Determination of the histological distribution of insulin like growth factor 1 receptors in the rat gut

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Abstract

The histological distribution of insulin like growth factor 1 (IGF 1) receptors in the rat gut was studied. Immunostaining of IGF 1 receptors identified localisation on the villus epithelium, in the crypts, and in Brunner's glands of the small intestine. These tissues represent areas of high cell growth/differentiation, division, and macromolecular synthesis respectively, which constitute biological activities long associated with IGF 1. Cellular localisation of IGF 1 receptors was seen in the lamina propria by IGF 1 receptor immunostaining and ligand binding of biotinylated IGF 1. IGF 1 receptor immunostaining in the spleen showed receptor localisation to the splenic pulp thus pointing to macrophages as the possible IGF 1 receptor positive cells in the lamina propria. The results further implicate IGF 1 as an important growth factor in gut maintenance.

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Insulin like growth factor 1 (IGF 1) is a low molecular mass peptide found in highest concentrations in serum. IGF 1 acts through signal transduction by a cytoplasmic tissue kinase function associated with the cell surface membrane receptor.¹ Biological activities attributed to IGF 1 include stimulation of mitogenesis and macromolecular synthesis.^{2,3}

The gastrointestinal tract is an organ of rapid growth, the rate of cell division and differentiation in the gastrointestinal mucosa being one of the most rapid proliferative rates in the body.⁴ The factors promoting the rapid rate of cell division and differentiation in intestinal mucosa are largely unknown. In recent years attention has been focused on a possible role for IGF 1. IGF 1 activity is mediated through the IGF 1 receptor, and consequently IGF 1 receptor topology has been investigated. Laburthe *et al.*⁵ showed appreciable concentrations of specific IGF 1 binding in rat gut, while Pillion *et al.*⁶ showed IGF 1 binding on proximal colon epithelial apical membranes. More recently type 1 IGF receptors were seen in porcine small intestine.⁷ These studies used radioactively labelled IGF 1 in competitive binding to tissue membranes prepared from mucosal surface scrapings. These studies are thus limited in characterisation of the histological distribution of the IGF 1 receptor because of the difficulty in achieving homogeneity of membrane preparation from heterogeneous tissue sections. This study used biotinylated IGF 1 and anti-IGF 1 receptor antibody to determine the in situ distribution of type 1 IGF receptors in the gut.

Materials and methods

BIOTINYLATION OF IGF 1

IGF 1 was biotinylated using N-biotinyl- ω -aminocaproic-acid-N-hydroxy succinimide (Enzotint kit; Enzo Biochem, Inc). Biotinylation was assessed by SDS-PAGE⁸ followed by western blotting⁹ and visualisation with streptavidin conjugated horseradish peroxidase using 3-amino-9-ethylcarbazole as substrate.

Receptor binding capacity was assessed by competitive binding assay with radiolabelled IGF 1 to ovine intestinal mucosal membranes.¹⁰ Briefly, assays were performed in 5 ml polystyrene tubes. Membrane preparations, stored in liquid nitrogen, were allowed to thaw at room temperature and were homogenised with a glass on glass loose fitting homogeniser before use. Into each tube was placed 200 μ l of 25 mM TRIS, 10 mM MgCl₂, containing 0.1% bovine serum albumin followed by 100 μ l of membrane protein (1 mg/ml). [¹²⁵I]IGF 1, iodinated by the method of Zapf *et al.*,¹¹ was added to each tube in 100 μ l aliquots (30 000 cpm; 195 pg). Competitive ligands were added in 100 μ l volumes at a final concentrations range of 0.01 nM to 100 nM and the tubes were gently vortexed and left shaking at 4°C overnight. The following day 3 ml of ice cold 25 mM TRIS, 10 mM MgCl₂, pH 7.4, was added to each tube. Tubes were vortexed before centrifuging at 3000 $\times$ g for 30 minutes. The tubes were decanted and the resultant pellet was counted.

Total binding was determined using only [¹²⁵I]IGF 1 as ligand. Non-specific binding was determined by the addition of excess unlabelled ligand.

TISSUE SECTIONS

Tissue was obtained from male Sprague-Dawley rats of approximate weight 200–300 g. Animals were killed by cervical dislocation, and the gut and spleen were immediately removed into liquid nitrogen. Tissue was embedded in an ornithine carbamoyltransferase compound (BDH Ltd, Poole, Dorset, England) and serial 5 μ m sections were taken by cryostat set at –20°C.

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Tissue sections were fixed in acetone (10 minutes, 4°C) and endogenous peroxidase activity was blocked with methanol: H₂O₂ (99:1 ratio of a 33% solution) for 10 minutes at 4°C and subsequently with 10 mM sodium azide during 3,3'-diaminobenzidine (DAB; Sigma Chemical Co Ltd, Poole, Dorset, England) substrate development.

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Figure 1: IGF 1 receptor immunostaining in rat intestine with methyl green as counterstain. (A) Magnification $\times 160$ shows the mid gut topology of receptor immunostaining. Immunostaining at $\times 630$ magnification is shown: (B) on the villi surface epithelium (mid gut); (C) in the crypts (mid gut); (D) in the Brunner's glands (mid gut); and in the lamina propria of (E) distal gut and (F) proximal gut sections. A negative control of villi immunostaining in rat sections (G) at $\times 160$ magnification is shown.

HISTOLOGICAL LOCALISATION OF IGF 1 RECEPTORS

After treatment with 10% normal human serum for 60 minutes, sections were incubated with either rabbit anti-chromogranin A antibody at 1/100 dilution (Dako, High Wycombe, Bucks, England) or rabbit anti-IGF 1 receptor antibody 1/50 dilution for 48 hours at 4°C (Upstate Biotechnology, Lake Placid, NY). The anti-IGF 1 receptor antiserum is specific for the IGF 1 receptor α subunit, and does not cross react with IGF 2 receptor or insulin receptor. The primary antibodies were replaced with normal rabbit

serum for chromogranin A and IGF 1 receptor as negative controls. After incubation, tissue sections were washed three times (15 minutes each) with phosphate buffered saline (PBS), pH 7.4. Tissue sections were then treated with biotinylated swine anti-rabbit secondary antibody (Dako) at 1/400 dilution for one hour at room temperature. Sections were washed as described previously in PBS before immunostaining. Alternately, tissue sections were incubated with biotinylated IGF 1 (0.5 mg/ml) for 24 hours at 4°C. Sections were washed three times with ice cold PBS (five minutes each).

Figure 2: Western blot (12.5% SDS-PAGE electroblotted onto nitrocellulose) of biotinylated IGF 1. The blot was developed with streptavidin conjugated horseradish peroxidase with 3-amino-9-ethylcarbazole as substrate.

Localisation of immunostaining was shown by the avidin-biotin peroxidase complex method, using Dako ABC kits.

Results

IGF 1 immunostaining was seen in rat mid gut (Fig 1A). Immunostaining of IGF 1 receptors was seen on the villi of the small intestine (Fig 1B), the crypts (Fig 1C), and the columnar epithelia of Brunner's glands (Fig 1D). Cellular staining seen in the lamina propria of mid gut (Fig 1B) and distal gut sections (Fig 1E) was diminished in proximal gut (Fig 1F). A negative control for IGF 1 receptor immunostaining is shown (Fig 1G). The general pattern of immunostaining remained consistent from proximal to distal gut. The intensity of immunostaining on the villi surface epithelium, however, decreased in moving from proximal to distal gut. Conversely, the intensity of cellular staining in the lamina propria increased in moving from proximal to distal gut.

IGF 1 was biotinylated using N-biotinyl- ω -aminocaproic-acid-N-hydroxy succinimide (Fig 2). Biotinylation of IGF 1 resulted in decreased affinity of the ligand for its receptor, as assessed by competitive binding with [125 I]IGF 1 in membrane receptors prepared from ovine intestinal mucosal scraping¹⁰ (Fig 3). The comparative potencies of IGF 1 and biotinylated IGF 1 in competitive binding to ovine intestinal mucosal membranes were evaluated by the concentration required to decrease binding of radioligand by 50%. Half maximal inhibition of [125 I]IGF 1 with IGF 1 was seen at 0.1 nM compared with 1.0 nM when in competition with

Figure 3: Competitive binding of [125 I]IGF 1 with IGF 1 (●) and biotinylated IGF 1 (○), in ovine intestinal mucosal membranes, is shown.

biotinylated IGF 1. Biotinylated IGF 1 bound ovine intestinal receptor membrane preparations at roughly 10-fold lower concentrations than insulin, while [125 I]IGF 1 binding was completely inhibited with 500 nM insulin (data not included). The IGF 2 receptor does not recognise insulin,¹² thus inferring that biotinylated IGF 1 bound the IGF 1 receptor in ovine intestinal membranes. Biotinylated ligand staining of IGF 1 receptors was not seen in proximal gut sections, while staining in distal gut sections was seen as a cellular pattern limited to the lamina propria (Fig 4).

Chromogranin A immunostaining was used as a positive control for IGF 1 receptor immunostaining in the gut. Staining was restricted to the lamina propria. No immunostaining was seen in the mucosal epithelium (Fig 5). Immunostaining of IGF 1 receptors in spleen was used as a positive control tissue stain for comparison with gut. IGF 1 receptor immunostaining in the spleen was located in the splenic pulp (Fig 6).

Discussion

Insulin like growth factor 1 is a polypeptide, which has been shown to stimulate macromolecular synthesis, cell division, and differentiation. The biological activity of IGF 1, when acting independently, additionally or synergistically with other growth factors, such as platelet derived growth factor, is in raising the cell growth rate.^{13,14} The gastrointestinal tract constitutes an organ in a constant high state of tissue repair and renewal.⁴ This study identifies the histological distribution of IGF 1 receptor densities in the gut. It also discusses the potential role of IGF 1 comparative with the known functions of particular tissue types, which show positive IGF 1 receptor immunostaining.

IGF 1 receptor immunostaining was seen in the stem cells of the crypts of Lieberkühn and the epithelial layer of the villi. Crypts represent regions of rapid mitosis and differentiation, providing for a continual turnover of cells that differentiate into columnar and goblet cells as they migrate from the crypts towards the luminal

Figure 4: IGF 1 receptor staining in rat distal gut with biotinylated IGF 1 and methyl green as counterstain. Magnification $\times 160$.

Figure 5: Chromogranin A immunostaining in rat mid gut with methyl green as counterstain. Magnification $\times 160$.

Figure 6: IGF 1 receptor immunostaining in rat spleen with methyl green as counterstain. Magnification $\times 630$.

end of the villi.⁴ The rapid rate of cell division and differentiation is consistent with the known bioactivity of IGF 1.^{2,3} IGF 1 receptor immunostaining in these tissues is corroborated by previous membrane receptor binding studies³⁻⁷ and more recently by insulin like growth factor 1 autoradiographic characterisation in rabbit intestine.¹⁵ This study showed that a single class of specific, high affinity insulin like growth factor 1 receptors was distributed throughout the muscular and mucosal layers of the entire rabbit gastrointestinal tract. These data, however, were limited by the interior cellular detail afforded by in situ autoradiographic visualisation comparative with immunohistochemical staining. IGF 1 receptor immunostaining was seen in the columnar epithelium of Brunner's glands, and conceivably functions in stimulating the high rate of macromolecular synthesis in this active secretory tissue.

IGF 1 receptors in the lamina propria of rat distal intestine were shown both by immunostaining and receptor binding of biotinylated ligand, while IGF 1 receptors on the epithelial surface of the villi were only detectable by immunostaining. The diminished binding of biotinylated IGF 1 to membrane receptors, comparative with the native peptide (Fig 3), is possibly a result of steric hindrance caused by the addition of biotin molecules close to the receptor binding site. The discrepancy in measurement sensitivity between anti-IGF 1 antibody and biotinylated IGF 1 allows for comparison of the cellular staining in the lamina propria and epithelial staining on the villi. An absence of staining in the epithelial layer with biotinylated IGF 1, when staining within the lamina propria shows definite cellular positivity, points to increased receptor densities, or increased receptor affinity in these cells comparative with the villus epithelium and the crypts.

The level of cellular staining of IGF 1 receptors in the lamina propria would represent particularly active macromolecular synthesis.³ The cell populations in the lamina propria include fibroblasts, connective tissue fibres, lymphocytes, eosinophilic leucocytes, mast cells, and macrophages.¹⁶ Only mast cells and macrophages, however, fit the criteria of a compatible histological distribution comparative with the immunostained/biotinylated ligand bound cells, and retain a biological activity, which might require high level IGF 1 receptor expression; that is, mast cells and macrophages actively produce cell mediators such as leukotrienes¹⁷ and cytokines¹⁸ as well as other peptides. IGF 1 immunostaining on the reticuloendothelium of the spleen would support macrophage staining in the lamina propria.

The localisation of IGF 1 receptors on different tissues within the gastrointestinal tract suggests varied functions for IGF 1 within this organ. Receptor localisation within Brunner's glands, and possibly on macrophages in the lamina propria, implies a function for IGF 1 in regulating macromolecular synthesis of enzymes or cell mediators, or both. Certainly, the regulatory role of IGF 1 on RNA, DNA, and protein synthesis is well reported.^{19,20} Localisation of IGF 1 receptors on crypts and villi

epithelium suggests a functional role for IGF 1 in cell growth and differentiation, thus suggesting a possible role for IGF 1 in gut maintenance and repair. To date, there have been few investigations of the role of IGF 1 in the gut. Available data, however, implicate IGFs as having a possible pivotal role in gastrointestinal maintenance, repair, and possibly development.^{4-7 21 22} The histological distribution of IGF 1 receptors in the gut would support such a role for IGF 1.

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