Regulation of concentrations of mRNA for amylase, trypsinogen I and chymotrypsinogen B in rat pancreas by secretagogues

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Regulation of pancreatic gene expression by the secretory stimulants cholecystokinin-pancreozymin, caerulein and pilocarpine was studied in the rat, by using cloned cDNA probes to quantify concentrations of specific mRNAs (amylase, trypsinogen I and chymotrypsinogen B). It is concluded that long-term pancreatic stimulation results in pre-translational regulation of secretory-protein gene expression, with a preferential accumulation of RNA transcripts encoding serine proteinases, compared with that for amylase.

INTRODUCTION

The influence of cholecystokinin-pancreozymin (CCK-PZ) and CCK-PZ-related peptides on pancreatic function and growth is well established (Mainz *et al.*, 1973; Barrowman & Mayston, 1974). These hormones are potent secretory stimulants (Brooks, 1973), and repeated injections of CCK-PZ or caerulein (Barrowman & Mayston, 1974) result in pancreatic enlargement, with increased nucleic acid content and secretory-enzyme accumulation. In addition, chronically stimulated pancreas shows an increased responsiveness to secretagogues (Petersen *et al.*, 1978; Otsuki & Williams, 1983). Of interest is the demonstration by Barrowman & Mayston (1974) that long-term CCK-PZ treatment does not simply enhance pancreatic function, but also modulates the relative amounts of secretory enzymes, with a preferential accumulation of serine proteinases compared with amylase. Similar modulations occur in the pancreas of rats whose diet is enriched in protein (Reboud *et al.*, 1966), and CCK-PZ has been proposed as one of the effectors of pancreatic adaptation to a protein-rich diet (Dagorn *et al.*, 1984). In previous experiments, we have demonstrated that the diet-induced changes in pancreatic enzyme production were accompanied by changes in the concentrations of the corresponding mRNAs (Giorgi *et al.*, 1984, 1985). The present study examined the influence of long-term treatment with CCK-PZ, caerulein and pilocarpine, a cholinergic agonist with potent secretagogue properties, on the pancreatic concentrations of mRNAs encoding amylase, trypsinogen I and chymotrypsinogen B.

EXPERIMENTAL

Male Sprague–Dawley rats weighing 200–250 g received twice daily for 7 days an intraperitoneal injection of either commercial CCK-PZ (120 units/kg; 20% pure; Kabi-Vitrum), caerulein (6 µg/kg; Farmitalia, Milan, Italy), pilocarpine (60 mg/kg; Sigma) or saline. On day 8, they were killed by decapitation and their pancreases removed and weighed. RNA was prepared from half of the gland by the method of Chirgwin *et al.* (1979). The quality of the preparation was monitored by agarose-gel electrophoresis in the presence of methylmercury hydroxide (Bailey & Davidson, 1976). The other half was

homogenized in 10 mM-Tris/HCl, pH 8.0. Total DNA and RNA were assayed in the homogenate as described by Schneider (1957) and Burton (1967) respectively. Amylase was assayed as described by Noelting & Bernfeld (1948). Chymotrypsinogen and trypsinogen were activated and assayed by the method of Hummel (1959), as previously described (Giorgi *et al.*, 1985). Quantification of mRNA by filter hybridization has been previously described (Giorgi *et al.*, 1984). The probes used (generously given by Dr. Ray MacDonald) were cDNA fragments cloned into plasmid pBR322. The lengths of the inserts were: amylase, 1100 base-pairs; chymotrypsinogen B, 825 base-pairs; trypsinogen I, 870 base-pairs. RNA was spotted on to nitrocellulose in sequential dilutions (White & Bancroft, 1982). After hybridization (Favaloro *et al.*, 1980) with the nick-translated probes (Maniatis *et al.*, 1975), filters were autoradiographed and the autoradiograms scanned. Values from four animals per group were plotted as arbitrary scanner units per µg of RNA, and the slopes of the curves were deduced by least-squares regression analysis. The regression coefficient, *r*, was always > 0.98.

RESULTS

Enzyme contents in pancreas

Chronic stimulation of pancreatic secretion by CCK-PZ, caerulein and pilocarpine resulted in an increase in amylase, chymotrypsinogen and trypsinogen in pancreatic cells (Table 1). Amylase increase was moderate (31%, 29% and 21% respectively, compared with control), but chymotrypsinogen and trypsinogen were more markedly increased (91%, 96% and 63%, and 83%, 58% and 54%, respectively). As judged by the values of the amylase/chymotrypsinogen and amylase/trypsinogen ratios, the three secretagogues evoked similar changes in the relative pancreatic concentrations of the three enzymes.

Filter-hybridization analysis of amylase, chymotrypsinogen B and trypsinogen I mRNAs

Recombinant plasmids encoding part of the sequence of rat amylase, chymotrypsinogen B and trypsinogen I were used to quantify the corresponding mRNAs in filter-hybridization experiments (see the Experimental

Table 1. Enzyme contents in pancreas after treatment with CCK-PZ, caerulein or pilocarpine

Enzyme contents are expressed in units per μg of DNA. Values are arithmetical means $\pm$ S.E.M. ($n = 8$). All values were significantly different ($P < 0.05$) from the corresponding control (NaCl) values. Values in parentheses indicate the percentage increase compared with control (NaCl).

Enzyme	Treatment...	Enzyme content			
		NaCl	CCK-PZ	Caerulein	Pilocarpine
Amylase		177.0 $\pm$ 14.0	232.0 $\pm$ 18.0 (+31.0)	228.0 $\pm$ 23.0 (+29.0)	215.0 $\pm$ 16.0 (+21.0)
Chymotrypsinogen		5.6 $\pm$ 0.6	10.7 $\pm$ 1.0 (+91.0)	11.0 $\pm$ 1.1 (+96.0)	9.1 $\pm$ 0.8 (+63.0)
Trypsinogen		5.9 $\pm$ 0.6	10.8 $\pm$ 0.9 (+83.0)	9.3 $\pm$ 1.0 (+58.0)	9.1 $\pm$ 1.0 (+54.0)

Table 2. Quantification of specific mRNA in total pancreatic RNA from secretagogue-treated animals

Values (in arbitrary scanner units per μg of RNA) correspond to the slopes of the linear regression curves ($\pm$ S.E.M.) of the dot-blot experiments (for details, see the Experimental section). Values in parentheses indicate the percentage changes relative to the saline control.

mRNA	Treatment...	mRNA content			
		NaCl	CCK-PZ	Caerulein	Pilocarpine
Amylase		67.0 $\pm$ 2.0	63.0 $\pm$ 2.1 (-6.0)	70.0 $\pm$ 2.4 (+4.5)	50.0 $\pm$ 2.3 (-25.0)
Chymotrypsinogen		35.6 $\pm$ 0.4	48.1 $\pm$ 1.3 (+35.0)	43.5 $\pm$ 2.2 (+22.0)	53.7 $\pm$ 1.8 (+51.0)
Trypsinogen		38.1 $\pm$ 1.5	71.4 $\pm$ 3.5 (+87.0)	59.8 $\pm$ 2.4 (+57.0)	59.7 $\pm$ 2.4 (+57.0)

Table 3. Influence of treatment with CCK-PZ, caerulein and pilocarpine on pancreatic concentrations of total RNA, and on amylase, chymotrypsinogen B and trypsinogen I mRNAs

Results are expressed as arithmetical means $\pm$ S.E.M., in ng/ μg of DNA ($n = 7$) for total RNA, and in arbitrary mass units (scanning units)/ μg of DNA ($n = 4$) for the mRNAs. All values, except that with *, were significantly different from the corresponding control (NaCl) value ($P < 0.05$). Values in parentheses indicate the percentage changes relative to the saline control.

RNA species	Treatment...	RNA content			
		NaCl	CCK-PZ	Caerulein	Pilocarpine
Total RNA		220.0 $\pm$ 25.0	316.0 $\pm$ 24.0 (+44.0)	305.0 $\pm$ 30.0 (+39.0)	369.0 $\pm$ 32.0 (+68.0)
Amylase		14.6 $\pm$ 1.9	19.8 $\pm$ 2.1 (+36.0)	21.2 $\pm$ 2.0 (+45.0)	18.2 $\pm$ 2.0* (+25.0)
Chymotrypsinogen		7.9 $\pm$ 0.9	15.2 $\pm$ 2.0 (+92.0)	13.4 $\pm$ 2.1 (+70.0)	19.7 $\pm$ 2.5 (+149.0)
Trypsinogen		8.4 $\pm$ 1.3	22.5 $\pm$ 2.0 (+168.0)	18.2 $\pm$ 2.2 (+117.0)	22.1 $\pm$ 2.5 (+163.0)

section). Increasing amounts of total RNA blotted on to nitrocellulose filters bound increasing amounts of the probes, in a linear fashion. Table 2 shows the values of the corresponding slopes calculated by least-squares regression analysis. The slopes gave an estimate of the relative amount of amylase, chymotrypsinogen B and trypsinogen I mRNAs in total RNA. Pilocarpine induced

a 25% decrease in amylase mRNA, whereas CCK-PZ and caerulein had no significant influence. By contrast, the proportions of chymotrypsinogen B and trypsinogen I mRNA in total RNA increased with the three stimulants. Again, the related peptides CCK-PZ and caerulein had qualitatively the same influence, both increasing trypsinogen I mRNA 1.6 times more than chymotrypsinogen B

mRNA. Quantitatively, however, the increase in serine proteinase mRNA was higher with CCK-PZ (87% and 35% respectively compared with control) than with caerulein (57% and 22% respectively). This could reflect a difference in the efficacy of the doses used for the two hormones. With pilocarpine, similar increases were observed for trypsinogen I and chymotrypsinogen B (57% and 51% respectively).

RNA concentrations in pancreas

Tissue concentrations of specific transcripts can be obtained from hybridization data, in which the amounts of mRNAs are expressed per μg of total RNA, if the tissue concentrations of total RNA can be determined. To this end, we measured the pancreatic concentration of total RNA in the four groups of rats (Table 3) and found that it was augmented in rats treated with secretagogues, compared with controls. Similar increases were observed with CCK-PZ and caerulein (44% and 39% respectively), whereas RNA concentration was increased by 68% with pilocarpine. Table 3 shows the pancreatic concentrations of the three transcripts, calculated from those data and data in Table 2. Secretagogue treatment resulted in increased concentrations of the three mRNAs tested. Amylase mRNA showed the smallest induction, however, its concentration being increased by 36%, 45% and 25% with CCK-PZ, caerulein and pilocarpine respectively, whereas trypsinogen I mRNA, with increases of 168%, 117% and 163%, always showed the highest increase in concentration. A more moderate increase was observed for chymotrypsinogen B mRNA (92% with CCK-PZ and 70% with caerulein), except for pilocarpine (149%).

DISCUSSION

The expression of genes encoding pancreatic enzymes is not always regulated in a co-ordinate manner. Upon chronic stimulation by CCK-PZ, for example, there is a preferential accumulation in pancreas of serine proteinases compared with amylase (Barrowman & Mayston, 1974; Dagorn *et al.*, 1984; Table 1), owing to modifications in the relative rates of synthesis of those enzymes (Dagorn & Mongeau, 1977; Schick *et al.*, 1984). Using cloned cDNA probes, we show in the present paper that chronic CCK-PZ stimulation induced a preferential accumulation in pancreas of the mRNAs encoding the serine proteinases trypsinogen I and chymotrypsinogen B, compared with that for amylase (Table 3). This result demonstrates that the hormone regulates the expression of the corresponding genes at a pre-translational step. In addition, the extents of mRNA induction were comparable with the increases observed in the tissue concentrations of the corresponding enzymes, indicating that preferential production of proteolytic enzymes induced by CCK-PZ could be entirely regulated at the pre-translational step. Additional experiments are, however, needed to evaluate the possible role of translational regulation.

The effect of chronic caerulein administration on mRNA concentrations was very similar to that observed with CCK-PZ, as it was for enzyme concentrations (Table 1). Since caerulein is a decapeptide with a C-terminal octapeptide identical with CCK-PZ (Anastasi *et al.*, 1968), the effects observed in the present study with the commercial preparation of CCK-PZ are probably due not to contaminating impurities, but to the active

principle present in the preparation. Moreover, the similar effects of CCK-PZ and the cholinergic agonist pilocarpine are suggestive of a post-receptor mechanism common to hormonal and cholinergic stimulants. Release of digestive enzymes from pancreas by both secretagogues occurs through the same mechanism involving mobilization of intracellular Ca^{2+} (Schulz, 1980). This is achieved through inositol trisphosphate formation by receptor-coupled hydrolysis of phosphatidylinositol bisphosphate, inositol trisphosphate being the second messenger responsible for Ca^{2+} release from the endoplasmic reticulum (Streb *et al.*, 1985). Whether inositol trisphosphate, Ca^{2+} or a third messenger in the cascade is involved in regulation of gene expression is not yet clear.

Finally, demonstration of induction by secretagogues of genes encoding three of the most abundant secretory proteins, as well as the observed increase in total RNA concentration in pancreas (Table 3), supports the previous report of RNA polymerase I and II stimulation by pancreatic stimulants (Black & Webster, 1974) and suggests that their trophic influence on their target organ could be the result of a general induction of the genes involved in secretory-protein production, i.e. encoding rRNAs and secretory mRNAs.

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REFERENCES

- Anastasi, A., Erspamer, V. & Endean, R. (1968) Arch. Biochem. Biophys. **125**, 57–68
- Bailey, J. M. & Davidson, N. (1976) Anal. Biochem. **70**, 75–85
- Barrowman, J. A. & Mayston, P. D. (1974) J. Physiol. (London) **238**, 73P–75P
- Black, O. & Webster, P. D. (1974) Am. J. Physiol. **227**, 1276–1280
- Brooks, F. P. (1973) Am. J. Clin. Nutr. **26**, 291–310
- Burton, K. (1967) Methods Enzymol. **128**, 163–166
- Chirgwin, M. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) Biochemistry **19**, 5294–5299
- Dagorn, J. C. & Mongeau, R. (1977) Biochim. Biophys. Acta **498**, 76–82
- Dagorn, J. C., La Bella, A., Lahaie, R. G. & Sarles, H. (1984) Digestion **29**, 152–157
- Favaloro, J., Treisman, R. & Karmen, R. (1980) Methods Enzymol. **65**, 718–749
- Giorgi, D., Bernard, J. P., Lapointe, R. & Dagorn, J. C. (1984) EMBO J. **3**, 1521–1524
- Giorgi, D., Renaud, W., Bernard, J. P. & Dagorn, J. C. (1985) Biochem. Biophys. Res. Commun. **127**, 937–942
- Hummel, B. C. (1959) Can. J. Biochem. Physiol. **37**, 1393–1399
- Mainz, D. L., Black, O. & Webster, P. D. (1973) J. Clin. Invest. **52**, 2300–2304
- Maniatis, T., Jeffreys, A. & Kleid, D. G. (1975) Proc. Natl. Acad. Sci. U.S.A. **72**, 1184–1188
- Noelting, G. & Bernfeld, P. (1948) Helv. Chim. Acta **31**, 286–290
- Otsuki, M. & Williams, J. A. (1983) Am. J. Physiol. **244**, G683–G688
- Petersen, H., Solomon, T. & Grossmann, M. I. (1978) Am. J. Physiol. **234**, E286–E293

- Reboud, J. P., Marchis-Mouren, G., Pasero, L., Cozzone, A. & Desnuelle, P. (1966) *Biochim. Biophys. Acta* **117**, 351–367
- Schick, J., Kern, H. & Scheele, G. (1984) *J. Cell Biol.* **99**, 1568–1574
- Schneider, W. C. (1957) *Methods Enzymol.* **2**, 680–684
- Schulz, I. (1980) *Am. J. Physiol.* **239**, G345–G347
- Streb, H., Heslop, J. P., Irvine, R. F., Schulz, I. & Berridge, M. J. (1985) *J. Biol. Chem.* **260**, 7309–7315
- White, B. A. & Bancroft, F. C. (1982) *J. Biol. Chem.* **257**, 8569–8572
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