Thrittene (S-13) Expression in the Gastric Mucosa.

Introduction:
Recently a peptide has been isolated from the mammalian GI tract that has considerable homology to the N-terminus of S-28 (1-2). This peptide, S-28 [1-13] (S-13), or thrittene, has not been previously described but is produced in endocrine cells in the stomach, intestine and pancreatic islets similar to the somatostatins (1-2). However, S-13 is also synthesized in GI neurons that do not contain somatostatin immunoreactivity. In addition, S-13 is produced in the GI tissues of mice with a targeted gene deletion of the prosomatostatin gene (1). These data indicate that S-13 is produced independently from prosomatostatin, possibly from a novel gene.

Rationale and/or Hypothesis:
It is important to determine thrittene's physiologic role. One of the ways in which this can be done is through examination of the tissue distribution. The stomach provides an important organ system because the gastric D endocrine cells are known to produce large amounts of somatostatin and it has previously been demonstrated that S-13 is produced in the gastric endocrine cells in prosomatostatin knockout mice. Since it has been shown that S-13 and the prosomatostatin active metabolites are produced independently we hypothesized that the two peptides may be produced in entirely different gastric endocrine cells.

Methods:
Immunofluorocytochemistry was used in order to test the hypothesis that S-13 and the products of prosomatostatin (S-14 and S-28) are produced and found in different cells. A dual-labeling experiment was set up using as primary antibodies MS12, a monoclonal mouse antibody that reacts with a C-terminal epitope on S-14 and S-28, but does not recognize S-13, and a polyclonal rabbit antisera that was raised against S-13. Fluorescently-labeled goat anti-mouse Cy3 (fluoresces red) and goat anti-rabbit AlexaFluor (fluoresces green) were added as secondary antibodies and the slides were analyzed using flourescent microscopy.

Results:
An S-13+ endocrine cell fluoresced green, an S-14/S-28+ cell fluoresced red, and using a dual filter co-localization of the two peptides showed as yellow. Images of the gastric mucosa were captured on a digital camera and analyzed. By visual examination, the majority of cells fluoresced yellow, with a small number showing only green-staining. A further quantitative analysis was done by capturing successive images of the gastric mucosa until 50 cells were counted. Quantitation was done by counting every cell as having co-localization (yellow) or S-13+ only (green with the absence of red). ~90% of the staining was co-localized and 10% of the cells expressed S-13 only without the somatostatins.

Conclusions & Significance:
Whenever a novel peptide is isolated it is important to understand its physiologic role, especially if it shares a sequence homology with part of a significant known peptide hormone such as somatostatin. From this experiment it has been shown that the majority of S-13 produced in the gastric mucosa does indeed come from the same endocrine cells that express prosomatostatin and synthesize S-14 and S-28. However, a small population of gastric mucosal cells exist that produce S-13 only, and do not contain the somatostatins. This finding provides further suuport for the independence of S-13 from prosomatostatin and may help to elucidate further functions of S-13 an