Human Chromogranin A Gene

MOLECULAR CLONING, STRUCTURAL ANALYSIS, AND NEUROENDOCRINE CELL-SPECIFIC EXPRESSION*

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Chromogranin A (CgA), an acidic glycoprotein, which is widely expressed in endocrine and neuroendocrine cells. It plays multiple important roles in the process of regulated hormone secretion. The single copy human CgA gene was isolated from a human fetal liver gene library. The gene spans 15 kilobases and contains 8 exons. Exon I encodes the 5'-noncoding region and the majority of the signal peptide coding region. Exons II-V collectively encode the highly conserved amino-terminal domain (the β-granin sequence). Exon VI encodes a variable domain within which is the chromostatin sequence, and exon VII encodes another variable domain, which contains the pancreastatin sequence. Exon VIII encodes the highly conserved carboxyl-terminal domain and the 3'-noncoding region. The human gene promoter has a consensus TATA box, CAMP response element, and Sp1 sequence. 2.3 kilobases of the upstream regulatory region of the human CgA gene directed efficient transcription of a reporter chloramphenicol acetyltransferase gene in several neuroendocrine cell lines, including human medul- lary thyroid C-cell tumor, mouse pituitary corticotroph, rat pituitary tumor, and rat pheochromocytoma. The promoter was virtually inactive in nonneuroendocrine cell lines. Transient transfection studies with deleted promoter constructs showed that sequences lying between −55 and +32 base pairs relative to the transcription initiation site, containing the consensus cyclic AMP response element and TATA box, were sufficient for neuroendocrine cell-specific expression.

Chromogranin A (CgA), an acidic glycoprotein first identified in chromaffin granules of the adrenal medulla (1), is the major member of the chromogranin/secreto- granin (granin) family of proteins, which are present in virtually all endocrine and neuroendocrine cells (2–4). These are costored within secretory granules and cosecreted with the resident hormone. Extracellularly, peptides formed as a result of proteolytic processing of granins regulate hormone secretion in an autocrine manner. Granins may also play a role in secretory granule formation and targeting of peptide hormones and neurotransmitters to granules of the regulated secretory pathway by virtue of their ability to aggregate in the low pH, high calcium environment of the trans-Golgi network.

The CgA cDNA has been cloned from several species. The gene encodes a preprotein of molecular mass 50 kDa. The signal sequence is 18 amino acids long, and the mature CgA protein is 430 amino acids in the porcine (5), 431 amino acids in the bovine (6–8), 439 amino acids in the human (9, 10), 444 amino acids in the rat (11, 12), and 445 amino acids in the murine (13) species. Mature CgA contains several conserved pairs of basic residues raising the possibility that biologically active peptides could be released from the molecule by precursor processing enzymes (14, 15). In fact, it has now been reported that several peptides encoded within the CgA molecule, such as vasostatin (16), β-granin (17, 18), chromostatin (19), pancreastatin (20), and parastatin (21), are biologically active, at least in vitro, and inhibit hormone or neurotransmitter release probably in an autocrine or paracrine fashion. Pancreastatin, a 49-amino acid peptide (encoded by porcine CgA-(240–288)) with a carboxyterminal amide, inhibits glucose-stimulated insulin release from perfused pancreatic islet cells (20). Chromostatin, which corresponds to a sequence beginning at bovine CgA amino acid 124, inhibits secretion from adrenal medullary cells apparently via a mechanism involving closing of a calcium channel (22). CgA may also be functional intracellularly and play a role in processing (23), sorting (2, 3, 24), and granule condensation (25). Some of these putative functions may be related to the molecule’s ability to bind calcium, which leads to changes in its structure and aggregation and membrane-association properties (26–30). Therefore, CgA and/or peptides derived from it may have multiple functions, both extracellular and intracellular. The relative importance of extracellular functions may depend upon the extent of processing of CgA, which is tissue-specific (2, 31).

The biosynthesis of CgA is regulated by many different factors, including steroid hormones and agents that modulate the protein kinase A and protein kinase C signaling pathways (see Ref. 2 for review). In theory, its biosynthesis could be regulated in concert with other components of the secretory granule of the particular cell involved. Alternatively, the biosynthesis of CgA and the resident hormone or neurotransmitter could be regulated differentially. Evidence for the latter type of regulation has been found in several systems. For example, in the parathyroid cell, whereas 1,25-dihydroxyvitamin D inhibits parathyroid hormone gene transcription, it stimulates CgA gene transcription (32–34).

The CgA gene is a single copy gene. The bovine and mouse...
CgA genes have been characterized and are comprised of eight exons (13, 35) with conserved exon-intron boundaries. There is some limited homology between the amino-terminal and carboxy-terminal domains of the CgA gene and the related chromogranin B gene, which has been cloned in the mouse (36). To further understand the regulation of CgA gene expression, we cloned the entire human CgA gene and delineated a minimal region of its promoter necessary for neuroendocrine cell-specific expression. We show that an 87-bp fragment of the promoter region containing the transcriptional start site, a TATA box homology, and a conserved cyclic AMP response element is sufficient to direct neuroendocrine-specific expression of a chloramphenicol acetyltransferase reporter gene after transient transfection into a variety of cell lines of both human and rodent origin.

EXPERIMENTAL PROCEDURES

Materials—Cell lines and the genomic library were from the American Type Culture Collection (Rockville, MD). Dulbecco’s modified Earle’s medium (DMEM), antibiotics, horse serum, and fetal bovine serum (FBS) were from Life Technologies, Inc. (Gaithersburg, MD). Normal bovine parathyroid glands, a human parathyroid adenoma, and the human medullary thyroid carcinoma (TT) cell line by the guanidium thiocyanate/cesium chloride method (39). Primer extension analysis was performed as described in Ref. 40, using as primer oligonucleotide a, described above under “Restriction Enzyme Mapping Analysis.” Ten μg of each total RNA and 25 μg of yeast RNA (as negative control) were hybridized overnight at 60°C in 10 mM Pipes, pH 6.4, 0.4 M NaCl, 1 mM EDTA. The RNA templates were then reverse transcribed using 200 units of murine mammary leukemia virus reverse transcriptase. The extension products were phenol-chloroform extracted, ethanol precipitated, and analyzed on a 6% acrylamide/7 M urea sequencing gel. As a DNA size marker, an unrelated sequencing reaction using pUC19 DNA (Pharmacia) and the universal primer, M13, was run. 

Human CgA Gene Promoter Analysis—A hCgA promoter construct (p2300CAT) was prepared by ligating a BamHI-Xhol restriction fragment spanning 2316 bp upstream of the cap site to 32 bp downstream of the cap site between the BamHI and Xhol sites of the promoterless CAT expression reporter vector, pBLCAT3 (41). The p700CAT and p540CAT constructs were prepared by ligating the HindIII-Xhol (-716 to +32) and XbaI-XhoI (-447 to +32) restriction fragments, respectively, into the pBLCAT3 vector. Constructs p900CAT and p550CAT were created by ligating BamHI-Xhol fragments generated by polymerase chain reaction into pBLCAT3. The forward primer for the p900CAT fragment, 5' - CCTTTGAGGATCTCAGGAATTCCGTTC - 3', is composed of six nucleotides flanking a BamHI site followed by the nucleotides -53 to -40. For both fragments the reverse primer was 5' - ACAGGGCTGCCTAGGAATCCGTC - 3', which is the complement of +25 to +41 and contains the XhoI site. Fragments were amplified by polymerase chain reaction, cloned with BamHI and XhoI, gel-purified, and ligated into BamHI-Xhol-cut pBLCAT3. All plasmid DNAs were prepared by the alkaline lysis/cesium chloride gradient method.

DNA Sequence Analysis—Seven restriction fragments of aghCgA (8) representing the entire human CgA gene were subcloned into pBluescript KS vector for further restriction mapping and nucleotide sequence analysis, which was carried out by the dideoxynucleotide chain termination method (38) using the Pharmacia T7 sequencing kit. Restriction fragments from selected subclones were 32P-labeled by the random primer method and used in Southern blot analysis of restriction enzyme-digested human leukocyte DNA.

Primer Extension Analysis of CgA mRNA—Total RNA was isolated from normal bovine parathyroid glands, a human parathyroid adenoma, and the human medullary thyroid carcinoma (TT) cell line by the guanidium thiocyanate/cesium chloride method (39). Primer extension analysis was performed as described in Ref. 40, using as primer oligonucleotide a, described above under “Restriction Enzyme Mapping Analysis.” Ten μg of each total RNA and 25 μg of yeast RNA (as negative control) were hybridized overnight at 60°C in 10 mM Pipes, pH 6.4, 0.4 M NaCl, 1 mM EDTA. The RNA templates were then reverse transcribed using 200 units of murine mammary leukemia virus reverse transcriptase. The extension products were phenol-chloroform extracted, ethanol precipitated, and analyzed on a 6% acrylamide/7 M urea sequencing gel. As a DNA size marker, an unrelated sequencing reaction using pUC19 DNA (Pharmacia) and the universal primer, M13, was run in parallel.

Characterization of Human Chromogranin A Gene

FIG. 1. Physical map of human DNA encoding the CgA gene. Panel A, two independent bacteriophage recombinant clones, aghCgA3 and aghCgA8, were isolated after screening a human fetal liver genomic DNA library as described under “Experimental Procedures.” Panel B, restriction map of the human CgA gene. Restriction sites shown are: B, BamHI; E, EcoRI; H, HindIII; F, Kpnl; P, PstI; X, XhoI. Solid boxes denote exons. The lower case letters refer to exon-specific oligonucleotide probes (a–i) used to construct the restriction map. See text for a detailed description of the oligonucleotides. Panel C, the seven restriction fragments of aghCgA8 that represent the entire human CgA gene. They were subcloned into the pBluescript KS vector for restriction mapping and nucleotide sequence analysis.
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Cell Culture—The neuroendocrine cell types used were the mouse pituitary corticort cell line (AT-T20), the rat pituitary cell line (GH1,c); the rat pheochromocytoma cell line (PC-12); and the human medullary thyroid carcinoma (TT) cell line. The nonendocrine cell lines used were the T-antigen-transformed monkey kidney cell line (COS-7), the mouse fibroblast cell line (NIH-3T3), and the rat fibroblast cell line (PC-12). All maintenance media contained 100 units/ml penicillin and 100 μg/ml streptomycin.

Cell Transfections—For transfections, cells were trypsinized, seeded at 5 x 10⁵ cells/35-mm tissue culture dish, and incubated overnight. Medium was aspirated, and cells were washed twice with serum-free DMEM. Transfections with 6 μg of the phCgACAT constructs, with the positive control SV40 early region promoter-driven plasmid pSV2CAT, and the negative control promoterless plasmid, pBLCAT3, were done by electroporation (42) in the majority of cells. How-ever, TT cells (10 x 10⁶ cells) were transfected by the electroporation method using the Bio-Rad Cell Porator with an extended capacitance unit. Forty-eight h after transfection, cells were washed once with ice-cold phosphate-buffered saline and harvested in 1 ml of phosphate-buffered saline by scraping into 1.5-ml Eppendorf tubes. Cells were spun at 500 x g at 4 °C for 7 min and resuspended in 250 μl of 0.5 M Tris-HCl, pH 7.5. Cells were lysed by three freeze-thaw cycles, and the DNA and cell debris were removed by centrifugation at 16 000 x g for 10 min at 4 °C. Cell lysates were assayed for protein using the Bio-Rad protein assay kit and assayed for β-galactosidase and CAT activity as described below. CAT activities were normalized to β-galactosidase activity.

RESULTS AND DISCUSSION

Identification and Restriction Mapping of Genomic Clones—Ten positive phage plaques were identified after the initial screening of the human fetal liver genomic library using an oligonucleotide complementary to the amino-terminal coding region of bovine CgA. These phage represented two independent clones designated AhgCgA3 and AhgCgA8 (see Fig. 1), which contained 16 and 20 kb of human genomic DNA, respectively. To facilitate restriction mapping and DNA sequence analysis, a variety of restriction fragments were subcloned. A
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physical map of the human CgA gene and flanking regions is
shown in Fig. 1.

Characterization of the 5' End of the Gene by Primer Extension—Primer extension analysis of oligomer a with RNA from human parathyroid adenoma and bovine parathyroid gland yielded a single major extension product for each RNA (Fig. 2). The largest extension product obtained with both human sources, parathyroid and thyroid 'M' cell line, was 213 bp, while a 193-bp extension product was obtained with bovine parathyroid RNA. The human CgA gene transcription initiation site is 29 bp downstream of a typical TATA box sequence, and the bovine CgA gene transcription initiation site is 30 bp downstream of the TATA box. The 5'-noncoding region of the human CgA mRNA is 213 bases and that of the bovine CgA mRNA is 193 bases. The data obtained for the transcription initiation site of the bovine gene in parathyroid are identical to
those reported by Iacangelo et al. (35) using bovine adrenal medulla RNA.

DNA Sequence Analysis and Structural Organization of the hCgA Gene—The nucleotide sequence of the human CgA gene including 2.3 kb of promoter sequence is shown in Fig. 3. All exons and exon/intron junctions were completely sequenced. The hCgA gene spans 15 kb and contains eight exons. Exon I being especially well conserved for their amino-terminal coding conserved amino-terminal domain (the P-granin sequence). peptide coding region. Exons 11-V collectively encode the highly conserved carboxyl-terminal domain and 5”noncoding region and the majority of the signal protein indicated by +1. Note that exon V of the mouse chromogranin A gene demonstrates variability in size because of the insertion of a polymorphic (CAG), polyglutamine tract, which is not present in either the human or bovine genes.

Comparison of the exon organization of the human, bovine, and mouse CgA genes. The relative sizes of the eight exons (roman numerals) of the human, bovine, and mouse CgA genes are shown. Filled boxes denote noncoding regions; open boxes indicate coding regions. The numbers at the intron/exon borders denote the amino acids of the preCgA protein with the first amino acid of the mature CgA protein indicated by +1. Note that exon V of the mouse chromogranin A gene demonstrates variability in size because of the insertion of a polymorphic (CAG), polyglutamine tract, which is not present in either the human or bovine genes.

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Fig. 4. Comparison of the exon organization of human, bovine, and mouse CgA genes. The relative sizes of the eight exons (roman numerals) of the human, bovine, and mouse CgA genes are shown. Filled boxes denote noncoding regions; open boxes indicate coding regions. The numbers at the intron/exon borders denote the amino acids of the preCgA protein with the first amino acid of the mature CgA protein indicated by +1. Note that exon V of the mouse chromogranin A gene demonstrates variability in size because of the insertion of a polymorphic (CAG), polyglutamine tract, which is not present in either the human or bovine genes.

Comparison of Gene Organization and Domains Encoding Regulatory Peptides—Fig. 6 emphasizes the relationship between CgA gene exons and regions that encode peptides that have been reported to be biologically active. Vasostatin is the term given to a mixture of NH2-terminal peptides, the shortest of which is bovine CgA(1-76), which were purified from bovine adrenomedullary granules (16). These peptides have a vasoinhibitory effect on isolated segments of human blood vessels (16). The CgA(1-76) peptide could potentially be released from CgA by cleavage at the dibasic Lys77-Lys78. The related peptide CgA(1-113) was first identified in rat insulinomas and pancreatic islets and was given the name β-granin (18). A function of this peptide in pancreas has yet to be described; however, it has been reported that the homologous bovine CgA peptide inhibits parathyroid hormone and CgA release from bovine parathyroid cells in culture (17). This peptide could be cleaved from CgA by proteolytic attack at the dibasic Lys114-Lys115. Vasostatin and β-granin are encoded by several gene exons (II–VI in the case of β-granin). This arrangement is absolutely conserved in all CgA genes characterized to date, as are the dibasic cleavage site at Lys114-Lys115 and a potential disulphide bridge encoded by exon III. A synthetic CgA(1–40) peptide has also been reported to be active in modulating hormone secretion in cultured cells (46, 47).

The term chromostatin refers to the sequence CgA(124–143), which was synthesized on the basis of the ability of tryptic and Lys-C-generated peptides of bovine CgA to inhibit chromafin cell secretion (19, 48). Chromostatin is encoded within exon VI. The precise amino-terminal and carboxyl-terminal ends of the “natural” chromostatin sequence are as yet unknown. However, binding and cross-linking studies suggest that chromostatin receptors are present in the adrenal medulla (22) and
that they mediate the closing of an L-type calcium channel via stimulation of a soluble protein phosphatase (49).

Pancreastatin, which is encoded by the 5’ end of exon VII, is a biologically active 49-amino acid amidated peptide from porcine pancreas (5, 20). Cloning of porcine CgA mRNA demonstrated that pancreastatin was encoded within its sequence (5). Pancreastatin’s biological activity resides in the carboxyl-terminal region where the sequence is most conserved (20). By contrast, the amino-terminal region is very variable (5). Konecki et al. (10) speculated on the basis of the hCgA cDNA sequence that cleavage could take place at the nonconserved Arg248-Lys249 to yield hCgA-(250–301) as the human pancreastatin molecule. However, human pancreastatin peptides isolated and characterized to date do not provide evidence of such a cleavage as they are either amino terminally extended or truncated relative to this putative cleavage site and to the known amino terminus of porcine pancreastatin (porcine CgA-(240–304)). In contrast to other prohormones, such as proparathyroid hormone and proparathyroid hormone-related peptide, in which a prohormone cleavage site is precisely bisected by intron/exon boundaries (50), in the hCgA the splice junction between exon VI and exon VII lies downstream of the Arg248, Lys249 sequence and upstream of the Arg253, which is another potential cleavage site. The amino acid sequence of this particular region of the CgA molecule, at the carboxyl-terminal end of exon VI and the amino-terminal end of exon VII, is the most variable between species. Knowledge of the exon/intron structure of this region of the porcine CgA gene may help to shed further light on the relationship between the placement of the exon boundaries and the cleavage site that releases the amino terminus of pancreastatin.

Several prohormone convertase dibasic cleavage sites occur in the carboxyl-terminal half of exon VII and the coding portion of exon VIII. A peptide derived from porcine CgA by Lys-C digestion, termed “parastatin”, porcine CgA-(347–419), which is homologous to hCgA-(356–428), has been reported to be in-

![Fig. 6. Relationship between human CgA gene structure and functional peptide coding regions. The exon/intron organization of the hCgA gene is related to the known CgA-derived peptides, which have been reported to be biologically active.](image)

![Fig. 7. Comparison of the promoters of the human, bovine, and mouse CgA genes. Panel A shows the alignment of the promoters of the human and bovine CgA genes. Panel B shows the alignment of the promoters of the human and mouse CgA genes. Nucleotides are numbered from the transcription initiation site (+1), which is indicated by an asterisk. TATA box motifs are boxed and CRE are underlined.](image)

![Fig. 8. Endogenous expression of CgA mRNA in neuroendocrine but not in nonendocrine cell lines. Cytoplasmic RNA was isolated from the neuroendocrine and nonendocrine cell lines shown and 10-μg aliquots of RNA examined by Northern analysis as described under "Experimental Procedures."](image)
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A: Neuroendocrine

**Fig. 9. Delineation of minimal region necessary for the neuroendocrine cell-specific activity of the human CgA gene promoter.** Neuroendocrine PC-12 and GH4C1 cells (panel A) and nonendocrine COS-7 and Rat-2 cells (panel B) were transfected with plasmid DNAs, and chloramphenicol acetyltransferase activity was determined as described under “Experimental Procedures.” DNAs transfected were: pBLCAT3, a promoterless CAT construct as negative control (not shown); pSV2CAT, in which the CAT gene is driven by the SV40 early region promoter as positive control; p2300CAT, p750CAT, p450CAT, p260CAT, and p55CAT, constructs that contain sequences from -2300, -750, -450, -260, and -55 bp to +32 bp relative to the hCgA gene transcription initiation site inserted upstream of the CAT gene in pBLCAT3.

B: Nonendocrine

Comparative analysis of the proximal promoter sequences of the human, bovine, and mouse genes—Alignment of the first approximately 200 nucleotides of the human CgA gene 5'-flanking region with the equivalent sequences of the bovine and mouse CgA genes is shown in Fig. 7. The human and bovine promoter regions are well conserved (76% identity), while the human and mouse promoters are somewhat less similar (49% identity). In all species there is a cyclic AMP response element (CRE) homology just upstream of the TATA box.

All of the promoters contain Sp-1 sites and possess a high GC content (human, 76%; bovine, 70%; mouse, 65%), which would be consistent with the wide distribution of expression of CgA. A highly conserved purine-rich region extending from -79 to -57 in the human, -80 to -58 in the bovine, and -101 to -83 in the
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Table I

Transcriptional activity of the human CgA promoter in neuroendocrine and nonendocrine cells

<table>
<thead>
<tr>
<th>Neuroendocrine</th>
<th>Cell line</th>
<th>Cell type</th>
<th>p2300CAT</th>
<th>p700CAT</th>
<th>p450CAT</th>
<th>p260CAT</th>
<th>p55CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AtT20</td>
<td>Pituitary</td>
<td>3.19 ± 0.27*</td>
<td>4.97 ± 0.92</td>
<td>6.15 ± 1.0</td>
<td>3.63 ± 0.2</td>
<td>1.58 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>Thyroid C cell</td>
<td>3.33 ± 0.33</td>
<td>3.35 ± 0.36</td>
<td>3.36 ± 0.18</td>
<td>2.67 ± 0.37</td>
<td>5.34 ± 0.79</td>
<td></td>
</tr>
<tr>
<td>GH3C1</td>
<td>Pituitary</td>
<td>10.07 ± 1.38</td>
<td>9.81 ± 1.06</td>
<td>15.4 ± 1.14</td>
<td>12.98 ± 0.73</td>
<td>11.55 ± 0.92</td>
<td></td>
</tr>
<tr>
<td>PC-12</td>
<td>Chromaffin</td>
<td>14.85 ± 0.44</td>
<td>14.41 ± 2.41</td>
<td>13.20 ± 1.29</td>
<td>7.86 ± 1.28</td>
<td>13.54 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>Nonendocrine</td>
<td>COS-7</td>
<td>Kidney</td>
<td>0.02 ± 0.001</td>
<td>0.04 ± 0.003</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.001</td>
<td>0.05 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>NIH-3T3</td>
<td>Fibroblast</td>
<td>0.13 ± 0.01</td>
<td>0.15 ± 0.001</td>
<td>0.14 ± 0.007</td>
<td>0.15 ± 0.02</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Rat-2</td>
<td>Fibroblast</td>
<td>0.06 ± 0.02</td>
<td>0.14 ± 0.007</td>
<td>0.11 ± 0.014</td>
<td>0.09 ± 0.006</td>
<td>0.09 ± 0.01</td>
</tr>
</tbody>
</table>

* Mean ± S.E. of triplicate or quadruplicate determinations.

The specificity of the hCgA promoter in directing expression in neuroendocrine cells and not in other types can be compared with data recently reported for the carboxypeptidase E gene promoter (52). Carboxypeptidase E is widely, although not exclusively, expressed in cells synthesizing peptide hormones and neurotransmitters. However, in contrast to the CgA gene promoter, the carboxypeptidase E gene promoter was found to be active in both neuroendocrine and nonneuroendocrine cell lines (52). Also, in contrast to the CgA gene promoter, the carboxypeptidase E gene promoter lacks both a TATA box homology and CRE. Therefore, in the CgA gene promoter, these elements possibly in combination with other cis-acting motifs may be critical in bringing about neuroendocrine cell expression and/or nonneuroendocrine cell repression. For example, it has been demonstrated that transcription can be negatively regulated through the CRE via members of the ATFKREB family such as CREM or CREB-2 (53). It is also noteworthy that all other granzin gene promoters characterized to date, i.e. the mouse chromogranin B gene (56) and the mouse secretogranin II gene (54), have TATA box homologies and CREs. Further deletion and mutational analyses will be needed to address these issues.

In summary, we have isolated and characterized the human CgA gene, which is similar in both size and exon/intron organization to the mouse and bovine CgA genes. The CgA gene flanking region within 55 bp of the transcription initiation site confers specific neuroendocrine cell expression. It will now be possible to elucidate the precise cis-acting elements involved in this neuroendocrine cell expression and those that bind other specific trans-acting factors such as steroid hormone receptors.

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Murine promoter is noteworthy. A similar, but not identical, purine-rich sequence, GGAAGG, is the recognition sequence for PU.1, a trans-acting factor that confers lymphoid cell-specific gene expression (51). Endogenous CgA mRNA Expression in Neuroendocrine and Nonneuroendocrine Cells—By Northern blot analysis (Fig. 8) CgA mRNA was undetectable in all nonneuroendocrine cells tested (Rat-2, NIH-3T3, and COS-7), whereas a single transcript was detectable in all neuroendocrine cell lines tested (PC-12, AtT-20, TT, and GH3C1). Although CgA mRNA was detected in GH3C1 cells it was expressed at a much lower level than in the other neuroendocrine cell lines.

Neuroendocrine Cell Specificity of Human CgA Promoter Activity—The 5′-flanking region of the human CgA gene exhibited neuroendocrine cell-specific promoter activity (Fig. 9). A plasmid (p2300CAT) containing a BamHI/Xhol fragment extending from 2.3 kb upstream to 32 bp downstream of the transcription initiation site inserted in the proper orientation upstream of the CAT gene demonstrated strong promoter activity after transfection into various neuroendocrine cell lines of different types and species including rat pituitary (GH3C1), mouse pituitary corticotrophs (AtT-20), adrenal chromaffin cells (PC-12), and human medullary thyroid C-cells (TT). The hCgA promoter fragment construct was some 3- to 15-fold more active, depending upon the cell line, than the positive control construct in which CAT expression was under the control of the SV40 early region promoter (Fig. 9 and Table I). In contrast, in nonneuroendocrine cell lines (COS-7, NIH-3T3, and Rat-2) the hCgA promoter had only very weak activity relative to the SV40 early promoter.

To localize the hCgA gene promoter region conferring specific neuroendocrine cell expression, deleted constructs containing approximately 700, 450, 260, and 55 bp of the 5′-flanking sequence were transfected into both neuroendocrine and nonneuroendocrine cell lines. All of the deletion constructs were just as active as the p2300CAT construct (Fig. 9 and Table I). All constructs demonstrated low activity in nonneuroendocrine cells.

Therefore, we have delineated a minimal region of the CgA gene promoter necessary for neuroendocrine cell-specific expression. An 87-bp fragment of the human promoter region containing the transcription start site, a TATA box homology, and a consensus cyclic AMP response element is sufficient to direct expression of a reporter gene in various neuroendocrine cell lines of rodent as well as human origin. This region does not contain the purine-rich GAGA sequence, similar to the PU.1 recognition motif, which confers lymphoid cell-specific gene expression (51), and which, it might have been speculated, played a role in the neuroendocrine cell-specific expression of the CgA gene. The role of this conserved motif in CgA gene expression remains to be elucidated. In addition, it remains to be determined if activation of the CgA gene involves enhancer action via specific positively acting neuroendocrine cell-trans-acting factors or release of negative repressor activity in nonendocrine cells.
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