Volume 309, number 1, 47-50 FEBS 11454 August 1992

Co-transcription of the gastrin and cholecystokinin genes with selective translation of gastrin mRNA in a human gastric carcinoma cell line

Wouter W. van Solinge and Jens F. Rehfeld

Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, DK-2100 Copenhagen, Denmark

Received 9 July 1992

So far, no cells have been found to synthesize both of the homologous hormones, cholecystokinin and gastrin. Northern analysis and reverse transcription PCR showed, however, that the human gastric carcinoma cell line (AGS) expresses both a gastrin mRNA of 0.7 kb and a cholecystokinin transcript of 0.8 kb. A library of sequence-specific radioimmunoassays, cleavage with processing-like enzymes and chromatography subsequently revealed that the gastrin mRNA was translated into progastrin that was constitutively secreted into the medium (45 ± 3 pmol/l). Neither procholecystokinin nor any of its processing products were detectable in cells and media. The results suggest that differentiation into gastrin- or cholecystokinin-producing cells may be regulated at the translational level. The gastric cell line, AGS, provides a model for studies of translational regulation of cell differentiation.

Cholecystokinin; Gastrin; Gene expression; PCR; Post-translational processing

1. INTRODUCTION

Most neuropeptides and peptide hormones occur in families of homologous members [1], the members of which are expressed in separate, highly differentiated neurons or endocrine cells. The cell-specific expression of peptide family members has been supposed to be regulated at the transcriptional level.

The homologous hormones, gastrin and cholecystokinin (CCK), constitute one peptide family. Accordingly, cellular co-existence of gastrin and CCK peptides has so far never been reported. Both hormones are synthesized as large propeptides that normally undergo extensive post-translational modifications [2]. Although transcriptional regulation of gastrin gene expression has been studied in certain rat cell lines [3–5], only little is known about tissue- and cell-specific factors that regulate the expression of both hormones in mammalian cells in general and, more specifically, in human cells.

In our studies of gastrin and CCK expression in human cell lines, we have now found a gastric cancer cell line (AGS), which transcribes both genes but translates only the gastrin mRNA. Such a cell line may provide general clues to the understanding of the differentiation of neuroendocrine cells.

Correspondence address: J.F. Rehfeld, Department of Clinical Biochemistry (KB-3011), Rigshospitalet, DK-2100 Copenhagen, Denmark. Fax: (45) 3537 5540.

Abbreviations: CCK, Cholecystokinin; RT-PCR, reverse transcription polymerase chain reaction.

2. MATERIALS AND METHODS

2.1. *Cell culture*

The gastric carcinoma cell line, AGS, was purchased from American Type Culture Collection (Rockville, Maryland, USA) and grown according to the instructions of ATCC. Cells were grown without serum and 0.2% crude bovine albumin (Sigma, St. Louis, USA) was added to the medium.

2.2. *Probes and oligodeoxynucleotide primers*

Gastrin and CCK oligonucleotides were synthesized according to published sequences ([6] and [7], respectively) and are complementary to the following positions: GAS 1, nucleotides −3670 to −3649 (exon 1); GAS 2, nucleotides 450–470 (exon 3); CCK 1, nucleotides 29–55 (exon 1); CCK 2, nucleotides 6829–6852 (exon 3). A human gastrin ^{32}P-labeled RNA probe was transcribed by SP6 RNA polymerase from a 261 bp *Hind*III human cDNA template. A human CCK cDNA fragment covering the coding region was ^{32}P-labeled using nick translation.

2.3. *Isolation of RNA and Northern analysis*

Total cellular RNA was isolated from cells according to Chirgwin et al. [8]. RNA was fractionated on 1% agarose-formaldehyde gels [9], transferred to Hybond-N nylon membranes, and UV crosslinked. Filters were hybridized with a human gastrin cRNA probe in 50% formamide, 600 mM NaCl, 8 mM Na-EDTA, 120 mM Tris-HCl with 1% SDS and 1% non-fat dried milk powder at pH 7.4 for 18 h at 65°C. The filters were washed for 3 × 20 min in 0.1 × SSC with 0.1% SDS at 75°C. When filters were hybridized with the nick-translated CCK probe, hybridization conditions were as described above. Washing of the filters was at 65°C for 3 × 20 min in 0.1 × SSC with 0.1% SDS. As positive controls both in Northern analysis and RT-PCR for gastrin and CCK mRNA, total RNA from human antral mucosa and the neuroepithelioma cell line, SK-N-MC [10], respectively, was used. The integrity and transfer of the RNA were confirmed by hybridization with a β-actin probe (data not shown).

2.4. *Reverse transcription and polymerase chain reaction*

The reverse transcription reaction and polymerase chain reaction were performed using a GeneAmp RNA PCR kit according to the

instructions of the manufacturer (Perkin Elmer Cetus, Norwalk, USA). Briefly, 1 μg of total RNA was reverse transcribed using an oligo $dT_{(16)}$ primer, before 30 pmol of each PCR primer per 100 μl total reaction volume were included. The cDNA was heated for 5 min at 95°C and amplified for 30 cycles using a Hybrid (Teddington, UK) thermocycler (1 min 94°C, 1 min 62°C, 2 min 72°C and finally 7 min at 72°C). For all mRNA samples amplified in a RT-PCR we routinely included controls where the reverse transcription step or input RNA were omitted.

2.5. *Radioimmunoassay*

Cell pellets (10^7 cells) were immersed in 2.5 ml boiling water (pH 6.6) for 20 min, homogenized and centrifuged at 10,000 × *g* for 30 min. The supernatant (neutral water extract) was stored at −20°C. The pellet was re-extracted in 0.5 M acetic acid, re-homogenized, left for 20 min at room temperature and centrifuged as above. The supernatants were measured using a library of specific radioimmunoassays. Antiserum 2604 is directed against the C-terminal part of the bioactive, carboxyamidated gastrins. The antiserum binds all bioactive gastrins (sulfated as well as non-sulfated) with equimolar potency. The cross-reactivity with cholecystokinin (CCK) is negligible [11]. Antisera 8017 and 2145 are specific for the N-terminal sequence of human gastrin-17 and -34, respectively [12,13]. They bind gastrin-17 and -34, as well as the corresponding C-terminally extended precursors. In order to measure N-terminal extended precursors as well, media were incubated with equal volumes of trypsin (0.1 mg/ml in 0.05 M sodium phosphate, pH 7.5) at 20°C for 30 min. The enzymatic reaction was terminated by boiling for 30 min. By the tryptic cleavage, the N-termini of gastrin-17 and -34 will be exposed for binding to antisera 8017 and 2145, respectively. Consequently progastrin and all its products are measured after trypsinization [14]. Glycine-extended precursors were measured using antiserum 3208 directed against the glycine-extended C-terminal sequence common to gastrin and CCK [15], and antiserum 5284 against glycine-extended unsulfated gastrins specifically [15]. After cleavage with trypsin (see above) and carboxypeptidase B (100 μl/ml sample) both CCK and gastrin precursors are bound to antiserum 3208, but only gastrin precursors to antiserum 5284. Human proCCK and CCK-22 was measured using antiserum 89009, which is directed against the N-terminus of human CCK-22. After tryptic cleavage, this antiserum will also measure otherwise unprocessed proCCK. When cell-line serum-free medium was assayed before enzymatic treatment it was first boiled for 20 min. Results for the cell-line media are given as mean ± S.E.M. of three separate experiments. The detection limit of the assays is 0.5 pmol/l in cell media and 0.1 fmol/10^6 cells in cell pellet extracts.

2.6. *Chromatography*

Samples (1 ml) were applied to a Sephadex G-50 superfine column (10 × 1,000 mm) and eluted with 0.125 M NH_4HCO_3 (pH 8.2) at 4°C with a flow rate of 4 ml/h in fractions of 1.0 ml. Void and total volumes of the columns were determined with ^{125}I-labeled albumin and ^{22}NaCl. Columns were calibrated with human gastrin-17, human gastrin-34, fragment 38–54 of human progastrin, human antral mucosa extract, human CCK-33 and human CCK-8. Serum-free cell medium was concentrated 20 times and applied to the column directly, or after treatment of the concentrate with trypsin and carboxypeptidase B (procedure as described above) and assayed with antisera 2145 and 3208.

3. RESULTS AND DISCUSSION

The expression of gastrin and CCK mRNA in the gastric carcinoma cell line, AGS, was characterized by RT-PCR and Northern analysis. Amplification of AGS cDNA with primers located in exons 1 and 3 of the gastrin gene yielded one specific PCR product of 423 bp (Fig. 1A, lane 3) corresponding to the product amplified from the human antrum RNA (Fig. 1A, lane 4). The 423 bp DNA hybridized to a human gastrin-specific cRNA probe at high stringency (Fig. 1B). Using the CCK primers the expected 421 bp fragment was detected in the AGS cell line (Fig. 1A, lane 1) which co-migrated with the fragment amplified from SK-N-MC RNA (Fig. 1A, lane 2). Both fragments hybridized under stringent conditions to the CCK probe (Fig. 1C). No products were detected when the reverse-transcription reaction or input RNA was omitted. Since PCR does not reveal the size of the mature mRNA transcript, the RNA was also examined by Northern analysis under stringent conditions. Although the intensity of the signals as detected by Northern analysis was weak, the mature transcripts of both gastrin and CCK were detectable in the AGS cells. The transcripts co-migrated with the control transcripts in the human antrum (0.7 kb) and in the SK-N-MC cell line (0.8 kb) (Fig. 2). The difference in intensity of the detected transcripts between the RT-PCR technique and Northern analysis is consistent with the significantly lower sensitivity of Northern analysis as compared to RT-PCR. This observation indicates that the amounts of mRNA for gastrin and CCK in the AGS cells are low. In conclusion, the results show that both gastrin and cholecystokinin mRNA are expressed in the AGS cells.

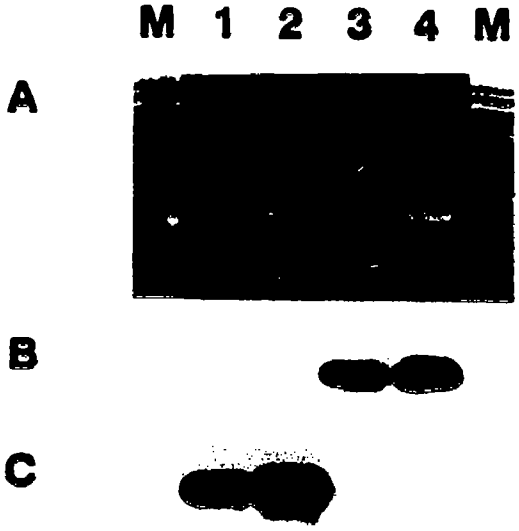

Fig. 1. CCK and gastrin mRNA from human antrum and the human cell lines, AGS and SK-N-MC, as detected by reverse-transcription polymerase chain reaction. 1 μg of total RNA was reverse transcribed and amplified using a polymerase chain reaction. Oligonucleotide primers CCK 1 and CCK 2 for CCK (lanes 1 and 2) and oligonucleotide primers GAS 1 and GAS 2 for gastrin (lanes 3 and 4) were used. 20 μl of sample was loaded and electrophoresed at 40 V for 6 h in a 3% Nusieve 3:1 Agarose gel (FMC BioProducts, Rockland, USA) in 1 × TBE buffer, and visualised by ethidium bromide staining (A). Lanes M, 1 kb DNA ladder (Gibco BRL) size marker; lane 1, AGS cells; lane 2, SK-N-MC cells; lane 3, AGS cells; lane 4, human antrum. Gels were denatured, blotted onto Hybond-N nylon membranes and hybridized with the gastrin (B) or CCK probe (C) as described in section 2.3. Exposure time was 30 min at −80°C.

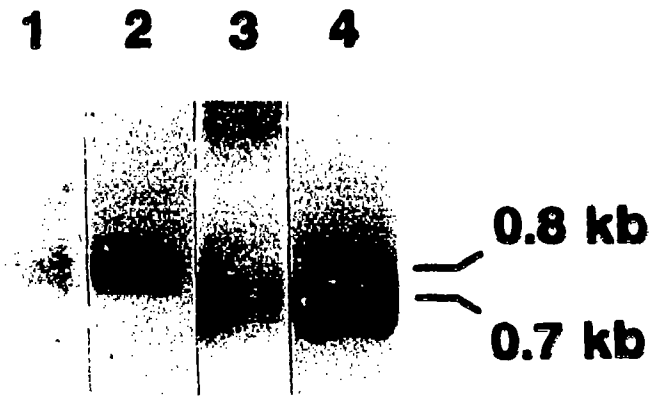

Fig. 2. Northern analysis of CCK and gastrin mRNA in human antrum and the human cell lines, AGS and SK-N-MC. 5 μg total RNA from SK-N-MC cells (lane 2) and human antrum (lane 4) or 25 μg total RNA from AGS cells (lanes 1 and 3) were electrophoresed at 40 V for 6 h and hybridized with a human CCK cDNA probe (lanes 1 and 2) or a human gastrin cRNA probe (lanes 3 and 4). Final washes were at 65°C (for CCK probe) or 75°C (for gastrin probe) in 0.1 × SSC/0.1% SDS. The filters were exposed as follows with intensifying screens: lanes 2 and 4, 18 h; lanes 1 and 3, 4 days.

Using a library of sequence-specific radioimmunoassays we measured progastrin in the serum-free cell medium (45 ± 3 pmol/l). The medium as such was devoid of progastrin, proCCK and any precursor fragments. The concentration of progastrin in the cells was low (13 ± 0.4 fmol/10^6 cells). Gel chromatography of concentrated medium, subsequent cleavage of the fractions with trypsin and measurement with antiserum 2145 showed one major component eluting in a position corresponding to the size of progastrin (K_d value of 0.20; Fig. 3, left panel). By gel chromatography of concentrated, enzyme-treated, serum-free AGS medium one component was detected with antiserum 2145 (K_d value of 0.58; Fig. 3, right panel), eluting at the position of fragment 38–54 of human progastrin [13]. The results indicate that the AGS cells synthesize progastrin which is constitutively released to the medium. Neither glycine-extended intermediates nor carboxyamidated gastrins could be detected. Thus, although the gastrin gene was transcribed and the mRNA translated, the functional mature peptide was undetectable (post-translational attenuation). Such mechanisms have been described earlier, both for gastrin and CCK [16,17]. Factors regulating the human gastrin gene have been investigated using human gene constructs in rat cell lines [3–5]. The AGS cell line may be a useful model to study the factors involved in the regulation of the human gastrin gene using a human system.

Neither procholecystokinin nor any of its processing products were detectable in the cells or medium. Although the CCK gene was transcribed, the translational phase of the expression is obviously inhibited. Recently,

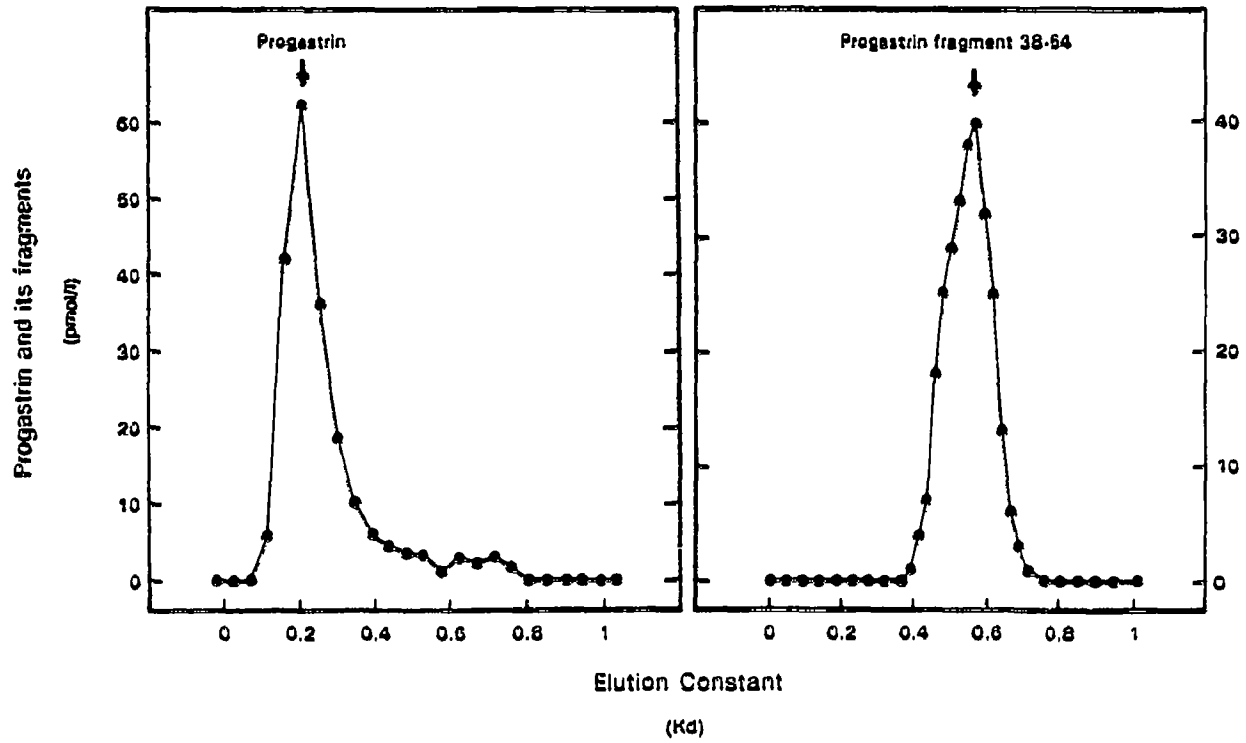

Fig. 3. Gel chromatography of an AGS cell line serum-free growth medium. A calibrated Sephadex G50 superfine column (10 × 1,000 mm) was eluted with 0.125 M NH_4HCO_3, pH 8.2 at 4°C with a flow rate of 4 ml/h. Fractions of 1.0 ml were collected. (Left panel) Fractions were incubated with trypsin (see section 2) and subsequently assayed with antiserum 2145, which is specific for the N-terminal sequence of gastrin-34. (Right panel) AGS cell medium was trypsin-treated prior to chromatography and applied to the column as above. The elution was monitored using antiserum 2145.

a discrepancy between significant expression of CCK mRNA and low concentration of its translation products in porcine cerebellum was reported, suggesting an efficient inhibition of CCK mRNA translation [16,18]. Translational control of gene expression has also been described for IGF-II mRNA [19] and other systems (for review see [20]). Using the AGS cell line as a model system, the mechanisms regulating translation of CCK mRNA may possibly be investigated.

Acknowledgements: The skillful technical assistance of Kirsten Culmsee and Cate Poulsen is gratefully acknowledged. We thank Karin Pedersen for providing the CCK primers and cDNA probe. The study was supported by grants from the Danish Medical Research Council, the Danish Cancer Union, the Alfred Benzon, Carlsberg, Gangsted, NOVO, and Vissing Foundations.

REFERENCES

[1] Rehfeld, J.F. (1984) in: Evolution and Tumour Pathology of the Neuroendocrine System (Falkmer, S., Håkanson, R. and Sundler, F. Eds.) Fernström Foundation Series, vol. 4, pp. 225–230, Elsevier, Amsterdam.
[2] Rehfeld, J.F. (1990) FEBS Lett. 268, 1–4.
[3] Wang, T.C. and Brand, S.J. (1990) J. Biol. Chem. 265, 8908–8914.
[4] Merchant, J.L., Demediuk, B. and Brand, S.J. (1991) Mol. Cell Biol. 11, 2686–2696.
[5] Godley, J.M. and Brand, S.J. (1989) Proc. Natl. Acad. Sci. USA 86, 3036–3040.
[6] Wiborg, O., Berglund, L., Boel, E., Norris, F., Norris, K., Rehfeld, J.F., Marcker, K.A. and Vuust, J. (1984) Proc. Natl. Acad. Sci. USA 81, 1067–1069.
[7] Takahashi, Y., Fukushige, S., Murotsu, T. and Matsubara, K. (1986) Gene 50, 353–360.
[8] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[9] Lehrach, H., Diamond, D., Wozney, J.M. and Boedtker, H. (1977) Biochemistry 16, 4743–4751.
[10] Verbeeck, M.A.E. and Burbach, J.P.H. (1990) FEBS Lett. 268, 88–99.
[11] Rehfeld, J.F., Stadil, F. and Rubin, B. (1972) Scand. J. Clin. Lab. Invest. 30, 221–232.
[12] Bardram, L. and Rehfeld, J.F. (1989) Scan. J. Clin. Lab. Invest. 49, 173–182.
[13] Van Solinge, W.W. and Rehfeld, J.F. (1990) Clin. Chim. Acta 192, 35–46.
[14] Rehfeld, J.F. and Bardram, L. (1991) Scand. J. Clin. Lab. Invest. 51, suppl. 204, 9–16.
[15] Hilsted, L. and Rehfeld, J.F. (1986) Anal. Biochem. 152, 119–126.
[16] Rehfeld, J.F., Mogensen, N.W., Bardram, L., Hilsted, L. and Monstein, H.J. (1992) Brain Res. 576, 111–119.
[17] Bardram, L., Hilsted, L. and Rehfeld, J.F. (1990) Proc. Natl. Acad. Sci. USA 87, 298–302.
[18] Gubler, U., Chua, A.O., Young, D., Fan, Z.W. and Eng, J. (1987) J. Biol. Chem. 262, 15242–15245.
[19] Nielsen, F.C., Gammeltoft, S. and Christiansen, J. (1990) J. Biol. Chem. 265, 13431–13434.
[20] Kozak, M. (1991) J. Cell Biol. 115, 887–903.