

The trophic action of growth hormone, insulin-like growth factor-I, and insulin on human duodenal mucosa cultured in vitro

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Abstract

Background—Experimental evidence suggests that hormones may regulate small intestinal adaptation after surgical resection.

Aims—To characterise the effect of recombinant human growth hormone (GH), insulin-like growth factor-I (IGF-I) and insulin on crypt epithelial cell proliferation in the human duodenal mucosa cultured in vitro.

Patients—Thirty nine adults had endoscopic duodenal biopsy specimens taken, which were histologically normal and pair matched specimens from each patient acted as their own control.

Methods—Paired biopsy specimens from patients were cultured in vitro, with or without the addition of GH (0.004 IU/ml), IGF-I (400 ng/ml) or insulin (50 µg/ml), alone or in combination. After 22 hours, vincristine sulphate was added to the cultures and three hours later specimens were removed and fixed, and DNA stained by the Feulgen method. Intestinal crypts were microdissected and crypt epithelial cell proliferation determined by estimating mean numbers of accumulated metaphase arrests/crypt present in tests and controls between 22–25 hours of culture.

Results—The addition of GH, IGF-I, and insulin, alone or in combination, significantly increased crypt epithelial cell proliferation in test explants compared with controls. IGF-I was most potent and its trophic effect was modified by insulin.

Conclusions—GH, IGF-I, and insulin are involved in the regulation of crypt cell proliferation in the human small intestine in vitro and possibly in vivo.

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Keywords: growth hormone, insulin-like growth factor-I, trophic action, small intestinal mucosa, organ culture.

Little has been reported of the actions of growth hormone (GH) or insulin-like growth factor-I (IGF-I) on the human small intestine until recently, when both hormones were shown to stimulate crypt epithelial cell proliferation (CECP) in duodenal explants cultured in vitro.^{1,2} GH has a mitogenic effect on crypt epithelial cells in the duodenum of hypophysectomised rats³ and when given alone causes hypertrophy of the rat gastrointestinal

tract to supranormal levels.⁴ GH increases body weight, distal ileal weight, and mucosal height in rats undergoing 75% small bowel resection⁵ as well as growth and differentiation in transplanted rat intestine in early neonatal life.⁶ GH receptors are present in crypt and villus epithelial cells of rats,⁷ suggesting a direct cellular effect of GH on small intestinal growth. GH stimulates the synthesis of IGF-I in the liver and other tissues,⁸ which mediates GH dependent cellular proliferation. IGF-I receptors have also been immunolocalised in the gastrointestinal tract of rats^{9,10} and IGF-I given subcutaneously to dexamethasone treated rats increases the total weight of all regions of the bowel¹¹ and improves mucosal adaptation after small bowel resection.¹² Insulin receptors are also present in the intestinal epithelium of rats^{13,14} and insulin stimulates epithelial cell proliferation in the suckling mouse intestinal mucosa.¹⁵

These findings suggest that GH, IGF-I, and insulin, alone or in combination, are involved in the regulation of CECP or epithelial cell differentiation, or both, in the human small intestinal mucosa in vivo. In this study, an organ culture technique¹ has been used to investigate the action and interaction of recombinant GH, IGF-I, and insulin, on CECP in explants of the human duodenal mucosa cultured in vitro in an insulin free medium, using a stathmokinetic technique with crypt microdissection.^{16,17}

Methods

Patients

Mucosal biopsy specimens obtained by fiberoptic endoscopy (Olympus GIF 1T) from the third or fourth part of the duodenum of 39 adults (21 men; median age 63, range 31–92) with upper gastrointestinal disorders were studied, with ethics committee approval. All specimens were expelled from biopsy forceps into cold Leibowitz L-15 medium, transported to the laboratory, and flattened serosal surface downwards, using aseptic techniques. Specimens for routine histopathology were fixed in 10% formal saline and the remainder bisected to provide six or seven explants of approximately 2 mm² for culture in vitro.

Organ culture

The organ culture technique¹ maintained the morphological integrity and proliferative viability of duodenal explants in vitro for at least

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48 hours, without added insulin. Biopsy specimens were placed mucosal surface upward on 1 cm×1 cm gelatin sponge squares (Gelfoam, Upjohn, Kalamazoo, USA), attached to the base of a 60 mm sterile plastic culture dish (Falcon). Paired biopsy specimens from each patient were their own controls and were cultured in basic medium with added rhGH (Genotropin, Pharmacia), rhIGF-I (Pharmacia) or rh insulin (Sigma), alone or in combination. Test and control specimens were cultured under identical conditions in separate culture dishes. The basic culture medium was both serum and insulin free and contained 9.65 ml CMRL 1066 medium supplemented with 0.2 ml L-glutamine (200 mM), 0.1 ml penicillin/streptomycin (10 000 U of each), 0.05 ml Fungizone, 10 mg glucose, 0.2 mg ascorbic acid, and 10 µg hydrocortisone hemisuccinate. The medium was sterilised by a 0.2 µm Acrodisc filter (Gelman Sciences). Culture

dishes were placed in a controlled atmosphere chamber (Bellco Glass Inc), sealed, and placed on a rocking apparatus in a 37°C incubator and rocked at 3 rpm in an atmosphere of 95% oxygen and 5% carbon dioxide.

Crypt epithelial cell proliferation (CECP)

Control and test explants were maintained in organ culture for 22 hours when 0.7 µg/ml of vincristine sulphate (Oncovin, Lilly) was added to start the stathmokinetic experiment. Explants were removed after three hours, fixed in Carnoy's fluid for four hours, and stored in 70% alcohol before rehydrating and staining DNA by the Feulgen method. Intestinal crypts were separated by microdissection in 45% acetic acid, squashed under a coverslip, and viewed by light microscopy. The mean number of metaphase arrests accumulating between 22–25 hours in 15 crypts from different parts of each specimen was determined and expressed the CECP. Statistical analysis of the difference between means in test and control explants was obtained using a paired Student's *t* test.

Dose/response studies

Dose/response studies were performed by adding rhGH (Genotropin, Pharmacia) 0.001–0.016 IU/ml of culture medium, rhIGF-I (Pharmacia) 50–800 ng/ml, and rh insulin (Sigma) 0.5–100 µg/ml respectively, to duodenal explants from three patients at the beginning of organ culture and estimating the CECP at 25 hours. From the results (Fig 1), 0.004 IU/ml of GH, 400 ng/ml of IGF-I, and 50 µg/ml of insulin were the doses selected for further studies.

Results

Histological appearances of the duodenal mucosa were initially normal by light microscopy and showed good preservation of villous architecture and epithelial cells for up to 25 hours. The addition of GH, IGF-I or insulin to the culture medium, alone or in combination, significantly increased the mean numbers of metaphase arrests/crypt accumulating between 22–25 hours in test specimens compared with controls ($p < 0.001$). The CECP in test specimens cultured with IGF-I alone, GH/IGF-I, or GH/insulin, was significantly higher than specimens cultured with insulin alone, GH alone, IGF/insulin, and GH/IGF-I/insulin ($p < 0.001$). (Table and Fig 2).

Discussion

In this study, insulin alone stimulated CECP in the duodenal mucosa and may have acted via insulin receptors or by cross reacting with IGF-I receptors.^{18–20} The dose of insulin (50 µg/ml) was the same as that used in Trowell's classic organ culture medium²¹ and was considerably higher than normal plasma insulin values in humans (0.42 µg/ml). High concentrations of insulin (1–10 µg/ml) probably cross

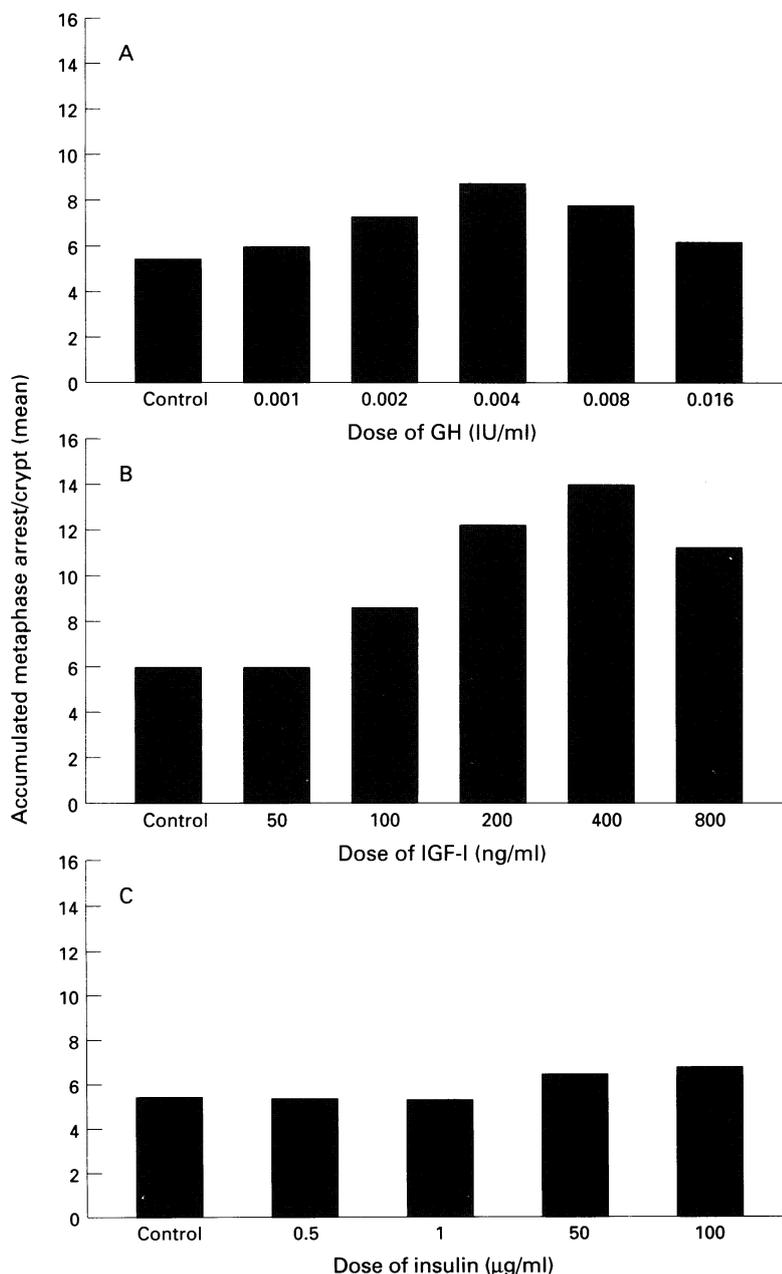


Figure 1: Dose/response studies of (A) GH, (B) IGF-I, and (C) insulin, on CECP in the duodenal mucosa.

Mean number of metaphase arrests/crypt – tests v pair matched controls

	Patients	Mean	SEM	t Test
Tests+insulin	8	7.3	0.44	
Controls	8	5.3	0.32	p<0.001
Tests+GH	8	7.6	0.43	
Controls	8	5.8	0.23	p<0.002
Tests+IGF-I	8	12.9	0.72	
Controls	8	5.8	0.18	p<0.001
Tests+GH+insulin	9	12.0	0.40	
Controls	9	5.8	0.40	p<0.001
Tests+GH+IGF-I	8	12.9	0.44	
Controls	8	5.8	0.23	p<0.001
Tests+IGF-I+insulin	8	8.3	0.59	
Controls	8	5.8	0.40	p<0.001
Tests+GH+IGF-I+insulin	9	7.6	0.43	
Controls	9	5.8	0.40	p<0.001

react with IGF-I receptors and concentrations of up to 6.5 µg insulin/ml have been shown to induce mitogenesis in cultured human skin fibroblasts via IGF-I receptors.²⁰ Insulin may induce a biphasic proliferative response on cultured skin fibroblasts, acting via insulin receptors at low concentrations and via IGF-I receptors at high concentrations.²² In studies on cultured small intestinal crypt cell lines (IEC-6), increased cell proliferation did not occur until high insulin concentrations were present (1–10 µg/ml), suggesting an action via IGF-I rather than insulin receptors.¹⁹ The need to use relatively high doses of hormones and other agents is a commonly observed phenomenon in organ culture experiments, as they reach cells by diffusion, in the absence of a blood supply.¹⁵ However even after making allowances for this phenomenon, the present findings showed that higher than normal tissue concentrations of insulin stimulated intestinal CECP, probably via IGF-I receptors, which may be of pathophysiological significance.

The dose of GH used to stimulate CECP (0.004 IU/ml; 1.6 µg/ml)¹ was higher than plasma concentrations in normal adults (<0.2–3.1 ng/ml). GH also has a trophic action on duodenal epithelial cells in experimental animals³ and overcomes gut hypoplasia induced by hypophysectomy in rats.⁴ Specific GH binding proteins and receptors are present in small intestinal columnar cells of adult rats

and GH could have a direct cellular action on CECP in the human intestinal mucosa. As IGF-I is also expressed in the small intestinal epithelium, GH could have stimulated CECP in this study by inducing local synthesis of IGF-I, which then acted in an autocrine/paracrine manner.

The potent action of IGF-I on CECP probably occurred via IGF-I receptors and the dose used (400 ng/ml) approximated normal plasma values in adults (315±27 ng/ml), which is mostly protein bound. IGF-I is mitogenic for many types of cultured cells in vitro, accelerating somatic growth in experimental animals and inducing the selective growth of tissues such as thymus, kidney, and spleen.²³ Free IGF-I is a potent trophic factor in the normal small intestinal mucosa of rats. In humans, circulating levels of free IGF-I have a half life of about 20 minutes,²⁴ increasing to 13–20 hours when bound to carrier proteins (IGFBPs).^{25, 26} Free IGF-I in exocrine secretions (saliva, gastric juice, jejunal chyme, pancreatic juice, and bile) could also influence growth in the human gastrointestinal epithelium in vivo, and the concentration in the gut lumen is approximately 180 ng/ml of jejunal chyme.²⁷

GH/insulin stimulated CECP and the result was higher than either hormone alone, suggesting a cumulative trophic response as described above. Furthermore as hepatic GH receptors in diabetic rats are insulin dependent,²⁸ high doses of insulin could have influenced the maintenance of GH receptors available for ligand binding in this study. The effect of GH/IGF-I on CECP was similar to IGF-I alone and greater than GH alone, suggesting an action mediated solely by IGF-I. The effect of IGF-I/insulin on CECP was lower than IGF-I alone, but slightly higher than insulin alone, suggesting that a high insulin dose may have decreased the trophic action of IGF-I by competing for IGF-I receptors. A combination of GH, IGF-I, and insulin stimulated CECP, but the result was significantly lower than that of IGF-I alone, of GH/insulin and of GH/IGF-I (p<0.001). These findings suggest that a high dose of insulin could have saturated IGF-I receptors and modified the action of IGF-I on CECP, but partial suppression of the trophic action of GH/insulin cannot be explained.

By studying cultured explants of the human duodenal mucosa in vitro, isolated from biological control of the intact organism, it has been possible to investigate actions of hormones regulating CECP more directly than would have been ethically possible in vivo. With the exception of hydrocortisone, the culture medium was free of known hormones and other growth factors influencing CECP. Hydrocortisone enhances the preservation of human small intestinal explants in long term organ cultures and its presence maintains a relatively higher steady state of epithelial cell proliferation in vitro (EEW, personal observation). Any influence it may have had on the action of GH, IGF-I, and insulin in short-term organ cultures is unknown.

The results of this study have shown that insulin, GH, and IGF-I had a significant

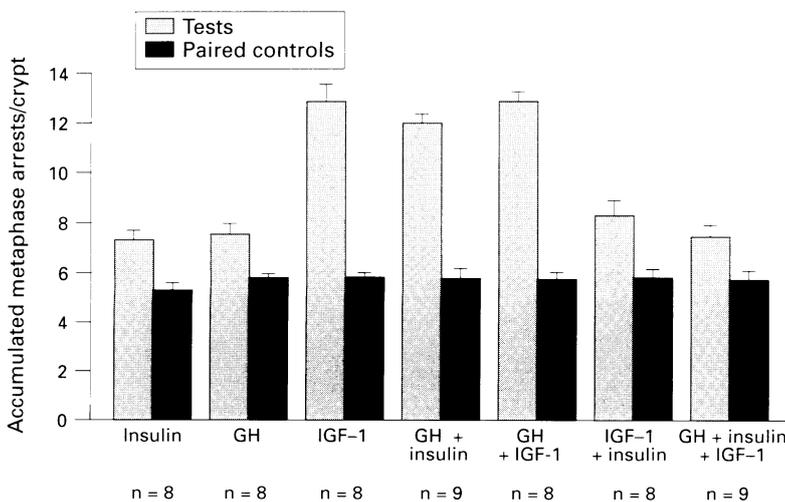


Figure 2: Mean number of metaphase arrests/crypt at 25 hours of culture after addition of insulin, GH, and IGF-I to the culture medium.

trophic effect on CECP in vitro. Growth control mechanisms responsive to GH, IGF-I or insulin in the human small intestinal mucosa will need further investigation, particularly to determine whether GH initiates an IGF-I autocrine/paracrine loop. Synthesis of IGF-I close to its locus of action in the bowel may have greater physiological significance than secretions of hormones from endocrine glands.⁸ Small bowel growth is increased in transgenic mice overexpressing bovine GH²⁹ and subcutaneous administration of IGF-I improves mucosal structure and absorptive function after small bowel transplantation in rats.³⁰ IGF-I and GH could be involved in the regulation of crypt cell proliferation in the human small intestinal mucosa in vivo and contribute to the adaptive changes occurring in the small intestine after surgical resection.

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