (data not shown).

A different pattern of inhibition of $^{125}$I-IGF II binding was observed (Fig. 3 right). Tracer binding was inhibited most potently by IGF II ($K_i = 1 \times 10^{-8}$ M), whereas IGF I was about 5-fold less potent at both the myotube and the myoblast stages of differentiation. Up to $1 \times 10^{-6}$ M insulin, as well as the chemically unrelated peptides glucagon and fetuin, were ineffective in competing with $^{125}$I-IGF II. This different specificity of peptide inhibition suggested that $^{125}$I-IGF I bound preferentially to a type I (insulin-inhibitable, IGF I-prefering) receptor, whereas $^{125}$I-IGF II was predominantly bound to a type II (insulin-noninhibitable, IGF II-prefering) receptor.

Affinity Labeling of Type I and Type II IGF Receptors—The presence of both type I and II IGF receptors in the L6 cells was further demonstrated by affinity cross-linking with disuccinimidyl suberate. Under nonreducing conditions, $^{125}$I-IGF I was cross-linked predominantly to a receptor complex with apparent $M_r > 300,000$ and to a lesser extent to a band at 220,000. After treatment with the reducing agent $\beta$-mercaptoethanol, the major band migrated at 130,000, similar to the subunit of the IGF I receptor observed in other studies (5), while the minor band moved to 250,000, similar to the previously described IGF II receptor (5) (Fig. 4, A and B). Unlabeled IGF I (1 $\times 10^{-7}$ M) inhibited the affinity labeling of the type I IGF receptor ($M_r = 130,000$), whereas unlabeled IGF II and insulin had no effect on this band at identical concentrations.

Using disuccinimidyl suberate, $^{125}$I-IGF II cross-linked to myoblasts and myotubes also yielded two bands. The major band had an $M_r$ value of 220,000 under nonreducing conditions and migrated with an $M_r$ value of 250,000 upon $\beta$-mercaptoethanol reduction (Fig. 4, C and D). IGF II interaction with this receptor species was inhibited by $10^{-7}$ M unlabeled IGF II, less potently by IGF I, and not at all by insulin. There was also a minor band which migrated at $M_r > 300,000$ under nonreducing conditions and 130,000 upon reduction. Cross-linking of IGF II to this species was inhibited by IGF I but not IGF II. Under our conditions of labeling and analysis, $^{125}$I-insulin was cross-linked to a receptor species which migrated with an apparent molecular weight of 120,000 under reducing conditions (not shown). This protein migrated further in the gel and was distinct from the 130,000 band labeled by $^{125}$I-IGF I.

Densitometric scanning of these autoradiographs was performed to allow for more precise calculations of the competition of each ligand for the different receptor types (Fig. 5). This revealed that IGF I was about 20-fold more efficient in inhibiting $^{125}$I-IGF I affinity labeling of type I receptor than $^{125}$I-IGF I labeling of the type II receptor (Fig. 5, left). In contrast, IGF II, up to $1 \times 10^{-7}$ M, did not significantly displace the labeling of the type I receptor by $^{125}$I-IGF II but inhibited the labeling of the type II receptor with a half-maximal effective concentration of approximately $1 \times 10^{-8}$ M (Fig. 5, right). This concentration is similar to that required to inhibit $^{125}$I-IGF II binding by the same amount. This suggested that IGF I strongly interacts with both type I and type II receptors and that the ability of low concentrations of IGF II to compete with $^{125}$I-IGF I in the binding assay may be produced by displacement of the tracer from the type II receptor rather than the type I receptor. IGF II also binds to both the type I and II receptors but competes primarily for binding to the type II receptor, while IGF I can compete for both sites.

**IGF I and IGF II Stimulation of Hexose and Amino Acid Uptake**—Since the L6 muscle cells exhibit a considerable number of IGF receptors at every developmental stage, the ability of IGF I and IGF II to stimulate 2-DG and AIB uptake was investigated and compared to insulin. In agreement with our previous observations and with the work of others (12), prolonged incubation (8–10 h) with insulin or IGFs was required to maximally stimulate these processes (data not shown). The dose-response relationships of IGF I and IGF II binding and stimulation of 2-DG uptake in the myoblasts are shown in Fig. 6 with insulin data included for comparison. Based on half-maximally effective concentrations, the relative potencies of IGF I, insulin, and IGF II stimulation of 2-DG uptake were 6:2:1. These assignments are somewhat arbitrary since the insulin dose-response curve had a different slope and crossed both the IGF I and II dose-response curves. Similar results were obtained on AIB uptake. When compared to the ability of these hormones to saturate binding sites, there was a much closer relationship between IGF I and binding and effects as compared to insulin (compare Fig. 6, A and C). In the case of insulin, the dose-response curve for the stimulation of 2-DG and AIB uptake was shifted to the left of the binding curve by almost 2 logs. In the case of IGF I and II, however, the dose-response curves were only about 3-fold to the left of the binding saturation curves, and this difference was not evident in all the experiments.

To investigate further the roles of IGF receptors in mediating metabolic responses, binding to insulin receptors was specifically blocked with the Fab fragment of the B8 anti-insulin receptor antisera (Fig. 7). This serum contains antibodies which react with both insulin and IGF I receptors but not IGF II receptors (17). At 100 µg/ml, the anti-insulin receptor Fab inhibited insulin stimulation of 2-DG uptake by 50%, whereas there was no inhibition of the IGF II stimulatory action. This suggested that IGF II receptors rather than insulin or IGF I receptors mediate the IGF II effect. This conclusion was further supported by correlations between the biological actions of IGF I and II and their ability to inhibit the affinity labeling of the type I and the type II receptors. If IGF I effects were mediated via the type I receptors and IGF I...
and IGF II on the \( \text{\textsuperscript{125}I}-\text{IGF I} \) and \( \text{\textsuperscript{125}I}-\text{IGF II} \) to L6 myotubes examined without or with disulfide reduction. Labeled IGF I (A and B, left) or IGF II (C and D, right) were incubated with L6 myotubes at concentrations of 1 to 4 \( \times 10^{-10} \) M for 4 h at 15 °C as described under "Experimental Procedures." Unlabeled IGF I, rat IGF II, or insulin was added as indicated. Cross-linking with 0.1 mM disuccinimidyl suberate, solubilization, electrophoresis, and autoradiography were performed as described under "Experimental Procedures." The presence of an \( M_r \geq 250,000 \) band in lanes 5–10 was an inconsistent finding that might have been produced by partial renaturation of the 250,000 receptor protein. Labeling of the \( M_r \geq 300,000 \) receptor band in lanes 11, 12, and 14 was more evident after more prolonged exposure of the film.

**FIG. 5.** Differential inhibitory effects of unlabeled IGF I and IGF II on the \( \text{\textsuperscript{125}I}-\text{IGF I} \) and \( \text{\textsuperscript{125}I}-\text{IGF II} \) binding. A and B, left) and IGF II (C and D, right) and the inhibitory effects of IGF I and IGF II, at the indicated concentrations, were quantitated by bidimensional densitometric scanning of the autoradiographs shown in Fig. 4. The percentage of inhibition of type I and type II receptor labeling was calculated as the percentage of the difference between the intensity of the labeled bands in the absence (initial labeling) and the presence of unlabeled IGF I or IGF II, and expressed as percentage of initial labeling. The data shown are representative of at least three experiments.

II effects via the type II receptors, one would expect a close correlation between receptor occupancy and bioeffects. On the other hand, if the effects of IGF II were mediated through the type I IGF receptor as recently proposed in the isolated soleus muscle (8), one would anticipate a close correlation between the occupancy of the type I receptor by IGF II and its stimulatory effect on the uptake of 2-DG and AIB. As illustrated in Fig. 8 (top), the ability of IGF I to stimulate 2-DG uptake correlated closely with its ability to occupy the type I receptor. Likewise, there was an almost 1:1 relationship between IGF II effects and its inhibitory action on the labeling of the type II receptors (Fig. 8, bottom). Thus, either IGF II determines its insulin-like effects through the type II IGF receptors and exhibits a close relationship between receptor occupancy and bioeffects or its action is mediated by the type I IGF receptors, but exhibits more than 50-fold greater efficiency than IGF I, an unlikely possibility.

**IGF Responsiveness during Differentiation**—We have previously reported that insulin responsiveness increased during L6 cell differentiation and that this change was accompanied by a parallel increase in insulin receptors at the cell surface. After differentiation, there was also a 3-fold increase in the ability of IGF I to stimulate AIB uptake, while the IGF II effect was unchanged (Fig. 9). In contrast, IGF I stimulation of 2-DG uptake was only 30% increased in the myotubes compared to the myoblasts, while the IGF II effect was more than 2-fold greater. These differential changes in the IGF I and IGF II stimulation of hexose and amino acid uptake are consistent with the involvement of separate receptors mediating the actions of the two hormones.

To verify the effect of differentiation on IGF action, IGF I and II binding and stimulation of 2-DG uptake were compared in the myoblasts and in the myotubes, and the efficiency of coupling between receptor binding and effect was plotted as a function of receptor occupancy both before and after differentiation (Fig. 10). In the case of insulin, the same receptor occupancy generated identical responses at every differentiation stage. In the case of IGF I and II, however, half-maximal stimulation of 2-DG uptake required, respectively, 2- and 16-fold greater receptor occupancy in the myoblasts than in the myotubes, suggesting the development of more efficient receptor-effector coupling during differentiation.

**DISCUSSION**

In skeletal muscle, as in most other tissues, the biological functions of IGF receptors and their relationship to the functions of the insulin receptor are uncertain. This is an important subject for investigation because of the major involvement of muscle in normal and altered conditions of glucose metabolism (18). In a previous report, we described the development of increased binding and physiological responsiveness to insulin during differentiation in the L6 skeletal...
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Fig. 6. Relationships between IGF I, IGF II, and insulin binding and effects on the uptake of 2-DG and AIB in L6 myotubes. Cells were plated on 60-mm culture dishes and cultured for 18 days as previously reported. 2-DG (A) and AIB (B) uptake were then determined as described under “Experimental Procedures” in the presence or the absence of the indicated concentrations of IGF I, IGF II, or insulin. Binding (C) was performed after diluting tracer IGF I, IGF II, and insulin with the unlabeled hormones to give the indicated concentrations. The data represent the mean of duplicate determinations from at least three experiments.

Fig. 7. Effect of B8 FAb on insulin and IGF II stimulated 2-DG uptake in L6 myotubes. Cells were plated on 60-mm culture dishes and cultured for 18 days as previously reported. Stimulation of 2-DG uptake was then carried out with optimal concentrations of insulin or IGF II as described under “Experimental Procedures” in the presence or the absence of 100 μg/ml B8 FAb. An additional set of plates was challenged with 100 μg/ml B8 FAb alone. 2-DG uptake was subsequently determined and expressed as percentage of maximal insulin or IGF II-stimulated uptake. At optimal concentration, the stimulatory effect of insulin was similar in magnitude to that of IGF II. The data represent the mean ± S.D. of duplicate determinations from two experiments.

Fig. 8. Relationship between IGF I and IGF II stimulatory effects on 2-DG uptake and their inhibitory effects on 125I-affinity labeling of the type I and type II IGF receptors in L6 myotubes. Type I and type II IGF receptors were labeled by 125I-IGF I in the absence (initial labeling) or the presence of the indicated concentrations of unlabeled IGF I (top panel) or IGF II (bottom panel). Inhibition of receptor labeling was quantitated by densitometric scanning of the autoradiographs and expressed as percentage inhibition of initial labeling. IGF I and IGF II stimulation of 2-DG uptake was determined as described under “Experimental Procedures.”
were determined as described under "Experimental Procedures." Each bar represents the mean of experiments.

Concentrations of IGF I (2 × 10^{-7} M), IGF II (8 × 10^{-6} M), or insulin (1 × 10^{-7} M) on 2-DG uptake (left panel) or AIB uptake (right panel) were determined as described under "Experimental Procedures." Each bar represents the mean ± S.D. of duplicate plates from at least four experiments.

![Fig. 9. Changes in IGF I, IGF II, and insulin responsiveness during L6 cell differentiation.](image)

**Fig. 9.** Changes in IGF I, IGF II, and insulin responsiveness during L6 cell differentiation. Cells were plated on 60-mm tissue culture dishes and cultured for 4 days (myoblasts) or 18 days (myotubes) as previously described. The stimulatory effects of optimal concentrations of IGF I (2 × 10^{-8} M), IGF II (8 × 10^{-6} M), or insulin (1 × 10^{-7} M) on 2-DG uptake and AIB uptake were determined as described under "Experimental Procedures." Each bar represents the mean ± S.D. of duplicate plates from at least four experiments.

And type II (insulin-insensitive, IGF II-prefering) IGF receptors was demonstrated by equilibrium binding and affinity cross-linking experiments. In the L6 cells, 125I-IGF I binding was inhibited by IGF I 30-fold more potently than by IGF II, while insulin inhibited binding only at high concentrations. Conversely, 125I-IGF II binding showed a 5-fold greater sensitivity to inhibition by IGF II than IGF I and no sensitivity to even high concentrations of insulin. Disuccinimidyl suberate cross-linked both 125I-IGF I and 125I-IGF II to two proteins with the apparent molecular weights described for type I and type II IGF receptors in other cell models (5, 6, 20). As determined by scanning densitometry, IGF I had high affinity for (i.e. strongly inhibited) both type I and type II receptor affinity labeling, although its affinity for IGF I receptors was about 20-fold greater than for IGF II receptors. In contrast, IGF II showed a poor ability to inhibit type I receptor labeling, but inhibited type II receptor labeling with a potency similar to that exhibited in displacing 125I-IGF II binding. High concentrations of insulin inhibited type I but not type II receptor labeling. These observations are consistent with the peptide specificity described for the two types of IGF receptors in most tissues (6, 21). Most importantly, they document the distribution of IGF I and IGF II between the two types of IGF receptors in the L6 cells.

Both subtypes of IGF receptors, as well as insulin receptors, were expressed at the cell surface at the myoblast and the myotube stages of differentiation. However, in contrast to insulin receptors, which increase during differentiation, both types of IGF receptors decrease. The mechanisms determining these changes are unknown but could involve alterations of the plasma membrane resulting in different receptor exposure or alterations in the expression of specific genes. Both types of changes have been described during skeletal muscle differentiation (22–24).

The large number of IGF receptors compared to insulin receptors present in L6 cells led us to investigate their functional relationships. Although IGF stimulation of glucose metabolism in rat adipocytes occurred through insulin receptors (7), stimulation of glucose metabolism by IGF receptors has been described in skeletal and cardiac muscle (8, 25–27), in cultured fibroblasts (28), and more recently in the nonfusing muscle cell line BC3H-1 (20). As previously demonstrated, insulin appears to stimulate glucose and amino acid uptake in the L6 cells through its own receptor. The low IGF concentrations required to generate effects suggested that their action could not be mediated solely through the insulin receptors. This conclusion was supported by the inability of anti-insulin receptor antibodies to inhibit IGF II stimulation of 2-DG uptake. When the ability of IGF I and IGF II to compete for type I and type II receptor labeling was compared with their stimulatory effects on 2-DG and AIB uptake, a close relationship between their stimulatory effects and their respective inhibition of type I and type II receptor labeling was found. Thus, the data suggest that the action of each hormone is mediated through its own receptor in these cells. In further support of this conclusion, a variant subline of the L6 cells that has 10-fold reduced IGF II binding but normal IGF I binding, exhibits a 10-fold decrease in the ability of IGF II to mediate biological effects.

Based on the relationship between the biological action of IGF II and its ability to inhibit the affinity labeling of the two types of IGF receptors, Yu and Czech (8) concluded that IGF II acts through the type I receptor in skeletal muscle in stimulating hexose and amino acid uptake. In contrast to the stimulatory effects of IGF II in the L6 cells, IGF II effects in this soleus preparation occurred at hormone concentrations greater than 1 × 10^{-7} M. Mottola and Czech (9) also have presented data suggesting that IGF II exerts its effects on DNA synthesis in H-35 hepatoma cells via insulin rather than IGF II receptors. From these two studies, it was concluded that IGF II receptors may not play a role in transmembrane signaling, but rather serve some other physiological function (9). Using the same types of evidence, our data suggest that in the L6 cells the two different IGFs mediate their responses via their own receptors.

In contrast to the close relationships between the dose-response curves for binding and action of IGF I and II, the insulin dose-response curves showed about 50-fold higher sensitivity for insulin stimulation of hexose and amino acid uptake than for binding at both the myoblast and the myotube stages of differentiation. As previously noted in isolated soleus muscle (8, 29), this finding might reflect the presence of spare receptors for insulin but the absence of spare receptors for the IGFs in the L6 cells.

In spite of the decrease in IGF I and IGF II receptor number during differentiation, hormone responsiveness increased or remained unchanged. In the case of IGF II, identical stimulation of 2-DG uptake required 16-fold greater receptor occupancy than the myoblasts. Similar, smaller changes were also observed for IGF I but not for insulin. This may reflect differences in the development of regulatory mechanisms for these three receptors during differentiation. It is noteworthy that both the level of receptor occupancy and the curvilinear

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coupling of binding to response is similar for insulin and IGF I receptors but quite different for type II IGF receptors. Current evidence also suggests close structural similarities between insulin and type I IGF receptors (6, 21) and differences from IGF II receptors. It is possible that differentiation may affect insulin and type I IGF receptors differently than type II receptors in the L6 cells.

As we have described in the L6 cells, type I and type II IGF receptors have been demonstrated in the nonfusing BC3H-1 muscle cell line (20). These cells, which may be more representative of smooth muscle than skeletal muscle, differ from the L6 cells in that they are not responsive to even high concentrations of insulin at the myoblast stage (11). Thus, they cannot be utilized to study the relationships between insulin and the IGFs before differentiation.

In summary, the cloned L6 skeletal muscle cell line provides an excellent model for investigating the relationships between insulin and IGF receptor structure and function during differentiation. Because of their abundant expression of both types of IGF receptors, these cells offer unique opportunities for investigating the interaction of insulin, IGF I, and IGF II with these receptors. Since insulin, IGF I, and IGF II seem to produce their bioeffects through separate receptors, the L6 cells may represent a powerful tool for probing the specific roles of these hormones in skeletal muscle development.

REFERENCES