The second human calcitonin/CGRP gene is located on chromosome 11

J.W.M. Höppener¹, P.H. Steenbergh¹, J. Zandberg², A.H.M. Geurts van Kessel¹, S.B. Baylin⁵, B.D. Nelkin⁴, H.S. Jansz², and C.J.M. Lips³

¹Institute of Molecular Biology, ²Laboratory for Physiological Chemistry, ³Department of Internal Medicine, University Hospital, State University of Utrecht, The Netherlands
⁴Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands
⁵The Oncology Center and the Department of Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205, USA

Summary. A second human calcitonin/calcitonin gene related peptide (hCT/CGRP) gene has been identified. This second hCT/CGRP gene has been shown to contain sequences highly homologous to exons 3, 5 (CGRP-encoding), and 6 of the first hCT/CGRP gene, but sequences closely related to exon 4 (CT-encoding) could not be demonstrated. Southern blot hybridization analysis of DNA from human-rodent somatic cell hybrids showed that the second hCT/CGRP gene is located in the q12-pter region of chromosome 11. The first hCT/CGRP gene has previously been assigned to the p13-p15 region of chromosome 11.

Introduction

Alternative (tissue-specific) expression of the calcitonin (CT) gene by means of differential processing of the primary transcript, has been studied extensively in the rat (Rosenfeld et al. 1984). This regulatory mechanism involves the production of two different cytoplasmic mRNAs from a single multi-exonic gene. In the C-cells of the thyroid gland, CT gene expression results in the production of mRNA encoding the precursor of the 32-amino acid calcium-regulating hormone calcitonin (Amara et al. 1982). This mRNA contains exons 1, 2, 3, and 4 of the rat CT gene (Amara et al. 1984). In brain tissue, mRNA encoding the precursor of the 37-amino acid neuro-peptide calcitonin gene related peptide (CGRP) is produced from the rat CT gene (Rosenfeld et al. 1983). This mRNA is composed of exons 1, 2, 3, 4, and 5 of the rat CT gene (Amara et al. 1984). Both CT and CGRP mRNAs have been detected in C-cell derived medullary thyroid carcinomas (MTCs) (Rosenfeld et al. 1982). In poly(A)-rich RNA from rat brain tissue, an additional mRNA encoding a precursor peptide that contains a CGRP-related sequence was identified. The putative CGRP-like peptide, referred to as β-CGRP, differs in primary sequence from rat CGRP (or α-CGRP) by only a single amino acid residue (Rosenfeld et al. 1984). Whether this peptide is actually produced in brain tissue remains to be investigated. We have isolated the first CT/CGRP gene from a cosmid library of the human genome and analyzed the nucleotide sequence of hCT mRNA and part of hCGRP mRNA. These studies have shown that the first hCT/CGRP gene also is composed of six exons. As in the rat, exons 1, 2, 3, and 4 are present in CT mRNA, whereas exons 3, 5, and 6 have been identified in hCGRP mRNA (Steenbergh et al. 1984a; Nelkin et al. 1984).

Restriction enzyme analysis of chromosomal DNA has shown that the first hCT/CGRP gene has a polymorphic site for restriction endonuclease TaqI, with fragments of 8.0 and/or 6.5 kilobase pairs (kb) hybridizing to hCT gene-specific probes (Höppener et al. 1984). Restriction endonuclease analysis of a cosmid clone has revealed that the 6.5kb TaqI fragment contains exons 2 to 6 of this hCT/CGRP gene (Steenbergh et al. 1984a). The hCT/CGRP gene has been mapped in the p14-pter region of chromosome 11 by analysis of human-rodent somatic cell hybrids (Höppener et al. 1984) and has been assigned to region p13-p15 by in situ hybridization experiments (Przepiorka et al. 1984).

The existence of a second hCT/CGRP gene was suspected by the presence of genomic TaqI fragments of 3.0 and 2.3kb, hybridizing to a hCT/CGRP gene-specific probe, in addition to the polymorphic fragments of 8.0 and 6.5kb which are specific for the first hCT/CGRP gene (Höppener et al. 1984). From a cDNA library of human medullary thyroid carcinoma (MTC) poly(A)-rich RNA, a cDNA clone (phCGRP 4) was isolated, containing sequences 92% homologous to part of exon 3 and the entire exon 5, and 65% homologous to exon 6 of the first hCT/CGRP gene (Steenbergh et al. 1985). DNA from this clone hybridizes to the same TaqI fragment of 2.3kb mentioned above, but more strongly to a TaqI fragment of 2.6kb, confirming the existence of a second hCT/CGRP gene (Steenbergh et al. 1985). Here we present additional information on the structure of the second hCT/CGRP gene and report the chromosomal localization of this gene.

Materials and methods

Production and characterization of somatic cell hybrids

Human-rodent somatic cell hybrids were constructed by the fusion of human leukocytes with Chinese hamster cells (Geurts van Kessel et al. 1981a) or the mouse myeloid cell line WEHI-TG (Geurts van Kessel et al. 1984) according to the method of Harris and Watkins (Harris and Watkins 1965). Hybrid clones were selected in HAT (Littlefield 1964) supplemented medium. Details of the procedure have been reported previously (Geurts van Kessel et al. 1981b).
The hybrid cells were characterized by the analysis of human chromosome-specific isozyme markers and karyotyping (Geurts van Kessel et al. 1983). Chromosome analysis was performed by examining air-dried chromosome preparations using R-banding (with acridine orange). A hybrid clone was scored positive for the presence of a particular human chromosome if at least one out of ten metaphases contained that chromosome. Cells used for chromosome analysis and Southern blot analysis were derived from the same culture.

**DNA isolation and Southern blot analysis**

Chromosomal DNA from hybrid cells and from their parents, was isolated as described previously (Bartram et al. 1983). Human placenta DNA was isolated by the method of Gross-Bellard (Gross-Bellard et al. 1973). Each DNA sample (5μg) was digested with restriction endonuclease TaqI at 2 units per μg DNA for 5h at 65°C. DNA fragments were size-fractionated by electrophoresis in 0.8% agarose gels and transferred onto hybridization membranes (Gene Screen, New England Nuclear; nitrocellulose, Schleicher and Schüll). Double-stranded DNA probes were labeled by nick-translation with 32P-dCTP to a specific activity of 1-2 × 10⁹ dpm ³²P/μg DNA. Prehybridization and hybridization were carried out in the presence of 50% formamide at 42°C. Blots were washed at 65°C in 2 × SSC/0.5% sodium dodecylsulphate (1 × SSC = 0.15 M NaCl/0.015 M sodium citrate).

As exon 3 specific probe, we have used a BanH fragment of plasmid pTT 42, containing about 120 base pairs (bp) representing the 3'-terminal part of exon 3 of the hCT/CGRP gene (Nelkin et al. 1984). As intron 3 specific probe, we have used a 1 kb HindIII-PvuII fragment of the plasmid schCT 6, containing a 4.5kb BglII fragment of the hCT/CGRP gene, cloned in the BamHI site of pBR 322 (Steenbergh et al. 1984a). In addition to pBR 322 sequences, the 1 kb fragment contains 13bp derived from the 5'-end of exon 4 and about 700bp of the bordering intron 3. As intron 4 specific probe, we have used a PstI-BglII fragment of phCGRP3, extending from 34 (BglII site) to 477 nucleotides upstream of exon 5 of the hCT/CGRP gene.

As probe for the chromosomal localization of the second hCT/CGRP gene we have used a 770bp SacI-PstI fragment of the plasmid phCGRP 4, containing a cDNA sequence that is highly (92%) homologous to exon 5 and to a lesser extent (65%) homologous to exon 6 of the first hCT/CGRP gene. (The synthesis, cloning, and characterization of this cDNA derived from the second hCT/CGRP gene has been published elsewhere (Steenbergh et al. 1985)).

**Results**

**Human CT/CGRP gene related sequences**

Previous experiments have shown that a cDNA probe containing exons 2, 3, and 4 of the first hCT/CGRP gene hybridizes with 6.5kb and 8.0kb TaqI fragments of human DNA, which are alleles of the restriction fragment length polymorphism of the first hCT/CGRP gene. In addition, TaqI fragments of 2.3 and 3.0kb, not present in this gene (Steenbergh et al. 1984a), hybridized to the same probe (Fig. 1, lane 1). In order to determine the origin of these additional fragments, we hybridized TaqI digested human DNA to probes specific for exons 3 and 4 (intron 3) and exons 4 and 5 (intron 4). The exon 3 specific probe detected both the 2.3kb and the 3.0kb TaqI fragment (results not shown). Using a cDNA containing only exon 4 (CT encoding exon) as probe, no additional CT-exon related sequences could be detected (Fig. 1, lane 2). The exon 5 specific probe hybridized to a 2.6kb TaqI frag-