Increased sensitivity of gastrin cells to gastric distension following antral denervation in the rat

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1. Secretion of the antral hormone gastrin is increased by protein in the gastric lumen and by nervous reflexes. We have examined the relative importance of luminal and neuronal mechanisms, by lesioning the antral innervation using benzalkonium chloride.

2. Benzalkonium chloride was applied to the serosa of the antrum in anaesthetized rats. In some animals, a stainless-steel cannula was also implanted in the corpus. Animals were allowed 10 days to recover. Plasma gastrin was measured by radioimmunoassay and mRNAs encoding gastrin, somatostatin and histidine decarboxylase were measured by Northern blot.

3. Antral denervation was associated with gastric retention after fasting, and elevated plasma gastrin (28·4 ± 7 pM compared with 7·6 ± 1·0 pM in controls). When fasted control or denervated rats were refed, plasma gastrin increased 3-fold in both cases. A gastrin-releasing peptide antagonist inhibited the post-prandial rise in plasma gastrin in control rats, but had no effect in antrally denervated rats.

4. In fasted, antrally denervated rats with a gastric fistula, basal gastric acid secretion was depressed 3-fold, and plasma gastrin concentrations were similar to controls.

5. Distension of the stomach with peptone via a barostat attached to the gastric cannula (5 cmH_2O, 30 min), produced 3-fold increases in plasma gastrin in both control and denervated rats. However, distension with a non-nutrient solution at pH 6·0 had no effect in controls, but increased gastrin to a similar extent to peptone in denervated rats; distension with 50 mM HCl had no effect in either control or denervated rats.

6. Somatostatin and gastrin mRNA abundances in the antrum were depressed by about 35% by antral denervation, but somatostatin mRNA in the corpus was unchanged; GAPDH mRNA abundance was unaffected by antral denervation.

7. The data suggest that luminal nutrient releases gastrin in the rat, *in vivo*, via activation of antral neurons secreting gastrin-releasing peptide, and that the antral innervation normally inhibits G-cell responses to non-nutrient distension of the stomach. After antral denervation, gastric distension with a non-nutrient solution is an adequate stimulus for gastrin release.

The release of the antral hormone gastrin is controlled by the luminal contents of the stomach and by neurohumoral agents acting at the basolateral membrane of the gastrin (G-) cell. The main luminal stimuli are protein and protein-digestion products, and the main luminal inhibitory factor is gastric acid (Dockray & Gregory, 1989; Walsh, 1994). It is generally thought that gastric acid releases somatostatin from antral D-cells, which in turn inhibits gastrin release by a paracrine mechanism (Chiba *et al.* 1980; Saffouri, Weir, Bitar & Makhlouf, 1980; Walsh, 1994). The mechanisms by which luminal nutrients stimulate the G-cell are less certain. The apical membrane of the G-cell consists of a tuft of microvilli that project into the lumen, and this has led to the idea that luminal nutrients might act directly on these cells (Lichtenberger, 1982; Lichtenberger, Delansorne & Graziani, 1982; Delvalle & Yamada, 1990). However, it is also clear that luminal stimulation of the G-cell can be mediated by nervous pathways (Saffouri, DuVal & Makhlouf, 1984; Schubert, Coy & Makhlouf, 1992).

It is well recognized that vagal efferent neurons stimulate gastrin secretion in the cephalic phase of digestion, but in addition there are local nervous reflexes controlling the G-cell. In the rat there is evidence for cholinergic muscarinic stimulation of gastrin release (Saffouri *et al.* 1984; Schubert *et al.* 1992; Schubert & Makhlouf, 1993), but in dog and man

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atropine typically has no effect on nerve-mediated gastrin release, or stimulates it – indicating the importance of non-cholinergic excitatory inputs to the G-cell (Farooq & Walsh, 1975; Feldman, Richardson, Taylor & Walsh, 1979; Dockray & Tracy, 1980; Schiller, Walsh & Feldman, 1980). The neuropeptide gastrin releasing peptide (GRP), which is well represented in antral mucosal nerve fibres (Dockray, Vaillant & Walsh, 1979), is thought to be the main non-cholinergic neurotransmitter stimulating gastrin secretion. In keeping with this, both antibodies to GRP and selective GRP antagonists have been shown to inhibit gastrin secretion in response to luminal peptone, electrical field stimulation of the antrum or vagal nerve stimulation (Schubert, Saffouri, Walsh & Makhlouf, 1985; Holst, Harling, Messell & Coy, 1990; Schubert *et al.* 1992). In the present study we sought to define the relative importance for gastrin release of neural mechanisms compared with direct stimulation by luminal factors using a preparation in which the entire antrum is denervated. The results indicate an unexpected increase in sensitivity to non-nutrient distension after antral denervation, and suggest the existence of an antral inhibitory innervation in the rat that suppresses G-cell responses to non-nutrient distension.

METHODS

Animals

Wistar rats were kept on a 12 h light–12 h dark cycle, and weighed approximately 250 g on the day of operation. Rats were fasted overnight before surgery but were allowed free access to water. Rats were anaesthetized by I.M. injection of a mixture of fentanyl citrate (0·32 mg kg^{-1}), fluanisone (4 mg kg^{-1}) and diazepam (2 mg kg^{-1}).

Operative procedures

Antral denervation. The stomach was exteriorized through a mid-line upper abdominal incision. The anterior and posterior surfaces of the antrum were covered in turn with a thin layer of gauze, which was soaked with benzalkonium chloride (BAC, 0·5% w/v in saline); the gauze was left in place for 3 min and additional BAC added as needed to keep it moist. The surface of the stomach was rinsed thoroughly with normal saline and returned to the peritoneal cavity, and the incision closed in layers. Control animals underwent a sham operation with serosal application of vehicle (normal saline). Previous studies have established that treatment of the antrum with BAC using this protocol results in loss of over 95% of antral neurons identified by histology or assay of neuropeptide markers (Higham, Vaillant, Yegen, Thompson & Dockray, 1997). The success of the denervation procedure in the present study was checked in every animal at the end of the experiment; antrum was extracted in boiling water as previously described, and GRP determined by radioimmunoassay as a marker of the intrinsic antral innervation (Dockray *et al.* 1979; Higham *et al.* 1997). In all cases, tissue GRP concentrations in the antrum of rats treated with BAC were 5% or less (< 1 pmol g^{-1}) of control values.

Gastric cannulation. For some experiments rats were fitted with an indwelling gastric cannula. Antral denervation, or sham denervation, were performed with the stomach exposed through a right paramedian incision as described above. A small stainless-steel Gregory cannula was then installed in the corpus as previously described (Dimaline, Carter & Barnes, 1986). The cannula was exteriorized through a mid-line stab incision and the paramedian incision closed in layers. Animals were allowed to recover for at least 10 days before experiments.

Refeeding experiments

Rats were fasted for 48 h on wire-bottomed cages, and were then allowed to refeed for 30 min. The GRP antagonist BW2258U89 (2 mg kg^{-1} in 0·5 ml of 0·14 M saline; a gift from Dr J. Leban, Burroughs Wellcome, Research Triangle Park, NC, USA; Moody, Venugopal, Zia, Patierno, Leban & McDermed, 1995), or saline, were injected subcutaneously 30 min before refeeding. Rats were killed by cervical dislocation followed by decapitation, and trunk blood was taken for assay of plasma gastrin. Samples of antral and corpus mucosa were taken for RNA extraction (see below), or assay of GRP (see above), and the contents of the stomach were removed. The latter were dried at 50 °C for 48 h and then weighed.

Gastric fistula rats

Prior to experiments the stomach was washed extensively with warm 0·14 M NaCl to remove solids. Gastric acid output was determined by collecting gastric secretion over four sequential 15 min periods and titrating to pH 7·0 with 20 mM NaOH using a pH autotitrator (Radiometer, Copenhagen, Denmark) as previously described (Dimaline *et al.* 1986). Plasma gastrin responses were determined after gastric distension with the following test solutions: 2% (w/v) methyl cellulose (2% MC), 2% MC at pH 6 (50 mM ammonium acetate), 2% MC with 50 mM HCl and 4·5% (w/v) peptone. In each case the test meal was delivered to the stomach for 30 min by connecting the cannula to a reservoir maintained at a pressure of 5 cmH_2O relative to the stomach. Trunk blood was then taken for gastrin radioimmunoassay.

Gastrin radioimmunoassay

The concentration of amidated gastrins in plasma was assayed using antibody L2, and synthetic human non-sulphated G-17 as standard and for labelling with ^{125}I, as previously described (Dockray, Hamer, Evans, Varro & Dimaline, 1991).

Quantification of mRNA

Tissue mRNA was extracted and quantified as described previously (Dimaline, Evans, Varro & Dockray, 1991). Briefly, samples of mucosa were homogenized in guanadinium isothiocyanate and centrifuged on a caesium chloride cushion. The pelleted RNA was dissolved in 0·1% SDS and precipitated with 0·3 M sodium acetate under 100% ethanol overnight. Total mRNA (10 μg) was denatured and applied to 1% agarose gels, electroblotted onto nylon membranes and cross-linked with ultraviolet light. Membranes were hybridized for up to 72 h, and then washed as described (Dimaline, Sandvik, Evans, Forster & Dockray, 1993). Membranes were sequentially hybridized with probes to gastrin, histidine decarboxylase (HDC), somatostatin or glyceraldehyde phosphate dehydrogenase (GAPDH) mRNAs using cRNA probes (a gift from Dr R. Dimaline) labelled with [^{32}P]UTP using T7 RNA polymerase (Promega, Southampton, UK) and the TA cloning vector pCRTM2.1 (Stratagene, Cambridge, UK) containing the appropriate template as described previously (Dimaline *et al.* 1991, 1993; Dimaline, Evans, Forster & Dockray, 1994). Signals were quantified using a Phosphor Imager (Molecular Dynamics, Sevenoaks, Kent, UK) and expressed with reference to controls (100%).

Statistics

Results are expressed as means ± S.E.M. Comparisons were made using Student's t test or Mann–Whitney U test where variances were unequal. Statistical differences were considered significant at $P < 0·05$.

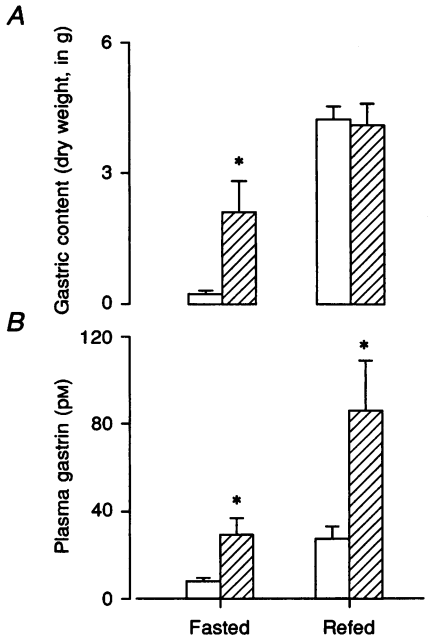

Figure 1. Plasma gastrin and gastric retention after antral denervation

Dry weight of gastric content (*A*) and plasma gastrin concentrations (*B*) in fasted and refed rats 10 days after either sham operation (□) or antral denervation by 0·5% BAC (▨). In fasted BAC-treated rats there was both retention of gastric content and elevated fasting plasma gastrin. After refeeding, plasma gastrin increased approximately 3-fold in both control and BAC-treated rats (means ± S.E.M., $n = 6$ for each group, * $P < 0·05$ *vs.* control, Mann–Whitney *U* test).

RESULTS

Hypergastrinaemia after antral denervation

Antral denervation was associated with fasting and post-prandial hypergastrinaemia. Thus basal plasma gastrin concentrations in antrally denervated rats fasted for 48 h were significantly elevated compared with control fasted rats (Fig. 1). Moreover, in rats treated with BAC to denervate the antrum and fed *ad libitum*, there were also elevated plasma gastrin concentrations (104 ± 8 pM) compared with intact control rats fed *ad libitum* (36 ± 5 pM; $P < 0·001$). We attribute the increased gastrin release from the denervated antrum to retention of food in the stomach, since after fasting for 48 h there was marked gastric retention in rats with a denervated antrum (Fig. 1); moreover, the fasting hypergastrinaemia in antrally denervated rats was corrected by gastric lavage. Thus in fasted antrally denervated rats with a gastric fistula, plasma gastrin concentrations after washing the stomach via the cannula (13·5 ± 3·2 pM) were not significantly different from those in fasted control rats with a gastric fistula (11·8 ± 2·0 pM).

Refeeding experiments

When control rats were fed for 30 min after fasting for 48 h, plasma gastrin increased approximately 3-fold. There was a comparable relative increase in plasma gastrin in BAC-treated rats, but the absolute increase after refeeding was higher than that in control rats, due to the elevated basal plasma gastrin concentration (Fig. 1). The GRP antagonist BW2258U89 (0·5 mg kg^{-1}) inhibited the rise in plasma gastrin concentrations in control rats seen after feeding for 30 min. In contrast, in antrally denervated rats, the post-prandial rise in gastrin was not dependent on GRP since there was no significant difference compared with denervated rats that received vehicle instead of GRP antagonist (Fig. 2).

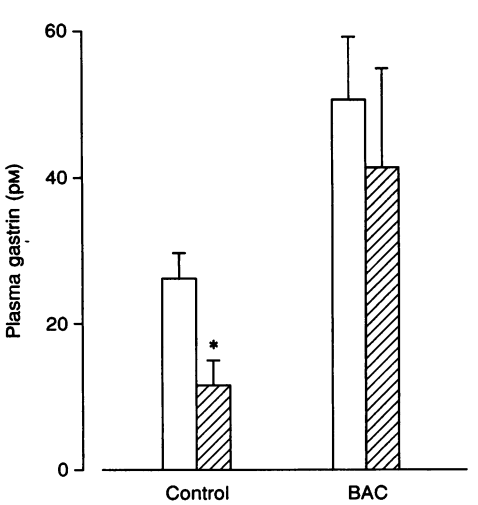

Figure 2. The effect of a GRP antagonist on the gastrin responses to food

Plasma gastrin concentrations in control (left) and antrally denervated rats (right) after administration of the specific GRP antagonist BW225U86 (▨) or saline (□) 30 min prior to feeding. In control, but not antrally denervated, rats the GRP antagonist significantly attenuated the post-prandial rise in plasma gastrin ($n = 6$ for all groups, * $P < 0·05$, Mann–Whitney *U* test).

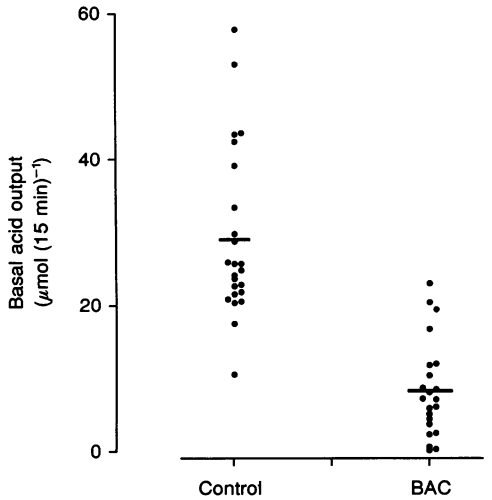

Figure 3. Basal acid output in control and antrally denervated rats

Mean basal acid output (μmol (15 min)$^{-1}$) in control and antrally denervated rats. Each data point represents the mean acid output of four sequential 15 min collections from one rat. In rats with antral denervation there was a significant reduction in basal acid output ($P < 0.05$).

Basal acid output

Because gastrin release is inhibited by luminal acid, we examined the possibility that antral denervation influenced acid secretion. The range of basal acid outputs in antrally denervated rats overlapped with that in normal rats, but the mean was approximately 3-fold lower (Fig. 3; 29·1 ± 2·4 *vs.* 8·3 ± 1·4 μmol (15 min)$^{-1}$; $P < 0.01$).

Distension experiments

The data outlined above suggest that changes in intragastric pH, intragastric pressure or nutrient (protein) might account for the increased gastrin release in rats with a denervated antrum following application of BAC. In order to assess separately the importance of these variables, we studied gastrin release after instillation of test meals via the gastric fistula in conditions in which each of them could be independently controlled. In both intact and antrally denervated rats, gastric distension at 5 cmH_2O with 4·5% (w/v) peptone for 30 min increased plasma gastrin about 3-fold, i.e. a similar increase to that produced by feeding (Fig. 4). There were, however, striking differences between control and antrally denervated rats in their responses to gastric distension by a non-nutritive solution. In initial studies, we found that distension with unbuffered methylcellulose stimulated gastrin release in antrally denervated rats, but not in controls. Because it seemed possible that acid inhibition of the G-cell might be reduced in denervated rats, these experiments were repeated using methylcellulose buffered to pH 6·0. In BAC-treated rats, the latter solution was again a good stimulus to gastrin release (the increase in plasma gastrin being comparable to that produced by peptone), but in control rats the same stimulus had no effect on gastrin release (Fig. 4). Finally, we examined the influence of intragastric acid (methylcellulose in 50 mM HCl) on gastrin release. In control rats there was a small decrease in plasma gastrin after distension at 5 cmH_2O for 30 min with HCl, which was not statistically significant. In antrally denervated rats there was no change in plasma gastrin after distension with acid, indicating that acid inhibits distension-evoked G-cell responses and this does not require the antral innervation.

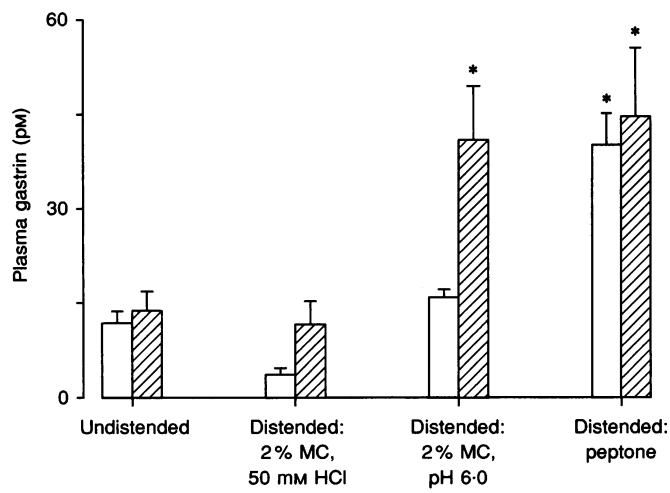

Figure 4. Plasma gastrin after gastric distension at controlled pressure and pH

Plasma gastrin responses after distension with 4·5% peptone were similar in control (□) and antrally denervated (▨) gastric fistula rats. In the latter, but not controls; plasma gastrin was also increased by distension with 2% MC buffered to pH 6·0. Neither group showed an increase in plasma gastrin after distension with 2% MC with 50 mmol HCl ($n = 6$–10 in each group, * $P < 0.05$ *vs.* control).

Gastrin, somatostatin and histidine decarboxylase (HDC) mRNA abundances

It is now well recognized that the physiological responses of gastric endocrine cells include modulation of hormone biosynthesis, and in part this is achieved by control of gene transcription (Dockray, Varro & Dimaline, 1996). We examined, therefore, whether antral denervation might alter the abundance of mRNA species encoding major endocrine cell products. In the antrum of BAC-treated rats, there was a significant (approximately 35%) reduction in somatostatin and gastrin mRNA abundances compared with controls (Fig. 5). In the corpus, however, somatostatin mRNA abundance was unchanged in antrally denervated rats compared with control rats, while HDC mRNA was slightly increased (Fig. 5). There was no difference in the expression of GAPDH between control and denervated rats, indicating that the changes in gastrin, somatostatin and HDC mRNAs were not a non-specific response.

DISCUSSION

The present study addresses the role of the antral innervation in controlling G-cell function. In previous work, the role of extrinsic neurons to the antrum has been examined by stimulation, lesioning or pharmacological blockade (Dockray & Gregory, 1989; Walsh, 1994), and the role of intrinsic neurons has been examined using neurotransmitter antagonists or immunoneutralization of neuropeptides (Schubert *et al.* 1985, 1992). This study used a chemical ablation method employing the detergent BAC, to lesion both the intrinsic and extrinsic innervation of the rat antrum. Our observations indicate that after antral denervation the post-prandial gastrin response is maintained, but it is no longer mediated by the peptide neurotransmitter GRP. Interestingly, we found that denervated, but not innervated, G-cells respond to gastric distension by non-nutritive solutions. The magnitude of the response to distension by non-nutritive solutions was sufficient to account for the post-prandial gastrin response after denervation. The results are unexpected since it is generally thought that G-cell responses to distension are mediated by nervous reflexes involving the antral innervation (Dockray & Gregory, 1989; Schubert & Makhlouf, 1993; Walsh, 1994).

The present data suggest that *in vivo*, antral neurons either directly or indirectly inhibit G-cell responses to increased intragastric pressure. Distension of the isolated perfused rat stomach is reported to inhibit gastrin release at low pressures, but stimulate it at high pressures (Schubert & Makhlouf, 1993). Both mechanisms are thought to be mediated by intrinsic antral neurons: there is cholinergic muscarinic mediation of the stimulatory pathway and vaso-active intestinal polypeptide (VIP) is thought to mediate the inhibitory pathway. In contrast in man, there appears to be a cholinergic inhibitory pathway mediating gastrin responses to distension (Schiller *et al.* 1980). Activation of antral inhibitory pathways would explain why gastric distension with methylcellulose did not stimulate gastrin release in the intact stomach. The novel finding in the present study is that there must exist a mechanism for distension-evoked release of gastrin, which is independent of the antral innervation.

Two types of mechanism might account for the observation that the denervated G-cell responds to non-nutritive distension of the stomach. First, the G-cell or a nearby cell in the antral mucosa might have an intrinsic mechanosensitivity (which is suppressed by the antral innervation). It has been recognized for many years that enterochromaffin (EC) cells are mechanosensitive (Bulbring & Lin, 1958; Kirchgessner, Tamir & Gershon, 1992; Wade, Chen, Jaffe, Kassem, Blakely & Gershon, 1996); denervated G-cells might therefore take on EC-cell-like properties, or alternatively antral EC cells might regulate G-cell function, for example through a paracrine mechanism. Second, non-nutritive distension of the stomach could release an endocrine factor from the gastric corpus, which increases gastrin release from the denervated,

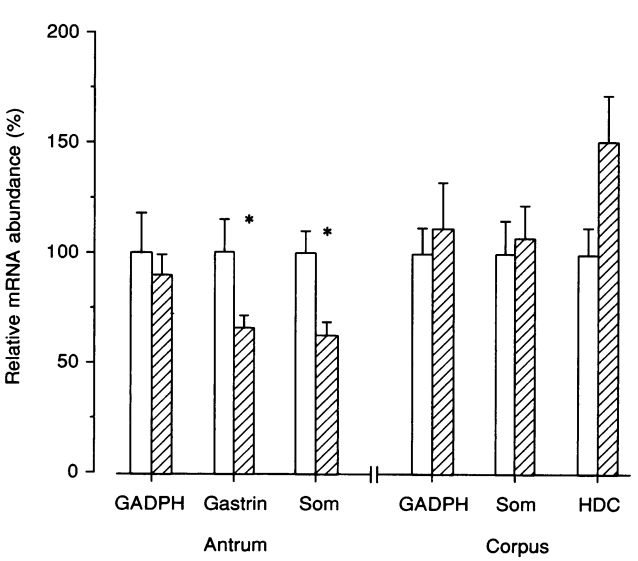

Figure 5. Relative abundance of mRNAs for gastrin, somatostatin and HDC in antrally denervated rats

Northern blots of antrum and corpus were separately prepared from total RNA recovered from control rats (□) and antrally denervated rats (▨). Membranes prepared from antral RNA were sequentially hybridized using probes for gastrin, somatostatin and GAPDH mRNA, and membranes prepared using corpus RNA were sequentially hybridized using probes for somatostatin (Som), HDC and GAPDH mRNA. The relative abundance of each mRNA species has been normalized by taking as 100% the abundance in control samples. Note that the abundance of GAPDH mRNA, which was used as a non-regulated marker, was similar in control and denervated rats. In the antrum, denervation decreased gastrin and somatostatin mRNA. Values are $n = 6$ in all cases; * $P < 0{\cdot}05$, Student's t test.

but not innervated, antrum. In the dog, Debas and co-workers showed that distension of the corpus increased gastrin release from an extrinsically denervated antral pouch, suggesting the existence of an oxynto-pyloric endocrine reflex (Debas, Walsh & Grossman, 1975). A similar reflex in the rat could explain the present findings, although it would appear that such a reflex is not normally activated in the innervated antrum.

In antrally denervated rats fed *ad libitum* there is persistent elevation of plasma gastrin that we attribute to the effects of gastric distension secondary to impaired emptying, which is a feature after antral denervation. The increased gastrin secretion must be supported by increased synthesis. We found that there was depression of gastrin mRNA abundance in antrally denervated rats, suggesting that the compensatory mechanisms controlling gastrin synthesis might operate at the level of mRNA translation; in this context it is interesting that other recent work suggests that gastrin synthesis can be independently regulated at both transcriptional and translational levels (Bate, Varro, Dimaline & Dockray, 1996). Generally, gastrin and somatostatin mRNA abundances change in opposite directions (Brand & Stone, 1988; Wu, Giraud, Mogard, Sunii & Walsh, 1990; Sandvik, Dimaline, Forster, Evans & Dockray, 1993). It was therefore interesting to find that after antral denervation both gastrin and somatostatin mRNA abundance decreased. These changes may be attributable to the loss of the antral GRP innervation since GRP stimulates both G- and D-cells (Schubert, Jong & Makhlouf, 1991). In the corpus, HDC mRNA abundance was slightly increased after antral denervation; this is in keeping with the observation of increased plasma gastrin concentrations given that gastrin is a major stimulant of enterochromaffin-like (ECL) cells, which are an important source of histamine and HDC (Dimaline *et al.* 1993). Increased histamine synthesis and secretion might be expected to stimulate increased acid output, and the reduced acid secretion we observed would therefore appear not to be secondary to inhibition at the level of the ECL cell.

The gastric responses to antral denervation exhibit some features that are similar to those seen after pyloric outlet obstruction, and others that are distinct. In both cases there is gastric retention secondary to decreased emptying. Chronic pyloric obstruction, like antral denervation, leads to hypergastrinaemia (Feurle, Tischbirek & Baca, 1988; Chen, Zhao, Nylander, Sundler & Hakanson, 1995). However, pyloric obstruction is associated with increased acid secretion, whereas antral denervation leads to decreased acid output. It appears that the increased acid secretion following obstruction is not due to enhanced production of histamine by ECL cells, since HDC enzyme activity is unchanged (Chen *et al.* 1995; Zhao, Chen, Monstein, Ding, Sundler & Hakanson, 1996), whereas after antral denervation we found a tendency for increased HDC mRNA abundance. It is thought that the increased acid secretion after obstruction is due to activation of a vago-vagal reflex originating in the pylorus (Brodie & Knapp, 1966). This pathway would plainly be eliminated by antral denervation; however, reflexes originating in the corpus should be preserved and might account for the depressed acid secretion in the presence of chronic retention (Dimaline *et al.* 1986).

It is well recognized that as a group, gut endocrine cells respond to a wide variety of different stimuli. Different populations of endocrine cells are stimulated by the major classes of nutrient and by a variety of non-nutrient factors (pH, mechanical stimuli). The relevant transduction mechanisms are not well understood, nor for that matter is it known what determines the adequate stimulus for a particular endocrine cell type. The present data show that G-cell responses to different stimuli are modulated by the antral innervation. There is therefore a plasticity in endocrine cell responsiveness that may be relevant both to an understanding of disease processes and to the mechanisms of adaptation in different physiological states. Further work will be needed to determine the cellular mechanism of the changed sensitivity.

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