Testosterone and Insulin-like Growth Factor (IGF) I Interact in Controlling IGF-Binding Protein Production in Androgen-Responsive Foreskin Fibroblasts*

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ABSTRACT

The growth of the male external genitalia is primarily regulated by androgens. However, human genital fibroblast growth is also stimulated by insulin-like growth factor (IGF) I. In this study, we report that IGF-binding protein (IGFBP) production in human foreskin fibroblasts is regulated by androgens and IGF-I. Human foreskin fibroblasts secrete IGFBP-3, IGFBP-4, and IGFBP-5. IGF-I increased the abundance of both intact IGFBP-3 and -5 in the culture medium. Testosterone increased IGFBP-3, and the combination of IGF-I and testosterone had an additive effect. Following its secretion, IGFBP-5 was degraded, but the effect of IGF-I on IGFBP-5 peptide abundance in conditioned media did not seem to be due to inhibition of proteolysis. Testosterone had no effect on IGFBP-5 degradation. Intact IGFBP-4 was decreased by IGF-I, and the combination resulted in a similar reduction. The mechanism seemed to be decreased synthesis, since IGFBP-4 messenger RNA was also decreased. The increase in IGFBP-5 synthesis was associated with an increase in the abundance of intact IGFBP-5 in the extracellular matrix. The combination of testosterone and IGF-I resulted in a synergistic stimulation of total protein synthesis by the fibroblast cultures, suggesting that a maximum anabolic response requires both hormones. These observations suggest that combined exposure to androgen and IGF-I altered the abundance of some forms of IGFBPs and that the IGFBPs that are regulated may play a role in modulating the effects of IGF-I on the anabolic response. (J Clin Endocrinol Metab 85: 1627–1633, 2000)

Peptide growth factors have been shown to mediate some of the growth-promoting effects of androgens in androgen-responsive tissues. Insulin-like growth factor (IGF) I has been shown to be synthesized by androgen-responsive tissues and to stimulate locally regulated growth. In addition to their growth-promoting actions, both androgens and IGF-I have metabolic actions in gonadal cells. Similarly, they have been shown to interact in controlling testosterone biosynthesis by Leydig cells. IGF-I has been shown to stimulate the testosterone production by testicular interstitial cells (1) and to enhance steroidogenesis by Leydig cells in response to human CG (2, 3). Further evidence for an interaction between these trophic factors comes from the clinical observation that patients with congenital GH deficiency (4) or those with GH receptor mutations (5–7) develop clinical observation that patients with congenital GH deficiency (4) or those with GH receptor mutations (5–7) develop.

The growth of the male external genitalia is primarily regulated by androgens. However, human genital fibroblast growth is also stimulated by insulin-like growth factor (IGF) I. In this study, we report that IGF-binding protein (IGFBP) production in human foreskin fibroblasts is regulated by androgens and IGF-I. Human foreskin fibroblasts secrete IGFBP-3, IGFBP-4, and IGFBP-5. IGF-I increased the abundance of both intact IGFBP-3 and -5 in the culture medium. Testosterone increased IGFBP-3, and the combination of IGF-I and testosterone had an additive effect. Following its secretion, IGFBP-5 was degraded, but the effect of IGF-I on IGFBP-5 peptide abundance in conditioned media did not seem to be due to inhibition of proteolysis. Testosterone had no effect on IGFBP-5 degradation. Intact IGFBP-4 was decreased by IGF-I, and the combination resulted in a similar reduction. The mechanism seemed to be decreased synthesis, since IGFBP-4 messenger RNA was also decreased. The increase in IGFBP-5 synthesis was associated with an increase in the abundance of intact IGFBP-5 in the extracellular matrix. The combination of testosterone and IGF-I resulted in a synergistic stimulation of total protein synthesis by the fibroblast cultures, suggesting that a maximum anabolic response requires both hormones. These observations suggest that combined exposure to androgen and IGF-I altered the abundance of some forms of IGFBPs and that the IGFBPs that are regulated may play a role in modulating the effects of IGF-I on the anabolic response. (J Clin Endocrinol Metab 85: 1627–1633, 2000)

In addition to production and responsiveness of IGF-I, androgen-sensitive tissues have also been shown to produce IGF-binding proteins (IGFBPs). IGFBP-2 to -4 are expressed in some androgen-sensitive tissues. For this reason, we studied IGFBP production by androgen-sensitive fibroblasts to determine whether IGF-I has the potential to regulate the responsiveness of androgen-sensitive tissues to this growth factor. For this reason, we studied IGFBP production by androgen-sensitive fibroblasts and correlated these findings with changes in anabolic responsiveness.

IGFBP-4 was decreased by IGF-I, and the combination resulted in a similar reduction. The mechanism seemed to be decreased synthesis, since IGFBP-4 messenger RNA was also decreased. The increase in IGFBP-5 synthesis was associated with an increase in the abundance of intact IGFBP-5 in the extracellular matrix. The combination of testosterone and IGF-I resulted in a synergistic stimulation of total protein synthesis by the fibroblast cultures, suggesting that a maximum anabolic response requires both hormones. These observations suggest that combined exposure to androgen and IGF-I altered the abundance of some forms of IGFBPs and that the IGFBPs that are regulated may play a role in modulating the effects of IGF-I on the anabolic response. (J Clin Endocrinol Metab 85: 1627–1633, 2000)

* Supported by NIH Grant AG02331.

Received September 5, 1997. Revision received June 2, 1999. Accepted December 28, 1999.

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Cell culture

Normal human foreskin fibroblasts (GM8333A) were obtained from Coriell Institute (Camden, NJ) and grown in MEM without phenol red (Life Technologies, Inc., Grand Island, NY) supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), 2 mM L-glutamine, and 15% FBS (Sigma, St. Louis, MO). The cells were plated on 10-cm plates (Falcon #3003; Division of Becton-Dickinson, Plymouth, UK), 48-well plates (#3548; Costar, Cambridge, MA). The medium was changed every 3 days until confluency was attained (usually 7–10 days). At that time, the experiments were initiated without a period of serum deprivation. These fibroblasts have been extensively characterized (14–16). We have shown that they express androgen receptors and respond to dihydrotestosterone (Yoshizawa, A., F. S. French, and D. R. Clemmons, unpublished observations).

Experimental Procedures

Cell culture

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Western ligand blots and immunoblots

Conditioned medium was collected by adding 4.0 mL serum-free MEM to fibroblast cultures with or without the listed treatments. The treatments included IGF-I (50 ng/mL, a gift of Genentech, Inc., South San Francisco, CA) or testosterone 10 nM (Sigma). These concentrations were chosen because, after testing concentrations between 5–100 ng/mL IGF-I and 1–100 nM testosterone, they were shown to give the greatest response. After 24 h, the medium was removed and centrifuged at 5000 x g for 10 min, then stored at –20°C until it was analyzed. In some experiments, 100 U/mL heparin (Sigma) was added to limit IGFBP-5 degradation. Each conditioned medium sample (100–150 μL) was lyophilized and reconstituted in 30 μL Laemmli sample buffer (17). The membranes were probed with 125I-IGF-I (specific activity 150–250 μCi/μg) using 600,000 cpm/membrane, as described previously (18). Immunoblotting was performed using a 1:500 dilution of a polyclonal rabbit antihuman IGFBP-4 antiserum and a 1:1000 dilution of a guinea pig or a 1:2000 NIH Image. Francisco, CA). The signal intensity of each band was quantified using NIH Image.

IGFBP-4 degradation assay

The conditioned medium (50 μL) that had been collected after a 24-h exposure to confluent, quiescent foreskin fibroblasts, was incubated with 50 ng pure human IGFBP-4 for 14 h at 37°C at 60 mmol; ICN Pharmaceuticals, Irvine, CA) to 0.5 ml low methionine (10^-6 M) medium and increasing concentrations of IGF-I or 50 ng/mL IGF-I plus increasing concentrations of testosterone for 6 h at 37°C. The medium was aspirated, and wells were rinsed twice with PBS containing 0.1% BSA (Sigma). Cells were lysed, and the total intracellular protein precipitated in 5% TCA then centrifuged at 14,000 x g for 10 min. The pellets were resuspended in 0.1 M NaOH with 1% SDS and counted in a β-scintillation counter.

RNA isolation and Northern blot analysis

RNA was isolated from cells using TriReagent (Molecular Research Center, Inc., Cincinnati, OH). Fifteen micrograms of total RNA were loaded to a 1.0% agarose formaldehyde gel and transferred onto a nylon membrane (ICN Biochemical, Inc., Irvine, CA). The membranes were hybridized with a 1125-bp [32P]-dCTP-labeled human IGFBP-3 probe (19), a 600-bp IGFBP-4 probe (19), or a 627-bp IGFBP-5 complementary DNA probe (19). The amount of radiolabeled probe that was hybridized with a 1125-bp [32P]-dCTP-labeled human IGFBP-3 probe, a 600-bp IGFBP-4 probe, or a 627-bp IGFBP-5 complementary DNA probe (19). The signal intensity of each band was quantified using NIH Image.

Preparation of extracellular matrix (ECM)

The cells were rinsed twice with phosphate-buffered saline (PBS), and the cellular membranes were removed by incubating for 5 min in 0.5% Triton X-100 in PBS (pH 7.4). The adherent nuclei and cytoskeletal proteins were removed by incubating for 5 min in 25 mM ammonium acetate (pH 9.0). After washing twice with PBS, the ECM was scraped from the plates into Laemmli sample buffer. The extracts, 0.05 cc, were loaded onto SDS polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Immobilon; Millipore Corp., Bedford, MA). The membranes were loaded to a 1.0% agarose formaldehyde gel and transferred onto a nylon membrane (ICN Biochemical, Inc., Irvine, CA). The membranes were hybridized with a 1125-bp [32P]-dCTP-labeled human IGFBP-3 probe (19), a 600-bp IGFBP-4 probe (19), or a 627-bp IGFBP-5 complementary DNA probe (19). The amount of radiolabeled probe that was hybridized with a 1125-bp [32P]-dCTP-labeled human IGFBP-3 probe, a 600-bp IGFBP-4 probe, or a 627-bp IGFBP-5 complementary DNA probe (19). The signal intensity of each band was quantified using NIH Image.

[35S]-methionine incorporation assay

The effects of the test substances on protein synthesis were determined by adding 20 μCi [35S]-methionine (specific activity 1218 Ci/
IGF-I or testosterone alone \((P < 0.02)\). IGFBP-5 was not significantly increased by testosterone. IGF-I stimulated a 415\% increase, but the response to testosterone + IGF-I was not significantly greater than IGF-I alone (Table I). Scanning densitometry of the band changes for IGFBP-4 showed very different results. Specifically, IGF-I reduced IGFBP-4 to 63\% of control, and testosterone resulted in no significant change. The combination of testosterone plus IGF-I reduced it by 63\%. IGFBP-1, -2, and -6 were not detected (data not shown).

To further analyze the mechanisms that accounted for these changes, Northern blotting was performed for each of the three mRNA species (Fig. 2). As shown in Table 2, IGF-I had no effect on IGFBP-3 mRNA, whereas it decreased IGFBP-4 to 73 \(\pm\) 6\% of the control culture value and increased IGFBP-5 to 193 \(\pm\) 33\% above control cultures. Similar findings have been reported for dermal fibroblasts that do not possess androgen receptors \((19, 21)\). Testosterone increased the abundance of IGFBP-5 mRNA and had no effect on IGFBP-3 and -4. The combination of IGF-I + testosterone also suppressed IGFBP-4 mRNA, but the degree of change was not different compared to IGF-I alone. In contrast, the combination of testosterone and IGF-I had an additive effect on IGFBP-5 mRNA \((i.e. 333\% stimulation)\). This response was significantly greater than the response to IGF-I or testosterone alone \((P < 0.05)\). The combination of testosterone and IGF-I decreased IGFBP-3 mRNA significantly. Because IGFBP-3 fragments were not detected by immunoblotting (data not shown), the increase in IGFBP-3 in media seen with testosterone plus IGF-I is probably due to either enhanced secretion or release of IGFBP-3 from the cell surface.

To further analyze the changes that were occurring in IGFBP-4, two types of experiments were conducted (Fig. 3A). Exposure of the cultures to testosterone alone resulted in no change in the amount of intact IGFBP-4, and there was no change in the abundance of IGFBP-4 fragment as compared with control. In contrast, when IGF-I was incubated with the cultures, almost no intact protein was detected and two IGFBP-4 fragment bands were present. The combination of testosterone and IGF-I gave similar results. This experiment was repeated nine times, and the blots were analyzed by scanning densitometry. The results showed that there was a 67\% \(\pm\) 9\% \((P < 0.05)\) decrease in intact IGFBP-4 band intensity with IGF-I and a 39\% \(\pm\) 17\% \((P < 0.05)\) increase in the intensity of the IGFBP-4 fragment bands (Table 3). The combination of testosterone plus IGF-I also resulted in a decrease in intact IGFBP-4 and in an increase in the fragments, but these changes were not different from those induced by IGF-I alone.

Table 1. Changes in IGFBP peptide abundance in fibroblast-conditioned medium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of control</th>
<th>% of control</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IGFBP-3</td>
<td>Intact IGFBP-4</td>
<td>Intact IGFBP-5</td>
</tr>
<tr>
<td></td>
<td>((n = 9))</td>
<td>((n = 9))</td>
<td>((n = 9))</td>
</tr>
<tr>
<td>IGF-I (50 ng/mL)</td>
<td>212 (\pm) 28\a</td>
<td>63 (\pm) 12\a</td>
<td>415 (\pm) 86\a</td>
</tr>
<tr>
<td>Testosterone (10 nmol/L)</td>
<td>145 (\pm) 13\a</td>
<td>106 (\pm) 12</td>
<td>169 (\pm) 37</td>
</tr>
<tr>
<td>IGF-I + testosterone</td>
<td>336 (\pm) 39\a,b</td>
<td>63 (\pm) 6\a</td>
<td>483 (\pm) 103\a</td>
</tr>
</tbody>
</table>

The band intensities were analyzed by phosphorimager. The values represent the mean percent increase above control \(\pm\) SE of nine independent experiments.

\(\text{a} P < 0.05\) compared to values derived from the control cultures, by Student’s t test.

\(\text{b} P < 0.02\) compared to the cultures exposed to IGF-I or testosterone alone.

To determine whether the appearance of IGFBP-4 fragments was due to a protease that was released into the medium or if degradation was occurring at an intracellular level or on the cell surface, 24-h conditioned medium was obtained, and the protease activity analyzed in vitro. It has been shown in several cell types that an IGFBP-4 protease that is released into medium is active in vitro, particularly if IGF-I or IGF-II is added to the incubation medium \((22)\). However, when we analyzed degradation of IGFBP-4 by the conditioned medium obtained in the presence of IGF-I, there was no additional detectable degradation (Fig. 3B). Taken

Fig. 2. Northern blotting analysis of IGFBP abundance after testosterone IGF-I stimulation. Fibroblast cultures were stimulated with the treatments listed for 24 h, then total RNA was extracted and analyzed by Northern blotting, as described in Experimental Procedures. Lane 1, control; Lane 2, 50 ng/mL IGF-I; Lane 3, 10 nm testosterone; Lane 4, IGF-I + testosterone. The bands shown represent IGFBP-3, -4, and -5 mRNA, as noted. The abundance of GAPDH was also determined, and the results were used to normalize the level of each mRNA loaded. The experiment was repeated nine times with similar results.

Table 2. Changes in IGFBP mRNA abundance in fibroblasts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of control</th>
<th>% of control</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IGFBP-3</td>
<td>IGFBP-4</td>
<td>IGFBP-5</td>
</tr>
<tr>
<td></td>
<td>((n = 9))</td>
<td>((n = 9))</td>
<td>((n = 9))</td>
</tr>
<tr>
<td>IGF-I (50 ng/mL)</td>
<td>120 (\pm) 27</td>
<td>73 (\pm) 6\a</td>
<td>199 (\pm) 33\a</td>
</tr>
<tr>
<td>Testosterone (10 nmol/L)</td>
<td>118 (\pm) 16</td>
<td>101 (\pm) 7</td>
<td>227 (\pm) 22\a</td>
</tr>
<tr>
<td>IGF-I + testosterone</td>
<td>77 (\pm) 7\a</td>
<td>64 (\pm) 6\a</td>
<td>333 (\pm) 70\a,b</td>
</tr>
</tbody>
</table>

Values are the mean percentage increase above control \(\pm\) SE. n, Number of independent experiments.

\(\text{a} P < 0.05\) vs. control.

\(\text{b} P < 0.05\) vs. IGF-I or testosterone alone.
The results strongly suggest that degradation is occurring either intracellularly or on the cell surface.

To definitively identify the 30-kDa protein as IGFBP-5 and to further analyze the changes that occurred in IGFBP-5 peptide, conditioned medium was analyzed by immuno-blotting. As shown in Figure 4A and Table 4, IGF-I induced a large increase in intact IGFBP-5, whereas testosterone had no effect, and the combination did not have a greater effect than IGF-I alone. To determine whether changes in IGFBP-5 degradation accounted for part of these changes, heparin was added to the culture medium to prevent IGFBP-5 degradation. IGF-I increased the abundance of intact IGFBP-5, whereas testosterone had no effect, and the combination of testosterone + IGF-I was no greater than the effect of IGF-I alone (Figure 4B, Table 4). Although heparin was added at a concentration that effectively inhibits proteolysis (23), a 14-kDa fragment was still detected in the medium.

Because IGFBP-5 is highly concentrated in the ECM of fibroblasts, and since matrix-associated IGFBP-5 can control the growth response of these cells to IGF-I (20), we also analyzed the effects of androgen and IGF-I treatment on the abundance of IGFBP-5 in the ECM. As can be seen in Figure 5, under basal conditions after 24 h IGFBP-5 could easily be detected in the ECM. IGF-I treatment alone did not alter IGFBP-5 abundance at 24 h, but after a longer incubation period (e.g. 72 h), IGF-I or testosterone alone induced significant increase in the amount of IGFBP-5 that was detectable in the ECM compared to control, nonstimulated cultures. Testosterone alone and the combination of testosterone plus IGF-I induced significant increases over control cultures at 48 h (Table 5). Testosterone plus IGF-I progressively increased the amount of IGFBP-5 from 24 to 72 h. At the 72-h point, the effect of testosterone plus IGF-I was not greater than IGF-I alone.

To determine the effects of testosterone and IGF-I on the cellular anabolic response, the cultures were exposed to increasing concentrations of IGF-I or to increasing concentrations of testosterone in the presence and absence of IGF-I, and the protein synthesis response was measured (Figure 6). There was a dose-dependent increase in protein synthesis in response to increasing concentrations IGF-I between 25 and 100 ng/mL. The maximum increase was 34 ± 3%, P < 0.001, compared with control. In contrast, increasing concentrations of testosterone between 1 and 1000 nM resulted in no change in the absence of IGF-I. However, a response to testosterone was easily detected if 50 ng/mL of IGF-I was also present, and increasing concentrations of testosterone stimulated protein synthesis from 25–57% above the value obtained with testosterone alone. Comparison of the result obtained with concentrations of testosterone between 10–1000 nM plus IGF-I with the value obtained with 1 nM testosterone plus IGF-I showed that these concentrations induced significantly greater increases, P < 0.05.

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### Table 3. Changes in intact IGFBP-4 and fragments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intact IGFBP-4 (n = 8)</th>
<th>Two major BP-4 fragments (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Testosterone (10 nmol/L)</td>
<td>92 ± 6</td>
<td>60 ± 28</td>
</tr>
<tr>
<td>IGF-I (50 ng/mL)</td>
<td>43 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>226 ± 39&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T/IGF-I</td>
<td>23 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>219 ± 45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values are expressed as the mean ± SE percentage of intact or fragment IGFBP-4 band intensities in control cultures from eight separate experiments.

<sup>a</sup> P < 0.001 compared to either the intact band intensities in the control cultures.

<sup>b</sup> P < 0.05 compared to fragment band intensities in control cultures.
Discussion

This study clearly demonstrates that there is an interaction between androgens and IGF-I in controlling IGFBP-3 and IGFBP-5 synthesis or secretion and in stimulating total protein synthesis by fibroblasts. Specifically, the abundance of IGFBP-3, the most abundant form of IGFBP that is secreted by fibroblasts, in the conditioned medium was stimulated by both compounds, and they seemed to have an additive effect. The changes in IGFBP-3 seemed to be due to release from either the cell surface or an intracellular compartment, rather than increased synthesis, because neither IGF-I nor testosterone increased IGFBP-3 mRNA abundance.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of control</th>
<th>Intact IGFBP-5 (n = 5)</th>
<th>Two major IGFBP-5 fragments (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>IGF-I (50 ng/mL)</td>
<td>298 ± 32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>174 ± 36</td>
<td></td>
</tr>
<tr>
<td>Testosterone (10 nmol/L)</td>
<td>109 ± 14</td>
<td>112 ± 15</td>
<td></td>
</tr>
<tr>
<td>T/IGF-I</td>
<td>309 ± 15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171 ± 40</td>
<td></td>
</tr>
</tbody>
</table>

The values are expressed as the mean ± SE percent of intact or the two major IGFBP-5 fragment band intensities in control culture medium from five separate experiments. The values in the lower part of the table were obtained from cultures that were exposed to the same treatments, except that they also contained heparin (100 µg/mL).

<sup>a</sup> P < 0.001 compared to the intact band intensities in the control cultures.

The changes in IGFBP-4 were complex. Specifically, IGFBP-4 mRNA was suppressed by IGF-I, but the abundance of an IGFBP-4 fragment in the media was also increased. The fragment was probably generated by an intracellular or plasma membrane-associated protease, since the medium did not contain IGFBP-4 proteolytic activity. There was an increase in the amount of fragment after exposure of the cells to IGF-I, but there is no direct evidence that IGF-I stimulated protease activity. Testosterone had no effect on IGFBP-4 mRNA and resulted in no change in intact peptide. IGF-I plus testosterone also decreased both IGFBP-4 mRNA and peptide abundance, but the effect was not greater than IGF-I alone. These results suggest that the decrease in IGFBP-4 that is induced by exposure to IGF-I is due to both decreased synthesis and enhanced degradation.

IGFBP-5 was regulated differently than IGFBP-3 or -4. Both testosterone and IGF-I increased its mRNA abundance, and their effects were additive. When immunoblotting was used to detect intact peptide, an increase could be detected in the conditioned medium with IGF-I alone or testosterone plus IGF-I. Testosterone alone had no effect. Exposure to IGF-I probably resulted in stimulation of IGFBP-5 synthesis, since steady-state IGFBP-5 mRNA levels were increased, as were intact IGFBP-5 peptide levels. In contrast to IGF-I, the testosterone-induced increase in IGFBP-5 mRNA abundance
TABLE 5. Changes in IGFBP-5 abundance in ECM

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% change 24 h (n = 4)</th>
<th>% change 48 h (n = 4)</th>
<th>% change 72 h (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (10 nmol/L)</td>
<td>-10 ± 1*</td>
<td>+17 ± 3*</td>
<td>+27 ± 2*</td>
</tr>
<tr>
<td>IGF-I (50 ng/mL)</td>
<td>-3 ± 7</td>
<td>+27 ± 12</td>
<td>+41 ± 3*</td>
</tr>
<tr>
<td>T/IGF-I</td>
<td>+8 ± 3*</td>
<td>+29 ± 9*</td>
<td>+44 ± 3*</td>
</tr>
</tbody>
</table>

The amount of IGFBP-5 in ECM was quantified using NIH Image after scanning the autoradiographs. n, Number of experiments. The results are expressed as the mean percent change ± SE compared to control cultures that were exposed to serum-free medium.

* P < 0.01 compared to control cultures.

** P < 0.05 compared to control cultures.

** P < 0.05 compared to control cultures.

The most interesting finding of this study was true synergism in the stimulation of protein synthesis by fibroblasts in response to testosterone/IGF-I. Increasing concentrations of testosterone up to 1000 nm had no effect on the protein synthesis response of these cells, but if IGF-I was added a testosterone concentration as low as 10 nm stimulated protein synthesis. Furthermore, the maximum effect that could be obtained with IGF-I was potentiated by testosterone, indicating a true synergism in the protein synthesis response. Dykstra et al. (25) had previously reported that IGF-I plus testosterone increased intracellular protein content in foreskin fibroblasts. However, they did not analyze the response to testosterone alone. Because the effects of testosterone and IGF-I on IGFBP-5 mRNA abundance and on IGFBP-3 peptide were additive, it is probable that these responses are related to the protein synthesis response of the cells. We have previously shown that enriching the fibroblast ECM in IGFBP-5 results in a potentiation of the fibroblast mitogenic response to IGF-I (20). Therefore, our finding of association between an enhancement of protein synthesis and the amount of IGFBP-5 in the ECM in these cells also suggests that it may have contributed to potentiation of the anabolic response.

The additive or synergistic responses between IGF-I and androgen suggests that there may be points in the IGF signal transduction pathway that may be influenced by activation of androgen receptor pathway. Specific genes whose transcription is activated in response to IGF-I include elastin (26–28), IGFBP-5 (29), crystallin (30), and a cholesterol sidechain cleavage enzyme (31). It will be important in future studies to determine whether increased IGFBP-5 synthesis in response to androgen is the result of a direct increase in transcription. Future studies should be directed toward determining whether the androgen/androgen receptor complex interacts with this 5’ flanking regulatory sequence in the IGFBP-5 promoter and whether or not this accounts for the ability of androgens to potentiate IGF-I-stimulated synthesis of this protein. The extent to which androgen receptor activity and IGFBP/IGF receptor occupancy are required for maximum stimulation of the protein synthesis in this cell type is also worthy of further study, as is understanding the point at which the signal transduction pathways interact, and the genes that are coregulated by both stimuli that result in an enhanced protein synthesis response.
Acknowledgments

We gratefully acknowledge the assistance of Mr. George Mosley in preparing this manuscript.

References


