Subcellular fractionation studies of human gastric antrum: localization of the mucosal peptide hormones

J. DAWSON, M. G. BRYANT, S. R. BLOOM AND T. J. PETERS
Division of Clinical Cell Biology, Clinical Research Centre, Harrow, Middlesex and Department of Medicine, Royal Postgraduate Medical School, London

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Summary
1. Analytical subcellular fractionation techniques have been applied to endoscopic human gastric antral biopsies to study the localization of gastrin, somatostatin, vasoactive intestinal peptide and the properties of the principal subcellular organelles.
2. The peptide hormones, detected by radioimmunoassay, showed particulate localizations with single peaks in the density gradients for somatostatin (modal density 1.23) and vasoactive intestinal peptide (modal density 1.17). Gastrin showed a more complex distribution with a distinct peak (modal density 1.18) and a substantial shoulder extending into the denser regions of the gradient.
3. The following organelles, characterized by their marker enzymes, were located in the density gradients: plasma membrane (5'-nucleotidase), mitochondria (malate dehydrogenase), peroxisomes (catalase), lysosomes (β-N-acetyl-D-glucosaminidase), endoplasmic reticulum (neutral α-glucosidase), cytosol (lactate dehydrogenase).
4. This technique can be applied to investigate disease of the gastric antrum at a subcellular level.

Key words: cytosol, endoplasmic reticulum, enzymes, gastric mucosa, gastrointestinal hormones, lysosomes, mitochondria, peroxisomes, plasma membrane, stomach, subcellular fractions.

Introduction
The gastric antrum is the major source of the peptide hormone gastrin and also contains abundant quantities of other peptides, particularly somatostatin and vasoactive intestinal peptide. This has been well documented by studies on tissue extracts (Berson & Yalow, 1971; Malmstrom & Stadil, 1975; McIntosh, Arnold, Bothe, Becker, Kobberling & Creutzfeldt, 1978; Bryant & Bloom, 1979) and by immunocytochemistry (McGuigan, 1968; Polak, Pearse, Grimelius, Bloom & Arimura, 1975; Buffa, Capella, Solcia, Frigerio & Said, 1977). Although there is good ultrastructural evidence that gastrin is localized to the secretory granules of the G cells (Greider, Steinberg & McGuigan, 1972), the subcellular distribution of these three peptides in the human stomach has not been systematically investigated by biochemical techniques. In this study we have used analytical subcellular fractionation techniques to investigate the localization of these peptides and to compare their distributions in the cell with the principal organelles. These data will provide base-line information for future studies into storage granule or organelle abnormalities in a variety of gastric disorders.

Methods
Biopsies were obtained after a 12 h fast from five subjects undergoing endoscopy (Olympus GIFD2 Gastroduodenoscope) for non-specific abdominal symptoms, in whom no pathology was subsequently found. Six biopsies were taken from the gastric antrum in each subject at a constant site on the lesser curve 1 cm from the pylorus.
Two of the biopsies were placed in formalin/sodium chloride solution (150 mmol/l) (1:9, v/v), examined histologically and in each case found to be normal. The remaining four biopsies were immediately placed in 3 ml of ice-cold sucrose medium (0-3 mol/l) containing disodium EDTA (1 mmol/l), pH 7-4, and ethanol (22 mmol/l) (SVE medium). These biopsies were then disrupted with 10 strokes of a loose-fitting (type A) pestle in a Dounce homogenizer (Kontes Glass Co., Vineland, N.J., U.S.A.) and centrifuged at 800 g for 10 min. The pellet was then resuspended in a further 2 ml of SVE medium with three strokes of the loose-fitting pestle and centrifuged again. The low-speed pellet consisting of nuclei and undisrupted cells was resuspended in 2 ml of SVE medium with a tight-fitting (type B) pestle. The postnuclear supernatants were combined and 3-5 ml of this supernatant was layered on a 28 ml sucrose density gradient extending linearly with respect to volume from a cushion of ice-cold sucrose medium (0.3 mol/l to 1.05 mol/l) in the Beaufay small-volume automatic zonal rotor, as described previously (Peters, 1976). The rotor was accelerated to 35 000 rev./min and run for 35 min with an integrated force of 3-3 \times 10^{16} \, \text{rad}^2 \, \text{S}^{-1}. The rotor was then slowed to 8000 rev./min for automatic unloading and 15 fractions were collected into tared tubes. After re-weighing and mixing, the densities of the fractions were determined indirectly with an Abbé refractometer.

Enzyme activities of the gradient fractions, postnuclear supernatants and nuclear fractions were determined by microanalytical methods previously described (Peters, 1976). The total activities in the biopsy homogenates were calculated from the activities in the postnuclear supernatants and the nuclear fractions. The results were expressed as m-units of enzyme activity/mg of protein in the biopsy homogenates, where 1 unit is equal to 1 \, \mu mol of substrate transformed/min. Immediately after fractionation aliquots of the gradient fractions, postnuclear supernatants and nuclear fractions were mixed with equal volumes of hydrochloric acid to a final concentration of 0-1 mol/l to prevent proteolytic degradation of the hormones and the samples were deep-frozen at -20°C. These samples were then assayed with radioimmunoassays especially modified and optimized to the small quantities of tissue available (Bryant, Dawson, Bloom & Peters, 1980). The gastrin assay was developed to the pure porcine hormone and was C-terminally directed (Mitchell & Bloom, 1978). The antisera were tested for specificity by addition, of up to 2 mmol/assay tube, of bombesin, cholecystokinin, gastrin, glucose-dependent insulin-releasing peptide, glucagon, insulin, secretin, somatostatin, substance P or vasoactive intestinal peptide. In no case was any significant degree of cross-reactivity observed. The results were expressed as pmol/mg of protein in the biopsy homogenates. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard. The enzyme and hormone distribution results are expressed as frequency-density histograms. All calculations, plots and fraction recoveries (the percentage of the total homogenate content of the sucrose-density-gradient fractions) were performed by computer as described previously (Peters, 1976). These studies were approved by the local Ethical Committee.

Results

In all studies the immunoreactive hormone content of the sucrose-density-gradient fractions diluted in parallel to the respective standard curves. Sucrose solutions at concentrations found in the density gradient fractions were found to have no effect on the assays. Addition of HCl to a final concentration of 0-1 mol/l was not found to affect the recovered immunoreactive hormone content of any of the peptides in homogenates or
Table 1. Enzyme and hormone concentrations in gastric antral biopsy homogenates and post-nuclear supernatants

<table>
<thead>
<tr>
<th>Enzyme or Hormone</th>
<th>Concentration/activity in biopsy homogenates</th>
<th>Activity in postnuclear supernatant (% of total homogenates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrin</td>
<td>21.6 ± 3.4</td>
<td>96.2 ± 2.6</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>4.53 ± 1.36</td>
<td>81.7 ± 6.2</td>
</tr>
<tr>
<td>Vasoactive intestinal peptide</td>
<td>3.72 ± 0.81</td>
<td>63.3 ± 3.9</td>
</tr>
<tr>
<td>5'-Nucleotidase (EC 3.1.3.5)</td>
<td>3.91 ± 1.44</td>
<td>55.5 ± 4.1</td>
</tr>
<tr>
<td>Malate dehydrogenase (EC 1.1.1.37)</td>
<td>1744 ± 178</td>
<td>73.9 ± 3.0</td>
</tr>
<tr>
<td>Catalase (EC 1.11.1.6)</td>
<td>48.35 ± 7.63</td>
<td>64.9 ± 4.8</td>
</tr>
<tr>
<td>Lactate dehydrogenase (EC 1.1.1.27)</td>
<td>67.74 ± 9.25</td>
<td>76.1 ± 3.8</td>
</tr>
<tr>
<td>β-N-Acetyl-d-glucosaminidase (EC 3.2.1.30)</td>
<td>3.21 ± 0.78</td>
<td>71.1 ± 1.4</td>
</tr>
<tr>
<td>Neutral α-d-glucosidase (EC 3.2.1.20)</td>
<td>0.41 ± 0.13</td>
<td>61.1 ± 5.2</td>
</tr>
<tr>
<td>Total protein</td>
<td>5.43 ± 1.50</td>
<td>62.2 ± 4.7</td>
</tr>
</tbody>
</table>

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Table 1 shows the specific enzyme activities and hormone concentrations in the homogenates. The percentage activity in the postnuclear supernatant is also listed, reflecting the proportion of cells disrupted and the amount of enzyme or hormone activity not sedimented by the low-speed centrifugation.

Fig. 1 shows the distribution of the individual peptide hormones in the sucrose density gradients and represents averaged data from five patients. The distribution of protein is shown for comparison. In each case a small amount of peptide is found in the soluble fraction, but most of the hormone is present in particulate form in the density gradients. Gastrin shows a distinct particulate component, modal density 1.18, but has a shoulder extending into the denser regions of the gradient. Somatostatin and vasoactive intestinal peptide each have a single distinct particulate component with modal densities 1.23 and 1.17 respectively.

Fig. 2 shows the density distribution of the principal organelle marker enzymes in the sucrose density gradients. 5'-Nucleotidase, the plasma membrane marker, shows a major distinct peak of activity with modal density 1.13. The mitochondrial marker, malate dehydrogenase, has a dual localization with a large soluble component and a particulate component, modal density 1.17. Catalase shows a similar dual localization with a soluble component and a peak, modal density 1.20, reflecting the peroxisomes. The cytosol marker, lactate dehydrogenase, remains with the sample layer. The lysosomal marker β-N-acetyl-d-glucosaminidase shows a rather broad peak with a modal density of 1.19, and an appreciable soluble component. The endoplasmic reticulum marker neutral α-glucosidase shows a peak, modal density 1.18, with a small soluble component.

Discussion

This study is the first application of analytical subcellular fractionation to human gastric antral mucosa in which the principal organelles and also the secretory granules of three polypeptide hormones are characterized in terms of their isopycnic densities. Gastrin has, however, previously been shown to be associated with particulate fractions in chicken intestine (Blair, Sherratt & Wood, 1967), and in human gastrinomas and antral tissue (Track, Creutzfeldt, Junge & Creutzfeldt, 1975) by using differential pelleting centrifugation procedures, but the granules have not been fully separated or characterized. Vasoactive intestinal peptide has also been shown to have a particulate localization in rat brain (Giachetti, Said, Reynolds & Koniges, 1977; Besson, Rotsztejn, Laburthe, Epelbaum, Beaudet, Kordon & Rosselin, 1979) but has not been previously studied in gastric tissue.

In current experiments the distribution of gastrin, in contrast to vasoactive intestinal peptide and somatostatin, which each show a single sharp peak in the density gradient, is more complex, showing both a peak, modal density 1.18, and a substantial shoulder extending into the denser regions of the gradient. The modal density of the shoulder coincides with the peak of gastrin, previously found in the jejunum (Bryant et al., 1979; Dawson, Bryant, Gregor, Bloom & Peters, 1979). Recent morphological studies have shown that, whereas jejunal gastrin-secretory cells contain a single population of small electron-dense secretory granules, antral G cells contain,
FIG. 1. Isopycnic centrifugation of postnuclear supernatant from gastric antral biopsy homogenate from normal fasting subjects. Graphs show frequency–density histograms for gastrin, somatostatin, vasoactive intestinal peptide and protein and represent averaged results from five patients. Frequency (mean ± SD) is defined as the fraction of total recovered activity present in the individual fraction divided by the density span covered by that fraction. The activity present over the density span 1.05–1.10 represents, over an arbitrary abscissa interval, enzyme remaining in the sample layer and presumed to reflect soluble activity. Percentage recoveries (mean ± SE): gastrin, 107 ± 16; somatostatin, 80 ± 24; vasoactive intestinal peptide, 96 ± 6; protein, 74 ± 14.

in addition and in much greater numbers, large electron-lucent granules (Greider et al., 1972; Buchan, Polak, Solcia & Pearse, 1979). We could therefore speculate that the peak at 1.18 corresponds to the large electron-lucent granules, whereas the shoulder encompassing the jejunal peak corresponds to the small electron-dense granules, which are similar to those found in the jejunum. Furthermore, it should now be possible, by morphological studies and column chromatography of the gradient fractions, to test the hypothesis that the differential location of the two major forms of gastrin (G34, G17) in the jejunum and antrum respectively (Berson & Yalow, 1971) is due to location of these two molecular species in different granules (Buchan, Bryant, Timson, Polak & Bloom, 1979).

Somatostatin and vasoactive intestinal peptide each show a single sharp peak, indicating a single granule population for each which differs from that found for gastrin. The modal density for each granule corresponds to its jejunal counterpart (Bryant et al., 1979; Dawson et al., 1979). However, unlike gastrin, which is almost entirely released into the postnuclear supernatant by the homogenization procedure, a considerable quantity of each of these peptides remains in the low-speed pellet. This could be explained by a partial localization of each of these peptides to neural elements (Bryant, Bloom, Polak, Albuquerque, Modlin & Pearse, 1976; Hokfelt, Schultzberg, Johansson, Ljungdahl, Elfvin, Elde, Terenius, Nilsson, Said & Goldstein, 1978; Larsson, Polak, Buffa, Sundler & Solcia, 1979), which are not disrupted by the homogenization procedure designed to disrupt epithelial elements. Nevertheless these experiments indicate that a considerable proportion of the two peptides is
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![Graphs of enzyme density distributions](image)

**Fig. 2.** Isopycnic centrifugation of postnuclear supernatant from gastric antral biopsy homogenate from normal fasting subjects. Graphs show frequency–density histograms for organelle marker enzymes. For details see the legend to Fig. 1. Percentage recoveries (mean ± SE): 5′-nucleotidase, 85 ± 13; malate dehydrogenase, 77 ± 15; catalase, 91 ± 19; lactate dehydrogenase, 101 ± 27; β-N-acetyl-D-glucosaminidase, 66 ± 17; α-glucosidase, 108 ± 18.

present in readily liberated granules, which remain intact throughout the homogenization and fractionation procedures.

In studying the other principal subcellular organelles of the gastric antrum, a single well-characterized marker was chosen for each organelle. Thus 5′-nucleotidase was used as a plasma membrane marker (Solyom & Trams, 1972) and showed a similar density distribution to that previously shown in the jejunum with similar techniques (Peters, 1976). Similarly the mitochondria (malate dehydrogenase) showed a sharp peak similar in density to that found in the jejunum with equivalent proportion of soluble cytosolic enzyme. The presence of particulate catalase activity suggests the presence of peroxisomes in gastric antrum (Baudhuin, Beaufay, Rahman-Li, Sellinger, Wattiaux, Jacques & de Duve, 1964), and their density distribution is again similar to that found in the jejunum. Lactate dehydrogenase does not appear to adsorb to particles in the gradient and is thus a reliable cytosolic marker in this tissue. β-N-Acetyl-D-glucosaminidase was used as lysosomal marker (de Duve & Wattiaux, 1966) and also shows a similar density distribution to that found in the normal jejunum. Neutral α-glucosidase has previously been shown as a marker of the endoplasmic reticulum in human liver (Peters & Seymour, 1978) and human jejunum (Peters,
1976) and in these experiments shows a distinct peak with a slightly heavier modal density than that found in human jejunum.

These studies therefore have characterized the major organelles and secretory granules of three major regulatory peptides. In view of the profound ignorance regarding the underlying aetiology and pathogenesis of ulcer diathesis and processes involved in gastritis, extension of these studies to disease processes may begin to answer some of these more fundamental questions.

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References


