Biochem. J. (1992) 286, 737–741 (Printed in Great Britain)

Expression of ileal glucagon and peptide tyrosine-tyrosine genes

Response to inhibition of polyamine synthesis in the presence of massive small-bowel resection

Russell G. TAYLOR,* Dianne J. BEVERIDGE† and Peter J. FULLER†‡
*Department of Surgery, Royal Children's Hospital, Parkville, Australia
and †Prince Henry's Institute of Medical Research, Melbourne, Australia

Massive small-bowel resection results in a marked adaptive response in the residual terminal ileum. Increased polyamine synthesis is a necessary component of this response. The ileal L-cell-derived peptides enteroglucagon and peptide tyrosine tyrosine (PYY) have been implicated as humoral mediators of this response. We have previously reported a rapid and sustained increase in glucagon mRNA concentrations after massive small-bowel resection. In this study using an inhibitor of the rate-limiting enzyme in polyamine biosynthesis, ornithine decarboxylase, we have demonstrated that the response of the glucagon and PYY genes to massive small-bowel resection is dependent on polyamine biosynthesis. In addition, we have examined the response of both the ornithine decarboxylase and c-*jun* genes in this model of intestinal adaptation.

INTRODUCTION

Polyamine synthesis is thought to be required for the initiation of rapid cellular growth and differentiation as seen in the intestinal mucosa after massive small-bowel resection (MSBR) (Bristol & Williamson, 1988). The basal activity of ornithine decarboxylase (ODC), the first and rate-limiting enzyme in polyamine biosynthesis, is low in quiescent cells and tissues. Luk & Baylin (1983) have shown that increased mucosal ODC activity and increased polyamine content are intimately associated with the rapid increase in cell proliferation during the adaptive response of residual intestine after 50% intestinal resection. Inhibition of ODC activity by bis-α-difluoromethylornithine (DFMO) has been shown to decrease polyamine synthesis and mucosal hyperplasia after MSBR (Luk & Baylin, 1984).

The observed increase in polyamine synthesis, which appears critical to the mucosal hyperplasia, must itself be stimulated by factors such as luminal nutrition, pancreaticobiliary secretions or humoral/luminal growth factors. Savage *et al.* (1985) have reported increased plasma concentrations of both enteroglucagon and peptide tyrosine-tyrosine (PYY) after MSBR. Enteroglucagon and PYY are co-localized to the L-cells of the gastrointestinal tract (Ali-Rachedi *et al.*, 1984). Enteroglucagon is commonly considered to be the enterotrophic humoral factor involved in post-MSBR adaptation (Bristol & Williamson, 1988), although PYY has also been postulated to be a trophic factor (Adrian *et al.*, 1987; Goodlad *et al.*, 1989).

We have reported an increase in glucagon gene expression after MSBR, but, like other workers, have not been able to determine whether this initiated the adaptation (Taylor *et al.*, 1990).

In this study we have examined the effect of the inhibition of polyamine biosynthesis by DFMO on the response of the ileal glucagon gene to MSBR. In addition, we have defined the pattern of ODC gene expression in the gastrointestinal tract and compared the response to DFMO administration of the ODC gene with that of the glucagon gene.

MATERIALS AND METHODS

Surgical preparation of rats

An 80% resection of the small bowel was performed as described previously (Taylor *et al.*, 1990). In brief, female adult Sprague–Dawley rats were anaesthetized with halothane and oxygen, a midline laparotomy incision was made, and the bowel was exteriorized. Approx. 80% of the small gut was resected leaving 5 cm of jejunum distal to the ligament of Treitz and 5 cm of the ileum proximal to the ileocaecal valve. A single-layer anastomosis with interrupted 6/0 prolene was then completed. Experimental samples were obtained from the middle of the residual bowel at least 2 cm away from the anastomosis. During the postoperative period, the animals had free access to water and food. The DFMO (Merrell Down Research Institute, Cincinnati, OH, U.S.A.) was administered as a 2% solution in the drinking water (Luk & Baylin, 1984) beginning on the day before surgery. Animals were killed by cervical dislocation; the intestinal specimens were removed, washed in normal saline, snap-frozen in liquid nitrogen and stored at −70 °C.

mRNA analysis

Total RNA was isolated using the method of Chirgwin *et al.* (1979). Northern-blot analysis was performed as described previously (Taylor *et al.*, 1990). Briefly, 12.5 μg of total RNA was denatured in 1 M-glyoxal/50% dimethyl sulphoxide, electrophoresed in a 1.2% agarose gel and transferred to Hybond nylon membranes (Amersham, Bucks., U.K.). The membranes were baked at 80 °C for 2 h, u.v.-cross-linked for 10 min, prehybridized at 42 °C, and hybridized overnight at 60 °C in the hybridization solution of Leiter *et al.* (1987) [50% formamide, 5 × SSPE (1 × SSPE is 0.15 M-NaCl/0.010 M-sodium phosphate/0.001 M-EDTA, pH 7.4), 0.15 M-Tris/HCl, pH 8, 1% sodium SDS and 500 mg of heparin/ml] for complementary RNA (cRNA) probes. For the cDNA probe, prehybridization and hybridization was in a conventional hybridization solution at 42 °C (Taylor *et al.*, 1990). The blots were then washed once at room temperature in 2 × SSC (1 × SSC is 0.15 M-NaCl/0.015 M-sodium citrate, pH 7)/0.1% SDS, and then twice for 20 min at 60 °C (cRNA probe) or 50 °C (cDNA probe). Membranes were then blotted dry and exposed to Kodak X-AR (Eastman Kodak, Rochester, NY, U.S.A.) or Fuji (Fuji Photo Film, Osaka, Japan) X-ray film with a Cronex Lightening Plus intensifying screen (DuPont, Wilmington, DE, U.S.A.) at −80 °C.

Before rehybridization with a different probe, blots were

Abbreviations used: MSBR, massive small-bowel resection; ODC, ornithine decarboxylase; DFMO, di-α-difluoromethylornithine; PYY, peptide tyrosine-tyrosine; 1 × SSPE, 0.15 M-NaCl/0.01 M-sodium phosphate/0.001 M-EDTA, pH 7.4; 1 × SSC, 0.15 M-NaCl/0.015 M-sodium citrate, pH 7.

‡ To whom correspondence should be addressed.

placed in boiling distilled water for 2 min, re-exposed to determine the adequacy of removal of the previous probe, then pre-hybridized, and hybridized as described above. Relative mRNA concentrations were determined by scanning densitometry (ISCO gel scanner 1312, ISCO Inc., Lincoln, NE, U.S.A.).

Probes

The rat glucagon and PYY cRNA probes have been described previously (Leiter *et al.*, 1987; Taylor *et al.*, 1990). The ODC cRNA probe was constructed by subcloning an approx. 1 kb *Taq*I–*Pst*I fragment of the rat ODC cDNA clone, ODC-EIO (van Kranen *et al.*, 1987) into the plasmid pGEM-4Z (Promega Biotech, Madison, WI, U.S.A.). This construct was linearized at the *Bam*HI site in the vector for cRNA synthesis. The rat c-*jun* cRNA probe was constructed by subcloning an approx. 440 bp PCR product into pGEM-4Z for dideoxy sequencing and cRNA synthesis. The PCR reaction involved amplifying from rat genomic DNA the region of the rat c-*jun* cDNA from bases 892 to 1290 using four oligonucleotide primers corresponding to the published rat cDNA (Sakai *et al.*, 1989) and genomic (Kitabayashi *et al.*, 1990) sequences according to standard protocols (Sambrook *et al.*, 1989).

The cRNA probes were labelled using SP6 (glucagon and c-*jun*) or T7 (PYY and ODC) polymerases (Promega) and [α-^{32}P]UTP (> 400 Ci/mmol; BRESA, Adelaide, South Australia, Australia). The rat tubulin cDNA probe (Lemischka *et al.*, 1981) was labelled by nick translation (Amersham Nick Translation Kit, Amersham, Bucks., U.K.) with [α-^{32}P]dCTP (1800 Ci/mmol; BRESA).

RESULTS

As seen in Fig. 1, the marked increase in glucagon mRNA concentrations that occurs in the terminal ileum after MSBR is significantly attenuated by co-administration of the ODC inhibitor, DFMO. The pattern seen with the PYY cRNA probe (Fig. 1) is essentially the same as that for glucagon, suggesting that the response is an 'L-cell' rather than a specific 'glucagon gene' response. In Table 1 the results from the experiment shown in Fig. 1 are combined with those of other Northern-blot analyses from two separate experiments and the results quantified. The approx. 3-fold increase in glucagon mRNA concentrations after MSBR is decreased to less than a 2-fold increase in the presence of DFMO. As in our previous study (Taylor *et al.*, 1990), glucagon mRNA concentrations have been corrected for any potential loading artifacts or between-experiment variation by comparison with the tubulin cDNA probe; the results are expressed as a ratio of glucagon to tubulin mRNA concentrations.

The ODC gene is expressed widely though not uniformly in the gastrointestinal tract (Fig. 2). Three ODC mRNA species of approx. 2.6, 2.1 and 1.7 kb were observed as has been reported previously in other tissues (van Kranen *et al.*, 1987; Wen *et al.*, 1989). The 2.1 kb transcript was the predominant species in all tissues examined.

The observed increase in ODC mRNA concentrations after MSBR (Fig. 1) is surprisingly modest given the dramatic increases in ODC activity that are reported (Luk & Baylin, 1983). This increase is difficult to quantify because of the multiple mRNA species seen for ODC, though no change in the relative intensity of these various species was observed between groups. When these mRNA species are combined and analysed as for glucagon mRNA concentration, there is an increase in the ODC mRNA concentration after MSBR. This increase in ODC mRNA concentrations is present 18 h after MSBR, thereby preceding the rise in glucagon mRNA concentration (Taylor *et al.*, 1990), and remains constant at 1, 2 and 4 days after MSBR (D. T. Beveridge, P. J. Fuller & R. G. Taylor, unpublished work). Even more surprising than this relatively modest increase in ODC mRNA concentration after MSBR is the lack of a compensatory increase in ODC mRNA in either group receiving the enzyme inhibitor, DFMO (Table 1). In fact, the concentrations of ODC mRNA in the rats that received both MSBR and DFMO were significantly lower than in rats subjected to MSBR alone.

The above samples were also probed for the proto-oncogene c-*jun* mRNA. This was intended as a marker of cell proliferation. Curiously there is no significant response, after correction for tubulin mRNA concentration of c-*jun* mRNA concentration after MSBR, although a significant decrease was observed after

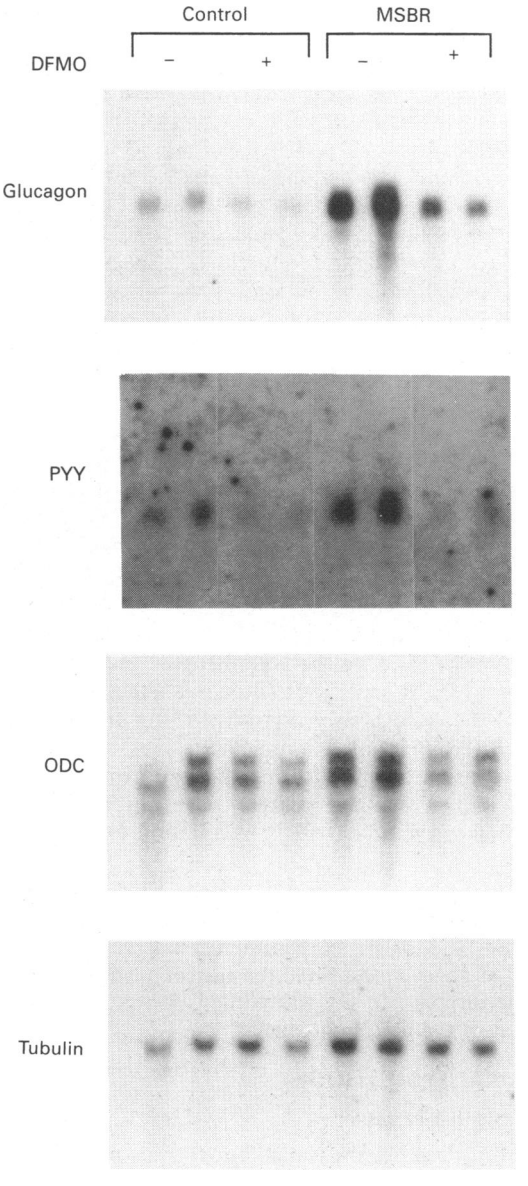

Fig. 1. Hybridization of glucagon, PYY, ODC and tubulin probes sequentially to a representative Northern blot of 12.5 μg of ileal RNA

Each lane is from an individual rat subjected to MSBR or control receiving either DFMO or drinking water alone as indicated.

Table 1. Effect of MSBR with or without DFMO treatment on ileal mRNA concentrations

Effect of DFMO on ileal glucagon, ODC and c-*jun* mRNA concentrations in control rats and rats 4 days after MSBR. The Northern blots were sequentially hybridized with glucagon, ODC or c-*jun* cRNA probes, autoradiographed, boiled, rehybridized with a tubulin cDNA probe and then re-autoradiographed. The mRNA concentrations were quantified by scanning densitometry. The ratio of glucagon, ODC or c-*jun* to tubulin concentrations for each individual rat was calculated. Results are expressed as means ± S.E.M. with the control taken as 100% ($n = 5$, $^*P < 0.05$, $\dagger P < 0.01$, $\ddagger P < 0.001$, compared with the control or as indicated.

	Control	Control +DFMO	MSBR	MSBR +DFMO
Glucagon	100 ± 5	86 ± 21	316 ± 25‡	183 ± 31*
ODC	100 ± 22	109 ± 24	128 ± 16	61 ± 5
c-*jun*	100 ± 10	64 ± 6*	110 ± 22	93 ± 12

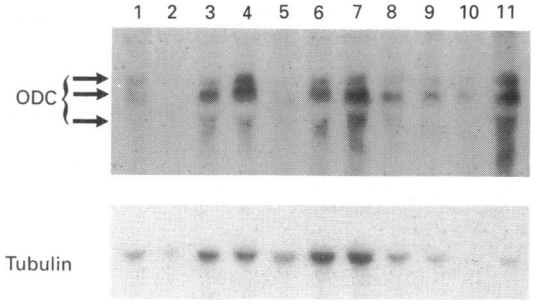

Fig. 2. Hybridization of the ODC and tubulin probes to the same Northern blot of approx. 12.5 μg of corpus (lane 1), antrum (lane 2), duodenum (lane 3), jejunum (lane 4), ileum (lane 5), caecum (lane 6), ascending colon (lane 7), transverse colon (lane 8), descending colon (lane 9), liver (lane 10) and kidney (lane 11) total RNA

DFMO treatment in the control group (Table 1); this is in contrast with the other genes analysed.

DISCUSSION

The intestinal adaptation that occurs after small-bowel resection is perhaps analogous to renal compensatory hypertrophy and hepatic regeneration; both of these are also models of growth and differentiation. Like these other models, however, there has been very limited molecular analysis of ileal adaptation. The structural changes that occur after MSBR (Booth *et al.*, 1959; Bristol & Williamson, 1988), which include dilatation, muscle-wall hypertrophy and mucosal hyperplasia, are well characterized, as is the increase in absorption per unit length which is aided by decreased motility (Dowling & Booth, 1967). The mediators of this response are not well characterized, although luminal nutrition, pancreatico-biliary secretion, humoral growth factors and luminal growth factors have all been implicated (Bristol & Williams, 1988; Sagor *et al.*, 1982; Al-Mukhtar *et al.*, 1983). Polyamine biosynthesis, as in most situations involving cell proliferation, appears to be important, although which of the aforementioned mediators is stimulating the increased polyamine biosynthesis is not clear.

Isolated loops of bowel normally become hypoplastic, but, after MSBR, this process is reversed and hyperplasia may occur (Hanson *et al.*, 1977; Williamson & Bauer, 1978), arguing strongly for the presence of a humoral mitogenic factor. Enteroglucagon has been identified as a potential humoral mediator of intestinal adaptation [for a review see Bristol & Williamson (1988)]. Numerous studies have reported increased plasma enteroglucagon concentrations in the context of intestinal adaptation and we have reported increased glucagon gene expression in the residual terminal ileum after MSBR (Taylor *et al.*, 1990). By measuring proglucagon mRNA concentrations we have used a direct measure of synthetic activity and have also avoided the confounding variable, differential processing of the pre-peptide (Mosjov *et al.*, 1986), which limits studies of peptide concentrations. The increase in proglucagon mRNA concentrations in the residual terminal ileum after MSBR is prompt and sustained; this increase is partially attenuated by starving and is specific to the ileum, there being no increase in the concentrations of proglucagon mRNA in either the jejunum or colon (Taylor *et al.*, 1990). Lund *et al.* (1990) have also reported an increase in proglucagon mRNA concentrations at 1 and 5 weeks after MSBR.

In the ileum, proglucagon is synthesized by a subpopulation of endocrine cells, the L-cells which also synthesize PYY. Plasma concentrations of PYY are also elevated after MSBR (Savage *et al.*, 1985). The cloning by Leiter *et al.* (1987) of the rat PYY cDNA has enabled us to demonstrate that PYY mRNA concentrations after MSBR parallel those of proglucagon, suggesting that the 'glucagon response' after MSBR should really be viewed as an L-cell response. PYY has been suggested as a possible humoral growth factor (Adrian *et al.*, 1987; Goodlad *et al.*, 1989); however, it appears more likely that its role in intestinal adaptation is to decrease intestinal motility, the so-called 'ileal brake' (Spiller *et al.*, 1988; Goodlad *et al.*, 1990), thereby allowing increased contact time between nutrients and the mucosa (Goodlad *et al.*, 1990).

Polyamines, the ubiquitous low-M_r organic cations, putrescine, spermidine and spermine, are required for cell growth (Scalabrino *et al.*, 1991). Luk & Baylin (1983, 1984) have demonstrated that polyamine biosynthesis is increased in the first 48 h after MSBR and that inhibition of ODC, the rate-limiting enzyme in polyamine biosynthesis, by DFMO inhibits the adaptive response. In control rats, an effect of DFMO on the small intestine has been reported in weaning but not adult rats (Alarcon *et al.*, 1987). Administration of putrescine *in vivo* (Seidel *et al.*, 1985) and *in vitro* (Ginty *et al.*, 1989) increase intestinal epithelial cell proliferation. Similarly when endogenous putrescine concentrations are increased by using aminoguanidine to block diamine oxidase, enhanced ileal proliferation after MSBR results (Erdman *et al.*, 1989).

The ability of DFMO administered in the drinking water to block ileal adaptation after MSBR has been demonstrated by several groups (Luk & Baylin, 1984; Kingsnorth *et al.*, 1986; Hosomi *et al.*, 1987). Using this experimental approach the response of the glucagon gene was examined. As expected, DFMO administration did not alter ileal glucagon mRNA concentrations in the control rats, which is congruent with previous reports indicating little or no effect in normal adult small bowel (Alarcon *et al.*, 1987). By contrast, mRNA concentrations of the proto-oncogene c-*jun* were depressed to approx. 64% of control. Our working hypothesis was that if the L-cell response was a primary one, then DFMO administration should either fail to affect, or perhaps enhance, the increase in glucagon mRNA concentration after MSBR, particularly as the L-cell

response does not appear to depend on L-cell proliferation (P. J. Fuller, D. J. Beveridge & R. G. Taylor, unpublished work). Conversely, inhibition of the L-cell response would suggest that the changes are secondary to the increase in polyamine biosynthesis after MSBR. The observed attenuation of both the glucagon and PYY mRNA concentrations after MSBR in the DFMO-treated rats argues that the L-cell response plays a secondary role, albeit an early one (Taylor *et al.*, 1990) in the adaptive response.

In contrast with the aforementioned studies examining the effect of DFMO administration on intestinal adaptation, the availability of an ODC probe (Van Kranen *et al.*, 1987; Wen *et al.*, 1989) has enabled us to examine the distribution of ODC gene expression in the rat gastrointestinal tract and to determine the concentrations of ileal ODC mRNA after MSBR. ODC mRNA is distributed throughout the gastrointestinal tract with the exception of the antrum of the stomach where, even allowing for a loading artifact, the concentrations are low or absent (Fig. 2).

Consistent with the studies of ODC activity, ODC mRNA concentrations were increased relative to tubulin after MSBR. We postulated that the DFMO administration would result in increased ODC synthesis via either product depletion or substrate accumulation. The converse is true in that putrescine administration has been shown to decrease ODC activity, although not ODC mRNA concentration (Ginty *et al.*, 1990). The observed fall in ODC mRNA concentration after MSBR in the presence of DFMO was unexpected, but may occur via two possible mechanisms. First, ODC synthesis appears to be primarily regulated at a translational level; this is reflected in our observation of a modest increase in ODC mRNA concentration after MSBR whereas, under the same conditions, ODC activity is increased more than 100-fold (Luk & Baylin, 1983). A similar pattern of dissociation is observed *in vitro* (Ginty *et al.*, 1990), whereas a good correlation is observed between ODC peptide concentration and activity (Ginty *et al.*, 1990). This translational regulation may be mediated via the 5′-untranslated region of the ODC mRNA (Grens & Scheffler, 1990; Ito *et al.*, 1990; Manzella & Blackshear, 1990), Van Steeg *et al.*, 1991), although the mechanism of polyamine-mediated regulation is controversial (Van Daalen Wetters *et al.*, 1989; Ito *et al.*, 1990; Van Steeg *et al.*, 1991). Secondly, much of the ODC activity has recently been localized to the differentiated non-proliferating villus-tip cells, the increased numbers of which after MSBR are inhibited by DFMO, presumably via a smaller (40-fold) but more critical pool of ODC activity in the proliferating crypt cells (Luk & Yang, 1988). These two pools of mucosal ODC activity appear to be differently regulated (Iwami *et al.*, 1990). Any increase in crypt-cell ODC mRNA concentration after MSBR may therefore be masked by changes in the considerably more numerous villus cells and their ODC mRNA content.

The assessment of c-*jun* mRNA concentration was intended as a simple relatively non-specific marker of cell proliferation. Proto-oncogene and/or early-response gene expression has not previously been examined in this model; the lack of an increase in c-*jun* mRNA concentration after MSBR was unexpected, although it may be that an increase occurs early after MSBR, as is seen in hepatic regeneration (Mohn *et al.*, 1990). We have not observed a significant increase in c-*jun* mRNA concentration at 18 or 24 h after MSBR (D. J. Beveridge, P. J. Fuller & R. G. Taylor: unpublished work). The decrease in c-*jun* mRNA concentration observed in control ileum after administration of DFMO indicates that, despite the earlier studies (Alarcon *et al.*, 1987), polyamines are active in normal ileum.

In conclusion, these studies provide further evidence that the response of the ileal L-cells to MSBR is secondary to increased polyamine synthesis. In addition, the lack of response of the ODC mRNA concentration to enzyme inhibition is consistent with recent studies which indicate that the regulation of ODC is translational. These findings do not of course exclude a role for L-cell-derived peptides in the adaptive response; indeed the increase in glucagon mRNA concentration after MSBR remains the most striking and robust molecular response to MSBR.

We thank Dr. H van Stegg and Dr. P. J. Blackshear for the gift of ODC cDNA clones and Dr. A. Leiter for the gift of the PYY cDNA clone. We also thank Judith Clements and Karen Verity for their critical reading of the manuscript, and Jeana Thomas, Sharyn Relf and Sue Panckridge for preparation of the manuscript. This work was supported by the Royal Children's Hospital Research Foundation. P. J. F. is a Wellcome Australian Senior Research Fellow in Medical Science.

REFERENCES

Adrian, T. A., Savage, A. P., Fuessl, H. S., Wolfe, K., Besterman, H. G. & Bloom, S. R. (1987) Surgery **101**, 715–719
Alarcon, P., Lebenthal, E. & Lee, P. C. (1987) Digest. Dis. Sci. **32**, 883–888
Ali-Rachedi, A., Varndell, I. M., Adrian, T. E., Gapp, D. A., van Noorden, S., Bloom, S. R. & Polak, J. M. (1984) Histochemistry **80**, 487–491
Al-Mukhtar, M. Y. T., Sagor, G. R., Ghatei, M. A., Bloom, S. R. & Wright, N. A. (1983) Br. J. Surg. **70**, 398–400
Booth, C. C., Evans, K. T. & Menzies, T. (1959) Br. J. Surg. **46**, 403–410
Bristol, J. B. & Williamson, R. C. N. (1988) Pediatr. Surg. Int. **4**, 233–241
Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) Biochemistry **18**, 4294–4299
Dowling, R. E. & Booth, C. C. (1967) Clin. Sci. **32**, 139–149
Erdman, S. H., Park, J. H. Y., Thompson, J. S., Grandjean, C. J., Hart, M. H. & Vanderhoof, J. A. (1989) Gastroenterology **96**, 1533–1538
Ginty, D. D., Osborne, D. L. & Seidel, E. R. (1989) Am J. Physiol. **257**, G145–G150
Ginty, D. D., Marlowe, M., Pekala, P. H. & Seidel, E. R. (1990) Am J. Physiol. **258**, G454–G460
Goodlad, R. A., Ghatei, M. A., Domin, J., Bloom, S. R., Gregory, H. & Wright, N. A. (1989) Experientia **45**, 168–169
Goodlad, R. A., Ghatei, M. E., Domin, J., Bloom, S. R. & Wright, N. A. (1990) Digestion **46** (Suppl. 2), 177–181
Grens, A. & Scheffler, I. A. (1990) J. Biol. Chem. **265**, 11810–11816
Hanson, W. R., Rijke, R. P. C., Plaisier, H. M., Van Ewijk, W. & Osborne, J. W. (1977) Cell Tissue Kinet. **10**, 543–555
Hosomi, H., Lirussi, F., Stace, N. N., Vaja, S., Murphy, G. M. & Dowling, R. H. (1987) Gut **28** (S1), 103–107
Ito, K., Kashiwagi, K., Watanabe, S., Kameji, T., Hayashi, S. & Igarashi, K. (1990). J. Biol. Chem. **265**, 13036–13041
Iwami, K., Wang, J.-Y., Jain, R., McCormack, S. & Johnson, L. R. (1990) Am. J. Physiol. **258**, G308–G315
Kingsnorth, A. N., Abu-Khalaf, M., LaMuraglia, G. M., McCann, P. P., Dickema, K. A., Ross, J. S. & Malt, R. A. (1986) Surgery **88**, 721–727
Kitabayashi, I., Saka, F., Gachelin, G. & Yokoyama, K. (1990) Nucleic Acids Res. **18**, 3400
Leiter, A. B., Toder, A., Wolfe, H. J., Taylor, I. L., Copperman, S., Mandel, G. & Goodman, R. H. (1987) J. Biol. Chem. **262**, 12984–12988
Lemischka, I. K., Farmer, S., Racaniello, V. R. & Sharp, P. A. (1981) J. Mol. Biol. **151**, 101–120
Luk, G. D. & Baylin, S. B. (1983) Am. J. Physiol. **245**, G656–G660
Luk, G. D. & Baylin, S. B. (1984) J. Clin. Invest. **745**, 698–703
Luk, G. D. & Yang, P. (1988) Am. J. Physiol. **254**, G194–G200
Lund, P. K., Ulshen, M. H., Rountree, D. B., Selub, S. I. & Buchan, A. M. (1990) Digestion **46** (Suppl. 2), 66–73
Manzella, J. M. & Blackshear, P. J. (1990) J. Biol. Chem. **265**, 11817–11822
Mohn, K. L., Laz, T. M., Melby, A. E. & Taub, R. (1990) J. Biol. Chem. **265**, 21914–21921
Mosjov, S., Heinrich, G., Wilson, I. B., Ravazzola, M., Orik, L. & Habener, J. R. (1986) J. Biol. Chem. **261**, 11880–11889
Sagor, G. R., Al-Mukhtar, M. Y. T., Ghatei, M. A., Wright, N. A. & Bloom, S. R. (1982) Br. J. Surg. **69**, 14–18
Sakai, M., Okuda, A., Hatayama, I., Sato, K., Nishi, S. & Muramatsu, M. (1989) Cancer Res. **49**, 5633–5637

Sambrook, J. Fritsch, E. F. & Maniatis, R. (1989) in Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
Savage, A. P., Gornacz, G. B., Adrian, T. E., Ghatei, M. A., Goodlad, R. A., Wright, N. A. & Bloom, S. R. (1985) Gut **26**, 1353–1358
Scalabrino, G., Lorenzini, E. C. & Ferioli, M. E. (1991) Mol. Cell Endocrinol. **77**, 1–35
Seidel, E. R., Haddox, M. K. & Johnson, L. R. (1985) Am. J. Physiol. **249**, G434–G448
Spiller, R. C., Trotman, I. F., Adrian, T. E., Bloom, S. R., Misiewicz, J. J. & Silk, D. B. A. (1988) Gut **29**, 1042–1051
Taylor, R. G., Verity, K. & Fuller, P. J. (1990) Gastroenterology **9**, 724–729
van Daalen Wetters, T., Macrae, M., Brabant, M., Sittler, A. & Coffino, P. (1989) Mol. Cell. Biol. **9**, 5484–5490
van Kranen, H. J., van de Zande, L., van Kreijl, C. F., Bisschop, A. & Wieringa, B. (1987) Gene **60**, 145–155
van Steeg, H., van Oostrom, C. T. M., Hodenmaekers, H. M., Peters, L. & Thomas, A. A. M. (1991) Biochem. J. **274**, 521–526
Wen, L., Huang, J.-K. & Blackshear, P. J. (1989) J. Biol. Chem. **264**, 9016–9021
Williamson, R. C. N. & Bauer, F. L. R. (1978) Br. J. Surg. **65**, 736–739

Received 29 November 1991/20 February 1992; accepted 5 March 1992