Genetically Engineered Animal Models: Physiological Studies with Gastrin in Transgenic Mice

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INTRODUCTION

The role of gastrin as a regulator of acid secretion has been appreciated for almost a century. For many years it has been clear that while gastrin may act directly on parietal cells, it also acts on enterochromaffin-like (ECL) cells to control histamine release which in turn stimulates acid secretion. It is presently thought that this effect is of primary physiological importance in the control of post-prandial acid secretion. In addition, gastrin has long been considered to be a regulator of gastric mucosal proliferation, although it remains controversial as to whether or not proliferating cells in the stomach normally express the gastrin-CCK receptor, which is the main receptor mediating the effect of COOH-terminally amidated gastrins.

The gastrins are produced from G-cells in the pyloric antral part of the stomach. Multiple factors influence gastrin production at several different levels, including modulation of gene expression, mRNA translation, post-translational processing (see below), as well as secretion. Recent studies in transgenic mice indicate that the biology of gastrin is more complex than formerly supposed. Three issues are highlighted by these studies. First, biosynthetic precursors and intermediates in the production of the amidated gastrins appear to have their own characteristic spectrum of biological activities, raising the possibility that peptides derived from the gastrin gene influence more than just the acid-secreting epithelium. Second, gastrin increases the production of a variety of paracrine mediators that may account for at least some of the responses normally attributed to gastrin. Third, increased production of amidated gastrins is a feature in several genetically modified mice, which exhibit similar phenotypes characterised by gastric hyper-proliferation, foveolar hyperplasia and gland atrophy. Taken together these phenotypes raise other interesting questions regarding the biology of gastrin. The present account will review these issues.

GASTRIN BIOSYNTHESIS

The gastrin gene encodes a precursor peptide of 101-104 residues depending on the species. Removal of the signal peptide yields progastrin, which is characterised by three putative cleavage sites (Arg-57,58; Lys-74,75; Arg-94,95), putative phosphorylation (Ser-96), sulphation (Tyr-87) and amidation (Phe-92) sites. The results of pulse-chase
labelling studies in rat and human G-cells,(30-32) indicate that cleavage occurs after sequestration in secretory vesicles and is likely to be mediated by the prohormone convertases PC1/3 and PC2. It has been clear for many years that the events of post-translational processing may be functionally regulated. Aside from variations in expression of the prohormone convertases,(23) there are several factors may account for these differences. First, in cells that lack the regulated pathway of exocytosis eg non-endocrine cells, pro gastrin progresses directly from the trans-Golgi network (TGN) to the cell surface by the constitutive route of secretion. The main secretory product is therefore progastrin. In this case, control of secretion is exerted at the level of synthesis rather than exocytosis per se. Second, phosphorylation of Ser-96 inhibits the rate of subsequent cleavage at Arg-94,95.(3) Since the latter event occurs in secretory vesicles, reduced progastrin cleavage leads to release of precursor from the regulated secretory pathway. Third, secretory vesicle pH determines the cleavage of G34 to G17 and therefore the ratio of these two peptides secreted.(4) The t_{1/2} of circulating G34 is five times greater than that of G17,(34) and so modulation of this cleavage directly influences the plasma concentrations of amidated gastrin. Secretory vesicle pH may be modulated by activity of vesicular monoamine transporters: these act as amine-proton exchangers with a stoichiometry of two protons for one amine.(4) They account for the ability of endocrine cells in the gut and elsewhere to co-store amines such as 5HT, dopamine, noradrenaline, histamine together with active peptides. In addition, secretory vesicle pH in G-cells may be increased by weak bases including dietary amines delivered via the lumen.(18)

![Diagram of trafficking and processing of progastrin](image)

**Fig 1.** Left: schematic representation of trafficking and processing of progastrin in the secretory pathway. In cells without vesicles of the regulated secretory pathway progastrin is secreted by the constitutive route. Cleavage at pairs of Arg and Lys residues, and COOH-terminal amidation, occur in vesicles of the regulated secretory pathway and the main products are G34 and G17. Their ratio can be influence by vesicle pH. **Right:** schematic of major cleavage sites in progastrin, and relative position of the phosphorylation site. Physiological casein kinase phosphorylates Ser-96 which inhibits cleavage at Arg-94,95. Increased secretory vesicle pH inhibits cleavage at Lys-74,75, so the main product (following cleavage at pairs of Arg residues and COOH-terminal amidation) is G34.

**GENTICALLY MODIFIED ANIMAL MODELS**

Transgenic mice over-expressing progastrin, Gly-gastrin and amidated gastrin have been described. In addition mice have been generated in which the gastrin or gastrin-CCK\_\_ receptor genes have been deleted by homologous recombination. In each case, new issues regarding the biology of gastrin have been raised. In addition there are many genetically modified mice strains with elevated plasma gastrin, mostly this can be attributed to decreased acid secretion, leading to reflex elevation of gastrin release from antral G-cells.(25;26)