Endogenous IGF-I regulates IGF binding protein production in human intestinal smooth muscle cells

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Kuemmerle, John F. Endogenous IGF-I regulates IGF binding protein production in human intestinal smooth muscle cells. Am J Physiol Gastrointest Liver Physiol 278: G710–G717, 2000.—Human intestinal smooth muscle in culture produces insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3, IGFBP-4, and IGFBP-5, which modulate the effects of IGF-I. This study examined the regulation of IGFBP production by endogenous IGF-I. R3-IGF-I, an agonist unaffected by IGFBPs, elicited concentration-dependent increase in growth, measured by [3H]thymidine incorporation, and production of IGFBP-3, IGFBP-4, and IGFBP-5, measured by Western blot. Antagonists of the IGF-I receptor, IGF-I Analog or monoclonal antibody 1H7, elicited concentration-dependent inhibition of growth and decrease in IGFBP-3, IGFBP-4, and IGFBP-5 production, implying that endogenous IGF-I stimulated growth and IGFBP production. R3-IGF-I increased the production of IGFBP-3, IGFBP-4, and IGFBP-5 production was partially inhibited by a mitogen-activated protein (MAP) kinase or a phosphatidylinositol-3-kinase (PI 3-kinase) inhibitor and abolished by the combination. We conclude that endogenous IGF-I stimulates growth and IGFBP-3, IGFBP-4, and IGFBP-5 production in human intestinal smooth muscle cells. Regulation of IGFBP production by IGF-I is mediated by activation of distinct MAP kinase and PI 3-kinase pathways, the same pathways through which IGF-I stimulates growth.

The interplay between IGF-I and IGFBPs is bidirectional. Not only do IGFBPs modulate IGF-I-induced growth, two mechanisms by which IGF-I can regulate IGFBP levels have been identified. Specific proteolytic mechanisms for IGFBP-2, -3, -4, and -5 degradation have been identified that are regulated by IGF-I (5, 6, 21, 23). In addition, IGF-I can regulate directly the production of IGFBPs (6, 21). Regulation of IGFBP production by endogenous IGF-I has been examined in A549 adenocarcinoma cells (21), in aortic smooth muscle cells (5), and in osteoblasts (6). In A549 cells, endogenous IGF-I stimulated IGFBP-3 production. In porcine aortic smooth muscle cells, endogenous IGF-I increased IGFBP-5 production and decreased IGFBP-4 levels. The effect of IGF-I on growth, therefore, is determined by its direct effects and indirectly by its effects on IGFBP levels.

Within the gastrointestinal tract, IGF-I and IGFBP-4 and IGFBP-5 are expressed by smooth muscle cells of the muscularis propria in rat colon and IGF-I and IGFBP-3, IGFBP-4, and IGFBP-5 are expressed by smooth muscle cells cultured from the human intestine and rat colon (3, 12, 15a, 27–29). The importance of endogenous IGF-I in regulating smooth muscle cell growth is highlighted by the increase in size of the muscularis propria in transgenic mice overexpressing a human IGF-I cDNA (19). In cultures of human intestinal muscle cells, IGF-I-induced growth is inhibited by IGFBP-3 and IGFBP-4 and augmented by IGFBP-5 (3, 15). Growth of human intestinal muscle cells induced by IGF-I is mediated jointly by activation of two major intracellular signaling cascades, the Raf/mitogen-activated protein (MAP) kinase pathway and the phosphatidylinositol 3-kinase (PI 3-kinase) pathway (14).

The regulation of IGFBP-5 production by IGF-I has been examined in cultures of muscle cells isolated from the rat colon. In these cells, addition of exogenous IGF-I increased IGFBP-5 mRNA and protein production (29). The role of endogenous IGF-I in regulating IGFBP-3, IGFBP-4, and IGFBP-5 production in human intestinal muscle and the intracellular mechanisms mediating these effects in human intestinal smooth muscle have not previously been examined.

In the present study, the role of endogenous IGF-I in regulation of IGFBP-3, IGFBP-4, and IGFBP-5 production was examined in smooth muscle cells cultured from the human intestine. The effects of endogenous IGF-I were identified using the IGF-I receptor antagonist IGF-I Analog and the inhibitory IGF-I receptor...
METHODS

Preparation and culture of human intestinal muscle cells. Cells from normal human jejunum were isolated and cultured as described previously (3, 12, 14) from surgical specimens obtained by a protocol approved by the Institutional Committee on the Conduct of Human Research. Tissue slices from the circular muscle layer were digested in a medium consisting of (mM) 120 NaCl, 4 KCl, 2.6 KH2PO4, 2 CaCl2, 0.6 MgCl2, 25 HEPES, and 14 glucose and 2.1% Eagle’s essential amino acid mixture with 0.2% collagenase (CLS type II) and 0.1% soybean trypsin inhibitor. Partially digested muscle was dispersed in enzyme-free medium. Muscle cells were harvested by filtration (500-µm Nitex). The cells were washed twice by centrifugation for 5 min at 150 g and resuspended in Hanks’ medium containing 200 U/ml penicillin, 200 µg/ml streptomycin, 100 µg/ml gentamicin, and 2 µg/ml amphotericin B. Muscle cells were resuspended in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (DMEM-10) and antibiotics and plated at a density of 5 × 10^5 cells/ml. The medium was replaced every 3 days. Once confluence was attained, primary cultures were passed and seeded at a density of 2.5 × 10^6 cells/ml. All subsequent studies were performed on first-passage cultured cells on day 7 of culture, at which time the cells attained confluence. We have previously shown (12, 25) that these cells express a phenotype characteristic of intestinal smooth muscle as determined by immunostaining for smooth muscle markers and expression of γ-enteric actin. Endothelial cells, neurons, and interstitial cells of Cajal are not detected in these cultures (25).

Preparation of conditioned medium. Conditioned medium was prepared as described previously from confluent muscle cells for measurement of soluble IGFBPs and IGF-II (3). The cells were washed and incubated in serum-free DMEM for 48 h. Conditioned medium was used to measure IGF-II as described below. The effects of R3-IGF-I, an IGF-I receptor agonist (8), were examined by addition of increasing concentrations (1–10 nM) of the agonist during the 48-h period. The effects of an antagonist of the IGF-I receptor, IGF-I Analog (20), were examined by addition of increasing concentrations (1–1,000 pM) of the antagonist during the 48-h period. After removal of the supernatant after acid-ethanol extraction to remove bound IGFBPs as described previously (12, 14). Briefly, aliquots of conditioned medium were added to an acid-ethanol mixture (87.5% ethanol-12.5% (vol/vol) hydrochloric acid), vortexed, and centrifuged. A 100-µl aliquot of the resulting supernatant was neutralized with 40 µl of Tris (0.855 M), incubated at 4°C for 2 h, and recentrifuged. IGF-I was measured in the resulting supernatant by radiomunoassay using a polyclonal antibody raised in rabbits against human IGF-II (33–40). This antibody reacts fully with human IGF-II but has no cross-reactivity with IGF-I or insulin. The limit of detection was 1 pg/tube, and the I_C50 was 22 pg/tube.

Measurement of IGFBP production by Western blot analysis. IGFBP production was measured by Western blot analogous to conditioned medium or whole cell lysates were added to loading buffer [62.5 mM Tris (pH 6.8) with 2% SDS, 25% glycerol, 0.01% bromophenol blue and 5% β-mercaptoethanol] to provide samples derived from equal amounts of total cellular protein (conditioned medium: 200 µg/30 µl, cell lysates: 30 µg/µl). Proteins separated on 12% polyacrylamide gels were electrotransferred to nitrocellulose membranes in 25 mM Tris (pH 8.3), 192 mM glycine, with added 20% methanol and 0.1% SDS. Membranes were incubated overnight at 4°C with a 1:10,000 dilution of an antibody to IGFBP-3, IGFBP-4, or IGFBP-5, each displaying <1% cross-reactivity with other IGFBPs. Membranes were incubated in a 1:1,000 dilution of goat anti-rabbit IgG-horseradish peroxidase conjugate. Bands were visualized with chemiluminescence and quantitated by densitometry.

[3H]thymidine incorporation assay. Growth of cultured muscle cells was measured by [3H] thymidine incorporation as described previously (12, 14). Cells were incubated for 48 h in serum-free DMEM with R3-IGF-I (0.10–100 nM) in the presence or absence of the IGF-I receptor antagonist (1 µM) or for 24 h with antagonist alone (1–1,000 pM). [3H] thymidine (1 µCi/ml) was added for the final 4 h, and its incorporation was measured in the perchloric acid-extractable pools using 2 N perchloric acid (30 min at 60°C). After solubilization, protein in the cell residue was measured using the BioRad protein reagent. [3H] thymidine incorporation was expressed as counts per minute per microgram of protein.

Measurement of IGF-II. IGF-II was measured in supernatants after acid-ethanol extraction to remove bound IGFBPs as described previously (12). Briefly, aliquots of conditioned medium were added to an acid-ethanol mixture (87.5% ethanol-12.5% (vol/vol) hydrochloric acid), vortexed, and centrifuged. A 100-µl aliquot of the resulting supernatant was neutralized with 40 µl of Tris (0.855 M), incubated at 4°C for 2 h, and recentrifuged. IGF-I was measured in the resulting supernatant by radiomunoassay using a polyclonal antibody raised in rabbits against human IGF-II (33–40). This antibody reacts fully with human IGF-II but has no cross-reactivity with IGF-I or insulin. The limit of detection was 1 pg/tube, and the I_C50 was 22 pg/tube.

Statistical analysis. Values represent means ± SE of n experiments, where n represents the number of experiments on cells derived from separate primary cultures. Statistical significance was tested by Student’s t-test for either paired or unpaired data. Protein bands were measured with computerized densitometry, and results are reported in arbitrary units above background.

Materials. Collagenase and soybean trypsin inhibitor were obtained from Worthington Biochemical (Freehold, NJ); [3H] thymidine (6 Ci/mmol) was obtained from NEN (Boston, MA); DMEM and Hanks’ medium were obtained from Mediatech (Herndon, VA); fetal bovine serum was obtained from BioWhittaker (Walkersville, MD); protein assay reagent was obtained from BioRad Laboratories (Hercules, CA); R3-IGF-I and rabbit polyclonal antibodies to IGFBP-3, IGFBP-4, and IGFBP-5 were obtained from Upstate Biotechnology (Lake Placid, NY); IGF-I Analog was obtained from Bachem (Torrance, CA); mouse monoclonal antibody to the IGF-I receptor α-subunit, 1H7, was obtained from Santa Cruz Biotechnology (Santa Cruz, CA); IGF-I radiomunoassay kit was obtained from Peninsula Laboratories (San Carlos, CA); and plastic cultureware was obtained from Corning (Corning, NY). All other chemicals were obtained from Sigma (St Louis, MO).
RESULTS

Characterization of IGF-I Analog as an IGF-I receptor antagonist in human intestinal muscle. IGF-I Analog was used as an IGF-I receptor antagonist (20). The ability of IGF-I Analog to act as an antagonist of IGF-I receptor activation was confirmed by its ability to inhibit growth stimulated by R3-IGF-I. R3-IGF-I, an Arg3-substituted IGF-I derivative, was chosen as an agonist because of its greatly reduced affinity, ~1,000-fold, for IGFBPs compared with native IGF-I and also because it does not bind to the IGF-II/mannose 6-phosphate receptor (8). This eliminated the modulatory effects of IGFBPs on IGF-I-induced effects, the effects of IGF-I on degradation of IGFBPs, and possible effects mediated by activation of the IGF-II/mannose 6-phosphate receptor (8). Incubation of confluent human intestinal muscle cells with R3-IGF-I (0.1–100 nM) increased [3H]thymidine incorporation in a concentration-dependent fashion (Fig. 1). The increase was significantly greater than that elicited by native IGF-I (Fig. 1) at all concentrations tested, implying that IGFBPs modulate the interaction of IGF-I with the IGF-I receptor. The increase in [3H]thymidine incorporation elicited by R3-IGF-I was significantly inhibited at all concentrations tested (P < 0.05) in the presence of 1 µM IGF-I receptor antagonist (Fig. 1).

We have previously shown (12) that endogenous IGF-I stimulates growth in rapidly proliferating cells on day 3 of culture. The effects of endogenous IGF-I to stimulate growth of confluent cells on day 7 of culture and the ability of the IGF-I receptor antagonist to inhibit the effects of endogenous IGF-I on growth were also confirmed. Confluent muscle cell cultures were incubated with increasing concentrations of the IGF-I antagonist (1–1,000 pM). The IGF-I antagonist inhibited basal [3H]thymidine incorporation in a concentration-dependent fashion (53 ± 8% inhibition with 1 nM, P < 0.01; Fig. 2). The results implied the presence of an autocrine mechanism whereby endogenous IGF-I stimulated growth of confluent muscle cells in similar fashion as in rapidly growing cells.

To determine whether IGF-II is produced by these cells, IGF-II production was measured in medium conditioned by confluent muscle cells by radioimmunoassay. Immunoassayable IGF-II was detected at low levels (17.3 ± 3.8 pM). IGF-II stimulated [3H]thymidine incorporation in a concentration-dependent fashion with a threshold of 100 pM (14 ± 4% above basal, P < 0.05) and with maximum increase elicited by 10 nM IGF-II (82 ± 19% above basal, P < 0.01). The increase in [3H]thymidine incorporation elicited by IGF-II was not altered in the presence of the IGF-I receptor antagonist (1 µM) at any concentration tested.

The specificity of the IGF-I antagonist for the IGF-I receptor is implied by the ability of the antagonist to inhibit the effects of IGF-I but not IGF-II. Moreover, the low levels of IGF-II production by human intestinal muscle cells suggest that endogenous IGF-II is unlikely to stimulate growth of muscle cells.

R3-IGF-I augments IGFBP production. The role of IGF-I in the regulation of IGFBP production was investigated by measurements of IGFBPs in both conditioned medium (soluble binding protein) and in whole cell lysates (cell-associated/extracellular matrix-assoc-
ated binding protein). Cultures of confluent muscle cells were incubated for 48 h with R3-IGF-I (1 and 10 nM), and the levels of IGFBP-3, IGFBP-4, and IGFBP-5 production were measured by Western blotting with densitometric analysis.

The production of soluble IGFBP-3 was augmented by R3-IGF-I in a concentration-dependent fashion (162 ± 56% increase above basal at 10 nM, *P* < 0.01; Fig. 3). This was paralleled by a concomitant concentration-dependent decrease in soluble IGFBP-5 production. In the presence of 1 nM IGF-I receptor antagonist, basal IGFBP-5 production was inhibited 36 ± 14% (*P* < 0.05; Fig. 7).

A pattern similar to that of soluble IGFBP-3 and IGFBP-5 was observed for the levels of cell-associated IGFBP-3 and IGFBP-5. Levels of cell-associated IGFBP-3 decreased in a concentration-dependent fashion (28 ± 8% decrease from basal levels with 10 nM IGF-I receptor antagonist, *P* < 0.05; Fig. 7).

Analysis of the effects of endogenous IGF-I on IGFBP-4 production using the IGF-I antagonist was}

**Fig. 3. IGF-I receptor activation stimulates IGF binding protein (IGFBP)-3 production.** A: representative Western blot showing increase in IGFBP-3 induced by R3-IGF-I. B: densitometric analysis of IGFBP-3 protein levels. R3-IGF-I induced concentration-dependent increase in levels of both soluble and cell-associated IGFBP-3. Results are expressed as a percent increase in band density (42/44 kDa) above basal levels in arbitrary units. Values represent means ± SE of 5 separate experiments. *P* < 0.05, **P** < 0.01 vs. basal levels.

**Fig. 4. IGF-I receptor activation stimulates IGFBP-4 production.** A: representative Western blot showing increase in IGFBP-4 induced by R3-IGF-I. B: densitometric analysis of IGFBP-4 protein levels. R3-IGF-I induced concentration-dependent increase in levels of soluble IGFBP-4. Results are expressed as a percent increase in band density (24 kDa) above basal levels in arbitrary units. Values represent means ± SE of 5 separate experiments. *P* < 0.05 vs. basal levels.
not possible because we have previously shown (12) that the IGF-I antagonist also inhibits IGF-I-activated proteolysis of IGFBP-4 in human intestinal muscle cells. Therefore, the effects of endogenous IGF-I on IGFBP-4 production were determined using a monoclonal antibody, 1H7, that inhibits activation of the IGF-I receptor by binding to the receptor α-subunit (16). The production of IGFBP-4 was inhibited in a concentration-dependent fashion in the presence of increasing concentrations of 1H7, with a 50 ± 4% decrease in IGFBP-4 production in the presence of 100 nM 1H7 (P < 0.01; Fig. 8).

Inhibition of basal production of IGFBP-3 and IGFBP-5 in the presence of the IGF-I receptor antagonist and of IGFBP-4 in the presence of the inhibitory antibody 1H7 implied that an autocrine mechanism involving endogenous IGF-I was responsible in part for the regulation of IGFBP production.

IGF-I-mediated IGFBP production is MAP kinase dependent and PI 3-kinase dependent. We have previously shown (14) that IGF-I activates distinct MAP kinase-dependent, PI 3-kinase-independent and MAP kinase-independent, PI 3-kinase-dependent pathways that are coupled to growth. To determine whether the effects of IGF-I on IGFBP production were mediated by similar intracellular mechanisms, confluent muscle cells were incubated with R3-IGF-I in the presence of either the MAP kinase kinase (MEK) inhibitor (1) PD-98059 (10 µM), the PI 3-kinase inhibitor (26) LY-294002 (10 µM), or a combination of the two inhibitors. At the concentrations used, these inhibitors specifically abolish the activation of MAP kinase or PI 3-kinase without affecting activation of the other kinase (1, 14, 26).

The increase in soluble IGFBP-3 production elicited by 10 nM R3-IGF-I was partially inhibited by either the PI 3-kinase inhibitor (48 ± 9% inhibition, P < 0.01) or the MEK inhibitor (57 ± 7% inhibition, P < 0.01) and abolished by the combination of the two inhibitors (92 ± 12% inhibition, P < 0.01) (Fig. 9). The production of soluble IGFBP-4 elicited by 10 nM R3-IGF-I was also partially inhibited by either the PI 3-kinase inhibitor (51 ± 7% inhibition, P < 0.01) or the MEK inhibitor (48 ± 9% inhibition, P < 0.01) and was strongly inhibited by the combination of the two inhibitors (76 ± 9% inhibition, P < 0.01) (Fig. 8). A pattern similar to that of IGFBP-3 was observed for the production of soluble IGFBP-5 elicited by 10 nM R3-IGF-I; it was partially inhibited by either the PI 3-kinase inhibitor (77 ± 11% inhibition, P < 0.01) or the MEK inhibitor (68 ± 18% inhibition, P < 0.01) and was abolished by the combination of the two inhibitors (93 ± 5% inhibition, P < 0.01) (Fig. 8). The pattern of inhibition of IGFBP-3, IGFBP-4, and IGFBP-5 production by the selective PI 3-kinase and MEK inhibitors and the additive effects of the two inhibitors implied that the increase in IGFBP production was mediated by activa-

![Fig. 5. IGF-I receptor activation stimulates IGFBP-5 production. A: representative Western blot showing increase in IGFBP-5 induced by R3-IGF-I. B: densitometric analysis of IGFBP-5 protein levels. R3-IGF-I induced concentration-dependent increase in levels of both soluble and cell-associated IGFBP-5. Results are expressed as percent increase in band density (31 kDa) above basal levels in arbitrary units. Values represent means ± SE of 5 separate experiments. *P < 0.05, **P < 0.01 vs. basal levels.](http://ajpgi.physiology.org/doi/10.1152/ajpgi.00714.2017)

![Fig. 6. Endogenous IGF-I stimulates IGFBP-3 production. A: representative Western blot showing decrease in soluble IGFBP-3 in response to IGF-I antagonist. B: densitometric analysis of IGFBP-3 protein levels. IGF-I antagonist induced concentration-dependent decrease in levels of both soluble (○) and cell-associated (●) IGFBP-3. Results are expressed as percent increase in band density (42/44 kDa) above basal levels in arbitrary units. Values represent means ± SE of 5 separate experiments. *P < 0.05 vs. basal levels.](http://ajpgi.physiology.org/doi/10.1152/ajpgi.00714.2017)
tation of two distinct pathways, a MAP kinase-dependent, PI 3-kinase-independent pathway and a MAP kinase-independent, PI 3-kinase-dependent pathway.

DISCUSSION

This study shows that both growth and the production of IGFBP-3, IGFBP-4, and IGFBP-5 are regulated by endogenous IGF-I in confluent cultures of human intestinal muscle cells. The regulation of IGFBP production by IGF-I occurs by activation of the same intracellular mechanisms previously shown to mediate IGF-I-induced growth and activation of distinct MAP kinase-dependent, PI 3-kinase-independent and MAP kinase-independent, PI 3-kinase-dependent pathways (14).

The evidence supporting a direct role for IGF-I in growth of confluent cultures of human intestinal muscle cells can be summarized as follows: 1) R3-IGF-I elicited concentration-dependent proliferation; 2) proliferation induced by R3-IGF-I was inhibited by the IGF-I receptor antagonist; and 3) IGF-I receptor antagonist inhibited basal proliferation in a concentration-dependent manner. These results implied that endogenous IGF-I stimulates growth in confluent human intestinal muscle cells.

The evidence supporting a direct role for IGF-I in the regulation of IGFBP production by human intestinal smooth muscle cells in culture can be summarized as

Fig. 7. Endogenous IGF-I stimulates IGFBP-5 production. A: representative Western blot showing decrease in soluble IGFBP-5 in response to IGF-I antagonist. B: densitometric analysis of IGFBP-5 protein levels (○, soluble IGFBP-5; ●, cell-associated IGFBP-5). IGF-I antagonist induced concentration-dependent decrease in levels of both soluble and cell-associated IGFBP-5. Results are expressed as percent increase in band density above basal levels in arbitrary units. Values represent means ± SE of 5 separate experiments. *P < 0.05 vs. basal levels.

Fig. 8. Endogenous IGF-I stimulates IGFBP-4 production. A: representative Western blot showing decrease in soluble IGFBP-4 in response to inhibitory antibody 1H7 (IGF-IRα antibody). B: densitometric analysis of soluble IGFBP-4 protein levels. Inhibitory antibody 1H7 induced concentration-dependent decrease in levels of soluble IGFBP-4. Results are expressed as percent decrease in band density above basal levels in arbitrary units. Values represent means ± SE of 3 separate experiments. *P < 0.05, **P < 0.01 vs. basal levels.

Fig. 9. Mitogen-activated protein (MAP) kinase and phosphatidylinositol 3-kinase (PI 3-kinase) mediate IGF-I-induced stimulation of IGFBP-3, IGFBP-4, and IGFBP-5 production. The increase in soluble IGFBP-3, soluble IGFBP-4, and soluble IGFBP-5 elicited by a R3-IGF-I (10 nM) was partially inhibited by MAP kinase inhibitor PD-98059 (10 µM), and abolished or strongly inhibited by combination of the 2 inhibitors. Results are expressed as a percentage of 10 nM R3-IGF-I-stimulated IGFBP-3, IGFBP-4, and IGFBP-5 levels. Values represent means ± SE of 4 separate experiments. **P < 0.01 vs. control levels (10 nM R3-IGF-I).
follows: 1) R3-IGF-I elicited a concentration-dependent increase in both soluble and cell-associated IGFBP-3 and IGFBP-5 production and in soluble IGFBP-4 production; 2) the IGF-I receptor antagonist elicited a concentration-dependent decrease in basal soluble and cell-associated IGFBP-3 and IGFBP-5 production; and 3) an antibody inhibiting IGF-I receptor activation elicited a concentration-dependent decrease in basal soluble IGFBP-4 production. The results implied the presence of an autocrine mechanism whereby endogenous IGF-I regulates IGFBP levels.

The regulation of IGFBP production by IGF-I has been investigated in a number of smooth muscle cell types including vascular and visceral smooth muscle (5, 10, 21, 29). Mechanisms of IGF-I-dependent regulation of IGFBP gene transcription, IGFBP mRNA stability, translation, and posttranslational processing have been identified depending on the specific IGFBP. In rat aortic smooth muscle, activation of the IGF-I receptor elicits an increase in IGFBP-3 and IGFBP-4 protein levels without concomitant changes in either IGFBP-3 or IGFBP-4 mRNA levels (10). In rat colonic smooth muscle IGF-I increases IGFBP-5 expression and production (29). In many cell types, IGFBP-4 levels are influenced by proteolysis. In previous studies we have shown that in human intestinal muscle IGF-I regulates IGFBP-4 levels through an IGF-I-activated proteolytic mechanism. Changes in IGFBP-4 levels were not caused by IGF-I-induced changes in IGFBP-4 mRNA levels (15). The focus of the current study is on the regulation of IGFBP production. Transcriptional activity and mRNA stability were therefore not examined. We have previously shown (14), however, that maintenance of basal IGFBP levels was dependent on continual new protein synthesis.

The intracellular signal transduction cascades that are activated by IGF-I and mediate IGF-I-induced regulation of IGFBP production have not previously been identified in intestinal smooth muscle. Two signaling cascades activated by IGF-I and coupled to stimulation of growth of human intestinal muscle cells are the Raf/MAP kinase and the PI 3-kinase pathways (14). Both pathways contribute additively to IGF-I-induced growth. Similar mechanisms are involved also for the regulation of IGFBP-3, IGFBP-4, and IGFBP-5 production. IGF-I activates both the MAP kinase pathway and the PI 3-kinase pathway, which contribute additively to increased production of IGFBP-3, IGFBP-4, and IGFBP-5 in these cells.

Growth of human intestinal muscle in culture is determined by the interplay of at least two autocrine growth factors, IGF-I and transforming growth factor-β1 (TGF-β1), and is modulated by IGFBP-3, IGFBP-4, and IGFBP-5, which are also produced by the cells (3, 12–14). The production of IGF-I and TGF-β1 is time dependent and reciprocal in culture. IGF-I levels are high during the initial period of rapid growth and decrease as cells attain confluence. In contrast, TGF-β1 levels are low initially and increase when the cells reach confluence. IGFBP-5, which attains its highest levels during the initial period of rapid growth concurrently with IGF-I, augments IGF-I-induced growth, whereas IGFBP-3, which attains its highest levels concurrently with TGF-β1, inhibits IGF-I-induced growth. IGFBP-4 inhibits IGF-I-induced growth. IGFBP levels, therefore, are regulated by the interplay of several simultaneous processes that vary during growth in culture, IGF-I- and TGF-β1-regulated production, and IGF-I-regulated proteolysis. Integration of these regulatory stimuli determines the high levels of growth-promoting IGFBP-5 and low levels of growth-inhibiting IGFBP-3 during the period of rapid growth and the progressively diminishing levels of IGFBP-5 and increasing levels of IGFBP-3 as cells attain confluence. The interplay between IGF-I, TGF-β1, and IGFBP production integrates, in part, growth of human intestinal muscle from a proliferating to a confluent contractile state.

The importance of IGF-I in development of the intestine is exemplified by the increased size of the muscularis propria in transgenic mice overexpressing a human IGF-I cDNA (19) and in suckling rats infused with either IGF-I or R3-IGF-I (24). The observations that IGF-I and IGFBP-5 expression is increased in the intestine of patients with Crohn’s disease (15a) and in rat models of enterocolitis (27–29) suggest that the IGF-I system may participate in the response of the intestine to inflammation. In both cases, IGF-I mRNA expression was increased in the intestine and IGFBP-5 expression was increased specifically within the muscularis propria (28, 29).

In summary, this study shows that endogenous IGF-I stimulates the production of IGFBP-3, IGFBP-4, and IGFBP-5 in cultures of human intestinal smooth muscle cells. We have previously shown (12) that ambient endogenous IGF-I levels in confluent muscle cells are 29.7 ± 7.7 nM, which is within the range examined in these studies. This process is regulated by activation of distinct MAP kinase-dependent and PI 3-kinase-dependent pathways, the same pathways mediating the growth effects of IGF-I in human intestinal smooth muscle cells.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-49691.

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Received 8 August 1999; accepted in final form 6 November 1999.

REFERENCES


