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

J Ryan, D C Costigan

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.

Materials and methods
BIOTINYLYATION OF IGF 1
IGF 1 was biotinylated using N-biotinyl-ω-aminocaproic-acid-N-hydroxy succinimide (Enzotin kit; Enzo Biochem, Inc). Biotinylation was assessed by SDS-PAGE followed by western blotting 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. 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 μl of 25 mM TRIS, 10 mM MgCl2, containing 0-1% bovine serum albumin followed by 100 μl of membrane protein (1 mg/ml). [125I]IGF 1, iodinated by the method of Zapf et al, was added to each tube in 100 μl aliquots (30,000 cpm; 195 pg). Competitive ligands were added in 100 μl 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 3000xg 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 μm 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.
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).
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-o-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 \( ^{125}\text{I} \)IGF 1 in membrane receptors prepared from ovine intestinal mucosal scraping\(^8\) (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 \( ^{125}\text{I} \)IGF 1 with IGF 1 was seen at 0·1 nM compared with 1·0 nM when in competition with biotinylated IGF 1. Biotinylated IGF 1 bound ovine intestinal receptor membrane preparations at roughly 10-fold lower concentrations than insulin, while \( ^{125}\text{I} \)IGF 1 binding was completely inhibited with 500 nM insulin (data not included). The IGF 2 receptor does not recognise insulin,\(^1\) 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.\(^1\)\(^4\) The gastrointestinal tract constitutes an organ in a constant high state of tissue repair and renewal.\(^*\) 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...
end of the villi. The rapid rate of cell division and differentiation is consistent with the known bioactivity of IGF 1. IGF 1 receptor immunostaining in these tissues is corroborated by previous membrane receptor binding studies and more recently by insulin like growth factor 1 autoradiographic characterisation in rabbit intestine. 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. The cell populations in the lamina propria include fibroblasts, connective tissue fibres, lymphocytes, eosinophilic leucocytes, mast cells, and macrophages. 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 leukotrienes and cytokines as well as other peptides. IGF 1 immunostaining on the recticuloendothelium 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. 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. 

The histological distribution of IGF 1 receptors in the gut would support such a role for IGF 1.

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