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# Expression of the cholecystokinin gene in a human (small-cell) lung carcinoma cell-line

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Expression of the cholecystokinin (CCK), gastrin and enkephalin A genes were studied by Northern blot analysis and a library of sequence-specific radioimmunoassays in human cell lines. The human small-cell lung carcinoma line (SCLC) U-1690 expressed moderate levels of CCK mRNA as compared to the human neuroepithelioma cell line SK-N-MC. Neither gastrin nor (pro)enkephalin A mRNAs were detectable in the U-1690 cell line. In contrast, the SCLC-line H-69 expressed Enk A but no CCK mRNA. The radioimmunoassays showed that the CCK mRNA transcript in the SCLC line U-1690 also is translated, and that preproCCK is processed into bioactive, carboxyamidated CCK peptides. Thus, the human small cell carcinoma cell line U-1690 is a useful model for studies of cell-specific CCK gene expression.

Cholecystokinin; Gastrin; Opioid peptide; Gene expression; Neuronal cell-line; Northern blot

## 1. INTRODUCTION

Bioactive cholecystokinin (CCK) peptides are generated by multiple proteolytic cleavages and derivatizations of a precursor polypeptide [1,2]. The peptides are synthesized in a cell-specific manner in the intestinal tract, the central nervous system [3-5], the pituitary [6] and in male germ cells [7]. The biogenesis of CCK and its regulation are difficult to investigate in tissues as complex as the brain or endocrine glands. Thus, several groups have investigated clonal cell lines which express CCK and gastrin at the transcriptional and translational/posttranslational level [8-13]. In earlier studies we found that the human neuroepithelioma cell line SK-N-MC co-expresses cholecystokinin and proenkephalin A mRNA and that these mRNA levels are differently modulated by cAMP and noradrenalin [13]. ProCCK [11], proenkephalin A [12] and proNPY [14] prohormone processing however appears to be incomplete in this cell line.

More recently it has been suggested that cultured human colon tumour cells express and mature CCK/Gastrin peptides, which play an important role in autocrine growth stimulation [15], whereas opioid peptides such as Met-enkephalin inhibit cell proliferation [16,17].

In this study we report that the human small-cell lung carcinoma (SCLC) cell line U-1690 [18] expresses the CCK-gene both at the transcriptional and translational level. Moreover, the translational product appears to be fully processed to bioactive, carboxyamidated CCK peptides.

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## 2. MATERIALS AND METHODS

### 2.1. *Cell lines*

The cell lines investigated in this study were: SK-N-MC (human neuroepithelioma), SH-SY-5Y (human neuroblastoma), IM-9 (human lymphocytes), U-1752 (human squamous cell, SQC), U-1819 (human large-cell lung carcinoma, LCLC), H-69, U-1285, U-1906, U-2020 and U-2050 (human small-cell lung carcinoma, SCLS). SQC, LCLC, SCLC and IM-9 cell lines or cell pellets were obtained from Dr. J. Bergh, University Hospital Uppsala [18]. Cell lines were cultured in Falcon flasks either in RPMI-1640 medium (GIBCO) supplemented with 10% fetal calfserum (SEROMED), 1.76 g/l $NaHCO_3$; 2.38 g/l Hepes; 290 mg/l L-glutamine and 10 ml/l Penicillin-Streptomycin (10.000 U and 10.000 μg/ml) at 37°C under 5% carbon dioxide, grown to confluence and harvested prior to RNA extraction. SK-N-MC, SH-SY-5Y, U-1752 and U-1810 cells, however, were cultured in Eagle's medium supplemented as described above.

### 2.2. *Control material*

Rat brain and the rat medullary carcinoma cell line CA-77, which are known to express CCK mRNA were used in this study as positive controls.

### 2.3. *Preparation of RNA and Northern blot analysis*

Total RNA from $10^7$ cells was extracted by the method of Chomczynski and Sacchi [19] and quantitated by UV absorption at 260 nm. The RNA preparations were routinely checked for degradation on an agarose minigel. Poly $A^+$ RNA was prepared from total RNA according to the procedure of Aviv and Leder [20]. Electrophoretic separation of the RNA in a denaturating agarose gel was carried out according to Kroczek and Siebert [21], followed by transfer onto Amersham Hybond-N membrane in 15 mM Na-phosphate, pH 6.4. Filters were either fixed by UV illumination in a Stratalinker (Stratogene) U.V.-box (Autocrosslink) or by backing under vacuum at 80°C for 2 h.



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2.4. *Hybridization, DNA templates and cRNA probes*

Prehybridization, hybridization and subsequent autoradiography to Amersham MP X-ray films were carried out as reported [22]. The construction and in vitro transcription of the human ppENK-A and rat ppCCK DNA templates were recently described [12,13]. The human gastrin DNA template was constructed by subcloning a 280 bp *Hin*dIII/*Exon*III-DNA fragment [23] into the *Hin*dIII site of pGEM-3 vector (Promega Inc.). The β-Actin template was obtained by subcloning a ~1150 bp *Pst*-DNA fragment [24] into vector pGEM-3. For each hybridization probe, ~ 1 μg DNA template was in vitro transcribed either by RNA polymerase T7 or SP-6, using Promegas Riboprobe-Kit and 50 μCi [$^{32}$P]α-UTP (400 Ci/mmol).

2.5. *Radioimmunoassay*

In order to measure biologically active forms of CCK and gastrin, washed cell pellets were extracted and analyzed with a library of sequence specific antisera [25]. Antiserum 2609 is directed against the common α-amidated C-terminus of CCK and gastrin, whereas antiserum 2604 is specific for carboxyamidated gastrins. Thus, antiserum 2604 discriminates between CCK and gastrin peptides. Antiserum 8017 is directed against the N-terminal part of human gastrin-17. Finally, antiserum 3208 recognizes glycine-extended CCK and gastrin processing intermediates. By prior treatment of the samples with trypsin and carboxypeptidase B, antiserum 3208 also recognizes unprocessed proCCK peptides.

## 3. RESULTS AND DISCUSSION

The expression of the CCK, gastrin, and enkephalin A gene in established cell lines was investigated by Northern blot analysis using CCK, gastrin, and enkephalin A complementary RNA ([$^{32}$P]-labelled cRNA) hybridization probes. Initially, total RNA (15 μg) from SCLC cell lines U-1690 and U-1906, the human neuroepithelioma line SK-N-MC and the human neuroblastoma cell line SH-SY-5Y was analyzed for the expression of gastrin and CCK mRNA. As a positive control, the CCK mRNA expressing rat medullary thyroid carcinoma cell line CA-77 and rat brain were included. Northern blot analysis and subsequent exposure of the nylon membrane to an Amersham MP-X-ray film for 3 days revealed that beside the SK-N-MC cell line and rat brain only the SCLC line U-1690 expressed a ~750 nt CCK mRNA transcript (Fig. 1). It appears that CA-77 and SK-N-MC cells hyperexpressed CCK mRNA whereas the level of CCK mRNA seems to be more moderate in the U-1690 line.

In many cases, neuropeptide mRNA expression appears to be low and any specific transcript may only be detectable in poly A$^+$ selected mRNA fractions. To test this possibility, poly A$^+$ RNA from 11 different established cell lines, including eight lung carcinoma lines (see Materials and Methods) were analyzed. Thus, 3 μg poly A$^+$ selected mRNA from the indicated cell lines was electrophoretically separated and transferred by capillary blotting to HybondN membranes. Filters were stepwise hybridized with gastrin, CCK, enkephalin A and β-Actin $^{32}$P-labelled cRNA hybridization probes. A ~750 nt CCK mRNA transcript was found to be expressed only in SK-N-MC and U-1690 cells (Fig. 2), whereas none of the cell lines expressed gastrin mRNA.

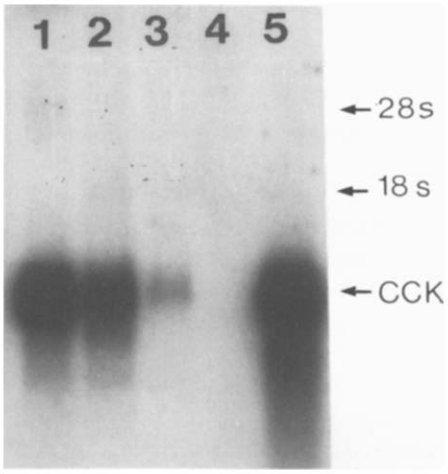


Fig. 1. Detection of ppCCK mRNA in rat medullary thyroid carcinoma cell line CA-77 (lane 1), human neuroepithelioma cell line SK-N-MC (2), human small cell lung carcinoma cell lines U-1690 (3) and U-1906 (4), rat brain (5). 15 μg total RNA was analyzed by Northern blot. Exposure was to Amersham MP X-ray film for 3 days at −70°C using a DuPont intensifier screen. The positions of 28s and 18s rRNA and ppCCK mRNA (CCK) are indicated.

In a recent study [26], we have shown that the gastrin gene rather than the CCK-gene is expressed in human bronchogenic carcinomas. This would suggest that established SCLC cell lines may express the gastrin gene.

Therefore, the findings that none of the investigated lung carcinoma cell lines expressed gastrin mRNA is rather unexpected. One explanation could be that the small quantities of somatostatin produced by the analyzed SCLC, and non-SCLC cell lines [18] inhibit the expression of the gastrin gene. In dog antral mucosa, somatostatin stimulates gastrin mRNA turnover drastically [27]. On the other hand, gastrin mRNA levels might be very low and nondetectable under the conditions used in this study. We therefore examined

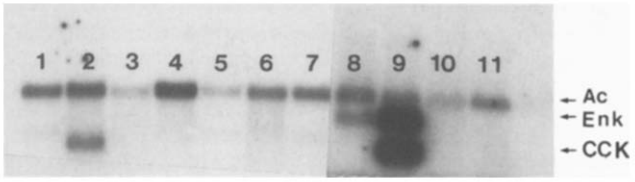


Fig. 2. Detection of ppCCK, ppENK-A, and β-Action mRNA. 3 μg poly A$^+$ mRNA was analyzed by Northern blot. The Nylon membrane was stepwise hybridized with human ppGastrin, rat ppCCK, human ppENK A, and mouse β-Actin cRNA probes. Exposure was to Amersham MP X-ray film for 3 days (lanes 1–7) and 2 days (lanes 8–11). The tested cell lines are; U-1285 (1), U-1690 (2), U-1752 (3), U-1810 (4), U-1906 (5), U-2020 (6), U-2050 (7), H-69 (8), SK-N-MC (9), SH-SY-5Y (10), IM-9 (11). The positions of ppCCK (CCK), ppENK A(ENK), and β-Actin(Ac) mRNA are indicated.

the occurrence of gastrin and CCK peptides in U-1690 cell pellets. Using a library of sequence-specific gastrin and CCK antisera (see Materials and Methods), we found in U-1690 cells that the CCK mRNA transcript is translated and processed into $\alpha$-carboxyamidated CCK (113,3 ± 17,0 pmol/l ($n = 3$) and glycine-extended CCK peptides (64,2 ± 11,9 pmol/l). Analysis of the U-1690 cell media revelaed that at least part of the cellular CCK peptides was released into the medium (29,4 ± 5,8 pmol/l, $n = 12$). We were not able to detect gastrin peptides, thus confirming the results obtained by Northern blot analysis.

In SK-N-MC cells, CCK and enkephalin A mRNA are co-expressed but differently modulated by noradrenalin and cAMP [13]. Rehybridization of a poly $A^+$ membrane with a human enkephalin A cRNA hybridization probe revealed that only the SK-N-MC cell line co-expressed CCK and enkephalin A mRNA (Fig. 2). Interestingly, the SCLC line H-69 expressed an ~ 1400 nt enkephalin A mRNA transcript but at a more moderate level as compared to the SK-N-MC cell line (Fig. 2). White et al. recently showed that human SCLC-lines not only express the POMC gene but also secrete ACTH into the medium [28]. Thus, it seems to be a common feature that many SCLC cell lines express different opioid peptide genes. Moreover, there is evidence that both CCK, gastrin as well as opioid peptides play an important role in autocrine growth regulation of colon cancer cells [15], neuroblastomas [16], and lung cancer cells [17]. Thus, it becomes evident that regulation and modulation of neuropeptide gene expression both at the transcriptional, translational and posttranslational level are indeed important steps in the whole cascade of neuropeptide gene expression.

In summary we find that the human SCLC cell lines U-1690 and H-69 as well as the epithelioma line SK-N-MC, all differently expressing either the CCK or the Enkephalin A gene, provide useful model systems to study cell specific neuropeptide gene expression at the transcriptional, translational and posttranslational level.

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