Proinsulin stimulates growth of small intestinal crypt-like cells acting via specific receptors

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Jehle, Peter M., Rolf D. Fussgaenger, Niklas K. O. Angelus, Robert J. Jungwirth, Bernhard Saile, and Manfred P. Lutz. Proinsulin stimulates growth of small intestinal crypt-like cells acting via specific receptors. Am J Physiol. 276 (Endocrinol. Metab. 39): E262–E268, 1999.—The mechanisms that regulate cell turnover in the intestinal epithelium are incompletely understood. Here we tested the hypothesis that proinsulin, present in serum and pancreatic juice in picomolar concentrations, stimulates growth of the rat small intestinal crypt-like cell line IEC-6 under serum-free conditions. Proinsulin binding was assessed by competitive ligand binding studies. Proinsulin and insulin-like growth factor I (IGF-I) stimulated cell proliferation up to threefold above controls, with half-maximal action already in the picomolar range and with additive effects. In early confluent cell monolayers, proinsulin bound with higher affinity (IC50 1.3 ± 0.05 nM) and capacity (87,200 ± 2,500 receptors/cell) than IGF-I (4.0 ± 0.6; 23,700 ± 2,200, P < 0.05). C-peptide competed with 10-fold lower affinity for binding of 125I-proinsulin but not for 125I-IGF-I or 125I-insulin, suggesting a specific binding epitope of the proinsulin molecule within or close to the C-peptide region. In contrast, insulin showed ~100-fold lower binding affinity and growth-promoting potency than proinsulin or IGF-I. We conclude that proinsulin stimulates growth of small intestinal crypt cells through specific binding and may play a physiological role in the regulation of intestinal epithelial cell proliferation.

cell growth; C-peptide; insulin-like growth factors

EPITHELIAL CELLS of the small intestine represent one of the most rapidly dividing cell populations, with a turnover of ~72 h. Within this system, mitotically active cells are located in the crypts and migrate to the villus tip while differentiating into functionally mature absorptive cells (6). The local and humoral factors that control the precisely balanced ratio of proliferating and differentiating intestinal epithelial cells are not characterized completely. In human and in rodent species, the insulin-like growth factor (IGF) system is thought to be the most important growth regulator (15, 19–22, 24, 25, 31, 32). Experiments separating proliferating crypt cells from mature villus cells in rat gastrointestinal epithelium showed a gradient of IGF receptors along the crypt-villus axis, with the highest expression in crypt cells (15). The brush-border location of IGF receptors suggests that IGFs in the intestinal lumen may initiate signal transduction events by binding to receptors on the luminal plasma membrane (4). Luminal IGFs may be supplied by apical secretion from epithelial cells or by other sources such as pancreatic juice, saliva, or bile (22). In addition, infusion of IGF-I improves mucosal structure and function after small bowel transplantation and thus may gain therapeutic importance (33).

Proinsulin, the precursor of insulin, has high homology in amino acid sequence and three-dimensional structure to insulin and IGF-I (27). Although proinsulin is present in concentrations between 10 and 40 pM in pancreatic and duodenal juice (17) and therefore may act in a similar manner to IGF-I, there is no study investigating its biological effect on intestinal epithelial cell growth. All members of the IGF family exert a comparable spectrum of biological activities through interactions with distinct cell surface receptors. Distinct receptors for both IGF-I and insulin have been differentiated on the basis of relative ligand affinity and have been confirmed by protein sequence and structural analysis (8). The affinity of the insulin receptor for insulin is two orders of magnitude higher than for IGF-I. On the other hand, the IGF-I receptor binds IGF-I with 100-fold higher affinity than insulin. Proinsulin cross-reacts with 10- to 100-fold lower affinity at the insulin or IGF-I receptor in hepatocytes and adipocytes (3, 23). In addition to the above receptors, proinsulin-specific binding and action have been observed in several cell types. Proinsulin is one of the growth factors expressed in the developing chicken retina and appears to play an autocrine/paracrine stimulatory role in the progression of neurogenesis (10, 30). In endothelial cells, it has been shown that proinsulin stimulates the expression of plasminogen activator inhibitor type I and that this biological effect could not be attenuated by an inhibition of either insulin or IGF-I receptors (29). In chick embryo fibroblasts, proinsulin stimulates growth through specific receptor binding with a binding affinity in the nanomolar range (18). In human IM-9 lymphoblasts, we demonstrated specific proinsulin binding sites with clear differences from insulin receptors with respect to binding affinity, cross-binding of related peptides, downregulation, and signal transduction (13).

In the present study, we used IEC-6 cells, a rat small intestinal crypt-like cell line (26), to characterize proinsulin binding and growth-promoting action. In IEC-6 cells, IGF-I has been reported as a potent mitogen (14, 20, 31). As these cells express specific IGF-I receptors but lack insulin receptors, it has been suggested that both ligands mediate their effects via the IGF-I receptor (21). The present study extends these results by

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describing functional high-affinity proinsulin binding sites in IEC-6 small intestinal crypt-like cells.

MATERIALS AND METHODS

Reagents. Human recombinant proinsulin and human re-combinant C-peptide were generous gifts from Dr. B. H. Frank and Dr. R. E. Chance (Eli Lilly, Indianapolis, IN). Human recombinant [125]Tyr<sup>14</sup>-proinsulin (specific activity 233 µCi/mg) was labeled by the lactoperoxidase method and subsequently was purified by HPLC, as previously described (13). Human biosynthetic insulin was kindly provided by Dr. H. Feger (Hoechst, Frankfurt, Germany). Human [125]Tyr<sup>14</sup>-insulin (specific activity 360 µCi/mg) was labeled and HPLC purified by A. Liebe (Hoechst). Human recombinant IGF-I was a generous gift of Dr. Th. Mueller (Ciba-Geigy). [125]<sup>L</sup>-labeled IGF-I (specific activity 250 µCi/mg), [125]<sup>L</sup>-IGF-I (specific activity 250 µCi/mg), and human recombinant IGF-II were from Amersham (Braunschweig, Germany). All other reagents were of analytical grade.

Cell culture and growth studies. IEC-6 cells represent a small intestinal crypt-like cell line derived from the rat jejunum (26). IEC-6 cells between the 25th and 30th passages were grown in tissue culture dishes (10 cm; Nunc) at 37°C in a jejunum (26). IEC-6 cells represent a small intestinal crypt-like cell line derived from the rat jejunum (26). IEC-6 cells between the 25th and 30th passages were grown in tissue culture dishes (10 cm; Nunc) at 37°C in a humidified CO<sub>2</sub> incubator (92% air-8% CO<sub>2</sub>) in Dulbecco’s minimal essential medium (DMEM) containing 100 U/ml penicillin, 100 µg/ml streptomycin, 5 mM glucose, and 5% fetal calf serum (FCS). To study the effects of indicated peptides on cell proliferation, IEC-6 cells were cultured under defined serum-free conditions supplementing DMEM with Ham’s F-12 medium (50%), cortisol (1 nM), and transferrin (10 µg/ml). This basal medium could maintain IEC-6 cells in a viable, slowly dividing state. Cells were incubated for up to 10 days in the basal medium in the absence or presence of various concentrations of individual peptides (n = 7–10, mean ± SD). Medium was changed every second day. Viability as assessed by trypan blue exclusion was always >90%. To quantify cell number, cells were trypsinized, and cell numbers were determined using an electronic counter (Coulter Counter). To characterize DNA synthesis in the presence of 10 nM of proinsulin, IGF-I, or insulin, the relative proportion of IEC-6 cells in the S phase of the cell cycle was determined by flow cytometry as described previously (13). In addition, a non-adherent cell proliferation assay was used (CellTiter 96, technical bulletin no. TB112; Promega, Madison, WI) to measure the transformation of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide into formazan by mitochondrial dehydrogenases.

Receptor binding studies. Equilibrium competition binding assays (n = 3–6, means ± SD) were carried out in six-well plates using IEC-6 cells that had been cultured in DMEM plus 5% FCS for 7 days to early confluence (500,000 ± 50,000 cells/dish). Cell monolayers were washed two times with phosphate-buffered saline and one time with binding buffer consisting of 50 mM HEPES, pH 7.8/8.4, 120 mM sodium chloride, 5 mM potassium chloride, 1.2 mM magnesium sulfate, 10 mM calcium chloride, 10 mM dextrose, 15 mM sodium acetate, and 0.1% bovine serum albumin. After the addition of the indicated concentration of the unlabeled peptide, 10 pM tracer was given to each dish. Binding studies were performed under different conditions of time, temperature, and pH as indicated. Cell monolayers were washed three times with phosphate-buffered saline containing 0.1% bovine serum albumin at the end of each experiment.

To further characterize peptide binding, plasma cell membranes were prepared from confluent cell monolayers as described previously (16). Binding to isolated plasma membranes was performed in microcentrifuge tubes. For this, 0.1 mg membrane protein was incubated for 1 h at 23°C in 500 µl assay buffer (25 mM HEPES, pH 7.4, 104 mM sodium chloride, 5 mM potassium chloride, 1 mM potassium phosphate, 1.2 mM magnesium sulfate, 2 mM calcium chloride, 0.03% (wt/vol) soybean trypsin inhibitor, 0.2% (wt/vol) bovine serum albumin) containing 10 pM tracer and competitive ligand. To separate bound from unbound radioligand, 500 µl of ice-cold assay buffer were added to each tube, and plasma membranes were pelleted in a microfuge at maximum speed at 4°C. Supernatant was aspirated, and the pellets were washed two times with 500 µl of ice-cold assay buffer before counting. In cell and membrane binding studies, tracer degradation was measured by trichloroacetic acid (10%) precipitation of intact [125]<sup>L</sup>-labeled peptides. In all experiments, degradation of tracer was <10%, excluding that the differences in binding were caused by hormone degradation. Cell-bound and free intact activity was determined by γ-counting. Specific binding was determined by subtracting the amount of tracer unspecifically bound in the presence of 1 µM unlabeled analog peptide. All data are expressed as specific binding per 100,000 cells or per 0.1 mg membrane protein. Binding affinity and capacity were calculated using a computer-assisted curve-fitting program as described (13).

RESULTS

IEC-6 cell growth. Proinsulin, insulin, and IGF-I stimulated IEC-6 cell growth over basal medium with characteristic differences in time course, dose dependency, and efficacy. Proinsulin and IGF-I showed half-maximal effects already in the picomolar range, whereas insulin was 100- to 1,000-fold less potent. Three days after initial plating, IGF-I was most effective in stimulating DNA synthesis (5 phase cells: 39 ± 5%) and increased basal cell proliferation (5,900 cells/well) in a dose-dependent manner (EC<sub>50</sub>: 2 pM) with a 2.9-fold stimulation at 10 nM (163,700 ± 19,400 cells; Fig. 1A). Proinsulin also induced significant growth-stimulating effects when added in picomolar concentrations (EC<sub>50</sub>: 0.9 pM) with a 1.7-fold increase in cell number at 10 nM (99,000 ± 7,400 cells). Insulin showed a 1,000-fold lower potency (EC<sub>50</sub>: 1.0 nM) with a 1.6-fold increase in cell number at 10 µM (91,000 ± 5,000 cells). When added for 6 days (Fig. 1B), proinsulin and IGF-I yielded a comparable dose-dependent growth stimulation. The lower maximal effects [basal medium: 294,000 ± 4,500 cells; proinsulin (10 nM): 370,000 ± 7,000 cells; IGF-I (10 nM): 395,000 ± 5,900 cells] may be due to the lower number of cells undergoing DNA synthesis (20 ± 3%). A reduced growth velocity between days 5 and 7 was also observed frequently when cells were grown in the presence of FCS, indicating that this is not related to a lack of nutrients. Cell number of serum-free cultured IEC-6 cells did not significantly increase further up to day 10 (307,000 ± 4,000 cells). As
shown in Fig. 1C, during this late growth phase, proinsulin stimulated IEC-6 cell number 2.7-fold (10 nM: 820,000 ± 18,000 cells) and was significantly (P < 0.001) more effective than IGF-I (10 nM: 720,000 ± 8,200 cells). Both proinsulin and IGF-I showed a significantly higher potency (EC50: 1 pM) than insulin (EC50: 100 pM; P < 0.001), which was less effective than proinsulin (10 pM: 702,000 ± 8,000 cells, P < 0.001).

To exclude the possibility that the time-based differences between IGF-I, proinsulin, and insulin were caused by varying rates of degradation of each compound, we determined the degradation of native peptides by radioimmunoassay and that of 125I-labeled peptides by trichloroacetic acid (10%) precipitation of intact peptides. At the end of the 48-h incubation period, before media exchange, the residual amount of the peptides added in the concentration of 10 pM was higher than 75% for IGF-I and proinsulin and higher than 95% for insulin. Thus, in the nanomolar concentration range, the different growth-promoting activities were not caused by differences in the exposure to each hormone.

To further characterize the initiation of cell proliferation, IEC-6 cells were incubated for 24 h with 1 nM of proinsulin, IGF-I, insulin, C-peptide, or a combination of proinsulin and IGF-I. Cell proliferation was assessed by counting cells and by performing a nonradioactive cell proliferation assay. As shown in Table 1, both methods yielded comparable results. Proinsulin and IGF-I yielded the highest growth stimulation. Additive effects were observed when both peptides were combined. C-peptide yielded a weaker growth stimulation, whereas insulin had no significant effects.

Peptide binding capacity to cells and isolated membranes. Similar to our experience with IM-9 lymphoblasts (13), IGF-I and insulin reached their binding optimum at pH 7.8, whereas maximal proinsulin binding was at pH 8.4 (20% higher specific binding than at pH 7.8). For all three peptides, equilibrium binding of 10 pM labeled ligand was reached after 24 h (4°C), 2 h (23°C), or 0.5 h (37°C). Three days after initial plating, IGF-I bound to cells with significantly higher capacity than proinsulin (10 ± 2.4 vs. 2.5 ± 1.2%; P < 0.01). The opposite was found in early confluent cell monolayers after 7 days of culture (2.5 ± 0.5 vs. 5 ± 1.0%; P < 0.05 proinsulin vs. IGF-I). In membranes isolated from confluent cell monolayers (day 10), specific binding of 125I-proinsulin was significantly higher than that of 125I-IGF-I (2.2 ± 0.4 vs. 1.2 ± 0.3%; P < 0.01). In contrast, insulin binding in confluent cells and in isolated membranes was <1.5 and 0.3%, respectively.

Specificity of 125I-proinsulin binding. The specificity of 125I-proinsulin binding was assessed using increasing concentrations of related peptides as competitive ligands. C-peptide, IGF-I, and insulin competed with 125I-proinsulin (Fig. 2A and Table 2). With increasing temperature, binding affinity tended to increase (IC50 values: 2.2 nM at 4°C, 1.3 nM at 23°C, and 1.1 nM at 37°C; Fig. 2) without differences in the amount of specific binding (5 ± 1.2%). When the numbers of proinsulin binding sites were calculated by Scatchard analysis, two classes of binding sites were found at 4°C (150; Kd1: 65 ± 30 pM; R1: 560 ± 150

Table 1. Stimulation of IEC-6 cell growth after 24-h incubation with 1 nM of indicated peptides under serum-free conditions

<table>
<thead>
<tr>
<th>Cell Number, %</th>
<th>Cell Proliferation Assay, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum-free control</td>
<td>100</td>
</tr>
<tr>
<td>Proinsulin</td>
<td>131 ± 12*</td>
</tr>
<tr>
<td>IGF-I</td>
<td>135 ± 10*</td>
</tr>
<tr>
<td>Insulin</td>
<td>109 ± 20</td>
</tr>
<tr>
<td>C-peptide</td>
<td>120 ± 14*</td>
</tr>
<tr>
<td>Proinsulin + IGF-I</td>
<td>154 ± 12†</td>
</tr>
</tbody>
</table>

Mean values ± SD of cell number and results of the nonradioactive cell proliferation assay of 3–6 separate experiments are given. IGF-I, insulin-like growth factor I. Serum-free control without peptides is defined as 100%. * P < 0.05 and † P < 0.005 vs. control or insulin.
sites/cell; $K_{d1}: 8.8 \pm 1.4 \text{ nM}, R_1: 104,400 \pm 5,000$ sites/cell) or 23°C (Fig. 3B: $K_{d2}: 6.2 \pm 0.4 \text{ pM}, R_2: 100 \pm 15$ sites/cell; $K_{d2}: 8.6 \pm 0.5 \text{ nM}, R_2: 96,600 \pm 3,000$ sites/cell). The Scatchard plot of $^{125}$I-proinsulin binding data at 37°C was significantly better fitted by a one-site model (one-site model: $K_d: 5.4 \pm 0.2 \text{ nM}, R: 540 \pm 100$ sites/cell; $P < 0.05$ vs. two-site model: $K_{d1}: 0.9$ nM, $R_1: 6,500$ sites/cell; $K_{d2}: 8.6, R_2: 112,000$, respectively).

Table 2. Relative potencies of proinsulin, IGF-I, and insulin to compete for $^{125}$I-labeled peptide binding

<table>
<thead>
<tr>
<th>Competing Peptide</th>
<th>$^{125}$I-Proinsulin</th>
<th>$^{125}$I-IGF-I</th>
<th>$^{125}$I-Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proinsulin</td>
<td>1.3</td>
<td>135</td>
<td>1.4</td>
</tr>
<tr>
<td>IGF-I</td>
<td>172</td>
<td>4.0</td>
<td>68</td>
</tr>
<tr>
<td>Insulin</td>
<td>319</td>
<td>322</td>
<td>110</td>
</tr>
<tr>
<td>C-peptide</td>
<td>13</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Mean IC$_{50}$ values in nM of 3–6 separate experiments are given. ND, not detectable.

Specificity of $^{125}$I-IGF-I binding. $^{125}$I-IGF-I binding was competed with highest potency by IGF-I ($IC_{50}: 4 \pm 0.06$ nM; Fig. 4A), whereas insulin and proinsulin competed with 50- and 80-fold lower potency, respectively (Table 2). As shown in Fig. 4B, two classes of binding sites were obtained by Scatchard analysis, one with high affinity and low capacity ($K_{d1}: 54 \pm 10$ pM; $R_1: 540 \pm 100$ sites/cell) and the other with low affinity ($K_{d2}: 37 \pm 10$ nM) but high capacity ($R_2: 40,000 \pm 10,000$ sites/cell).

Specificity of $^{125}$I-insulin binding. $^{125}$I-insulin binding was inhibited by 50% at insulin concentrations of $110 \pm 15$ nM (Fig. 5A). IGF-I or proinsulin competed with 1.6-fold and 80-fold higher relative affinity than insulin (Table 2), indicating cross-binding of insulin to the proinsulin and IGF-I binding sites. Scatchard analysis of $^{125}$I-insulin binding revealed one class of low-affinity and high-capacity binding sites ($K_{d1}: 70 \pm 19$ nM; $R_1: 380,000 \pm 65,000$ sites/cell; Fig. 5B).
Specificity of 125I-IGF-II binding. 125I-IGF-II binding studies were performed to examine whether proinsulin binding might be explained by binding to IGF-II receptors. Specific IGF-II binding was demonstrated in both IEC-6 cell monolayers (5 ± 1.6%) and isolated plasma membranes (2 ± 0.4%). In accordance with typical IGF-II receptors, 125I-IGF-II binding was only displaced by IGF-II, whereas IGF-I, insulin, or proinsulin lacked any effects.

Specificity of 125I-C-peptide binding. To investigate whether C-peptide binds to the proinsulin binding site or to its own receptor, competitive binding studies using 10 pM of 125I-C-peptide and increasing concentrations of unlabeled C-peptide or proinsulin were performed. The total binding of 125I-C-peptide was very low, averaging 1%, and could not be specifically competed by unlabeled C-peptide. When proinsulin was added instead of C-peptide, 125I-C-peptide binding increased up to threefold at proinsulin concentrations between 10 pM and 1 nM, whereas higher concentrations of proinsulin induced a complete competition of 125I-C-peptide. Taken together, these studies support the hypothesis that C-peptide binds to the proinsulin receptor and not to its own binding site.

DISCUSSION

In the present study, we demonstrate for the first time specific binding of proinsulin in the small intestinal crypt-like cell line IEC-6. Our findings also demonstrate that proinsulin stimulates IEC-6 cell growth with similar potency and efficacy as IGF-I, whereas insulin was significantly less potent. The finding that IGF-I induced maximal stimulation of DNA synthesis and cell proliferation in the early growth phase implicates an important role as a growth-initiating factor. In contrast, proinsulin may be a growth-maintaining factor because maximal growth stimulation occurred in early confluent cell monolayers (10 days after plating).

The key question of the present study is whether proinsulin mediates its biological effects via its own specific binding site or via cross-binding to IGF-I or insulin receptors. Our data confirm previous studies demonstrating expression of typical IGF-I receptors but no high-affinity insulin receptors in IEC-6 cells (5, 21). Based on relative peptide affinity, proinsulin binding sites could be clearly distinguished from typical receptors for IGF-I, IGF-II, or insulin. The Scatchard plots of proinsulin binding indicate two sites at low temperature that shift to a single site with increased temperature. As proinsulin is composed of insulin and C-peptide, it might bind through the insulin or the...
C-peptide moiety or through a proinsulin-specific epitope. Binding through the insulin moiety seems highly unlikely since insulin competed for proinsulin binding with 250-fold lower affinity than proinsulin. On the other hand, C-peptide was only 10-fold less potent than proinsulin to compete for proinsulin binding, which is consistent with our recent findings in IM-9 lymphoblasts (13). Therefore, we suggest that binding of proinsulin occurs via a specific binding epitope of the proinsulin molecule within or close to the “connecting peptide backbone” (2). Although C-peptide has long been considered to possess little if any biological activity on its own, a recent study demonstrated prevention of vascular and neural dysfunction in diabetic rats by C-peptide (11). Our data support the hypothesis that C-peptide is able to induce biological effects, e.g., stimulation of cell proliferation, which in the case of IEC-6 cells may occur via binding to proinsulin receptors. Proinsulin may first be necessary to induce a conformational change of the receptor, which then may become capable of binding C-peptide, albeit with lower affinity than proinsulin itself. This would explain why lower concentrations of proinsulin increased C-peptide binding but higher concentrations were competitive. Further studies are needed to reveal the molecular structure of the proinsulin/C-peptide receptors and their signal transduction mechanisms. Among other cell types, IEC-6 cells could allow the cloning of the putative proinsulin receptor.

Our data support the hypothesis that proinsulin represents a highly potent growth-promoting factor for small intestinal crypt-like cells. The binding experiments suggest that proinsulin may stimulate IEC-6 cell proliferation through specific receptors located within the plasma membrane which, based on their relative peptide affinity and competitive action of C-peptide, can be clearly distinguished from the typical receptors for insulin, IGF-I or IGF-II. It could be speculated that an autocrine release of IGFs and IGF-binding proteins (IGFBPs) could have changed the effects of the peptides studied. In a recent study, we investigated the autocrine regulation of IEC-6 cell proliferation by IGF system components (12). These experiments revealed that, from day 6 to day 7 of serum-free culture, the autocrine release of IGF-I sharply increased from very low levels (0.02 ng/ml) to steady-state levels of 0.35 ng/ml (~0.05 nM), whereas that of IGFBP-2, which was low levels (0.02 ng/ml) to steady-state levels of 0.35 ng/ml (~0.05 nM). Thus the small amounts of IGFBP system components secreted by IEC-6 are unlikely to change the effects of the peptides studied.

The alkaline pH milieu of the small bowel in vivo corresponds to the pH optimum of proinsulin binding. Of interest, the concentrations of proinsulin necessary for half-maximal growth stimulation were comparable to those found in serum of healthy (12 ± 4 pM) and diabetic subjects (15–50 pM) and in human pancreatic juice (10 ± 40 pM; see Refs. 7 and 17). Studies in rats with streptozotocin-induced diabetes showed complex alterations in both growth and function of intestinal epithelium. Diabetic rats have ~80% more intestinal epithelial cells and crypt cells, and their nuclei are 40–50% bigger than in nondiabetic control animals (34). Taken together with the data presented in this paper and the clinical observation that subjects with type II diabetes frequently show elevated serum proinsulin levels (7), the increased intestinal growth in streptozotocin diabetic rats (28) may be secondary to pancreatic proinsulin secretion. However, in vivo, various other hormones such as growth hormone, glucagon, and glucagon-like peptides are involved in type II diabetes-associated bowel growth (1, 9, 28). Therefore, further studies, e.g., with either IGF-I and proinsulin receptor gene knockout animals or overexpression, are necessary to establish a role for proinsulin in the regulation of intestinal cell growth in vivo. Proinsulin may have a similar therapeutic potential as IGFs in stimulating the regeneration of small intestine (25, 33); however, its blood glucose-lowering effects might limit the clinical use.

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