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Sites of Synthesis of Chromogranins A and B in the Human Brain

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Abstract — The sites of synthesis of the chromogranins A and B, and their potential processed peptides, were examined by quantitating the levels of chromogranin A and B mRNA in various regions of the human brain by Northern blot analysis. Chromogranin A and B mRNA expression in the brain is region-specific and confined to grey matter. In situ hybridization histochemistry detected chromogranin A and B mRNA in pyramidal neurons of human cerebral cortex. Cell-specific expression in subpopulations of cerebrocortical neurons suggest that chromogranin A and B gene products may play a role in central neuronal function.

Introduction

Chromogranins A and B are two closely-related members of a family of abundant acidic glycoproteins found in intracellular secretory organelles and distributed widely throughout the diffuse neuroendocrine system and in the CNS (1-11). While chromogranin A (CGA) and chromogranin B (CGB) are co-expressed in several neuroendocrine tissues, they are also differentially expressed in others, and in several tissues are regulated by different second messenger and hormonal signals (12-15). In the human adrenal medulla, CGA and CGB are similarly abundant and co-stored in all cells (16, 17). Sympathetic neurons also contain both CGA and CGB (18-20). In the pituitary gland, CGA and CGB are co-localized in somatotrophs and thyrotrophs, yet only CGA is expressed in gonadotrophs, and only CGB in prolactinomas (21-26). CGA and CGB immunoreactivity exhibits an uneven distribution in the rat and bovine brain with cerebral cortical areas enriched (2-4 times) for CGA and cerebellum enriched (approximately 10 times) for CGB (15, 27, 28). In addition, the expression of CGA and CGB immunoreactivity in brain tissue may be restricted to certain neuronal subsets (29-33).

The localization of sites of synthesis of CGA and CGB in the brain may be important for determining the roles played by these two proteins in CNS function. Determining the differential location of CGA- and CGB-producing cells in the CNS is confounded at the protein level by two factors. First, CGA and CGB are presumably synthesized in neuronal cell bodies, packaged in storage organelles and transported to nerve endings. Processing CGA and CGB into polypeptide breakdown products may occur en

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route to the nerve ending. Thus, the presence of CGA and CGB immunoreactivity in a given brain region may indicate the presence of CG in cell bodies, fibers of passage and nerve terminals. Secondly, determining the distribution of CGA and CGB by immunochemical methods is complicated by the presence of multiple protolitically processed peptide fragments of each protein for which a given antibody or antiserum may have differential cross-reactivity (22, 34-40). Studies of the distribution of CGA and CGB mRNA may therefore be the most unambiguous means of determining the location of cells which express CGA, CGB and their processed peptide products in the brain (5, 15, 41, 42, 43). In the present report, an analysis of the regional expression of CGA and CGB mRNA gene products in the human CNS is presented.

Material and Methods

Human tissue was removed from cadavers 3.5 to 24 h postmortem, weighed, placed in aluminum foil, frozen in liquid nitrogen and stored at –70°C. Adjacent samples were fixed in Bouin's solution, embedded in paraffin and examined microscopically to verify their location and to evaluate the presence of any abnormalities. The brain samples were mostly neuron rich cerebral cortex and telencephalic nuclei, but associated white matter was also present. Cerebral grey matter was separated from white matter in one case. The tissues were grossly and microscopically normal and the patients had no evidence of neurological disorders.

The purification of mRNA was by the guanidinium isothiocyanate/cesium trifluoroacetic acid method followed by oligo-dT cellulose chromatography (44, 45). Approximately 10 μg of poly $(A)^+$ RNA per lane were dissolved in twenty microliters of a solution containing: 2.2 M formaldehyde/50% formamide/20 mM MOPS/5 mM sodium acetate/1 mM EDTA, pH 7.0/0.025% bromophenol blue and denatured at 60°C for 10 min. The RNA was electrophoresed in 1.0% agarose gels containing 2.2 M formaldehyde/20 mM MOPS/5 mM sodium acetate/1 mM EDTA. After transfer to GeneScreen (New England Nuclear) or Nytran (S & S) and crosslinked by UV irradiation (120,000 μJ), the RNA blots were prehybridized (12 h) and then hybridized with cDNA or oligonucleotide probes (10^6 cpm/ml) in a buffer containing: 4 x SSC (600 mM NaCl/60 mM sodium citrate, pH 7.0)/50% formamide/1 x Denhardts (0.5% ficoll, 0.5% polyvinylpyrrolidone, 0.5% bovine serum albumin)/200 μg/ml tRNA/250 μg/ml salmon sperm DNA (sheared and heat denatured) for 18 h at 42°C. The filters were washed in 0.2 x SSC/0.1% $NaDodSO_4$ at 45°C (oligodeoxyribonucleotides) or 55°C (CGA cDNA fragment) and exposed to x-ray film at –70°C.

In situ hybridization histochemistry was performed as described previously (26). Briefly, frozen post-mortem human brain sections (8 μm thick) from the cerebral cortex were thaw-mounted onto 3-aminopropylmethoxy silane-coated glass slides (Aldrich, Milwaukee, Wisconsin, USA). Warmed (24°C) sections were fixed in 4% paraformaldehyde and soaked in 0.25% acetic anhydride/0.1 M triethanolamine hydrochloride/0.9% NaCl. Following dehydration through a series of ethanol solutions the tissues were delipidated in chloroform and rehydrated in 95% ethanol and air-dried. Hybridization was performed in buffer containing: 4 x SSC (600 mM NaCl/60 mM sodium citrate, pH 7.0)/50% formamide/1 x Denhardts (0.5% ficoll, 0.5% polyvinylpyrrolidone, 0.5% bovine serum albumin)/1 mg/ml DTT/250 μg/ml tRNA/500 μg/ml salmon sperm DNA (sheared and heat denatured) and ^{35}S-labeled 24-mer (CGA) or 39-mer (CGB) oligodeoxyribonucleotide probes (600000 cpm) for 16 h at 44°C in a humid chamber. Slides were washed in 0.5 x SSC/0.1% $NaDodSO_4$ at 44°C, dehydrated in 95% and 100% ethanol with 0.3 M ammonium acetate, and air dried. Autoradiography was carried out by dipping in Kodak NTB2 emulsion diluted 1:1 with distilled water containing 0.3 M ammonium acetate. The slides were allowed to air dry for 1 h and then subsequently developed for 3 min in Kodak D-19 developer, washed in water for 1 min and fixed for 5 min in Kodak Fixer. After washing for 1 h they were stained with hematoxylin and eosin, and coverslipped.

Oligodeoxyribonucleotides complementary to the coding regions of human CGA (51-mer: cDNA base numbers 137-187; 24-mer cDNA base numbers 966-989) (46), CGB (39-mer: cDNA base numbers 3-42) (2) and β-actin (37-mer: cDNA base numbers: 1466-1502) were synthesized on an Applied Biosystems

380A DNA synthesizer and purified by electrophoresis in a preparative denaturing acrylamide gel. The oligonucleotide probes (5 pmol) were labeled to a specific activity of 3500 Ci/mmol, using terminal deoxynucleotidyl transferase (100 units; Bethesda Research Laboratories) and (α-^{32}P)-dATP (1 μM; 3000 Ci/mmol; New England Nuclear) or (^{35}S)thio-dATP (1 μM; 1300 Ci/mmol; Amersham). A cDNA fragment complementary to CGA (cDNA base numbers 1004-1246) was prepared as described previously (26). The cDNA probe was nick-translated to a specific activity of 10^8 cpm/μg using (α-^{32}P)-dATP and Klenow polymerase. The RNA blots were sequentially hybridized to CGA, CGB and β-actin probes, and stripped of the preceding probe with 95% formamide/1 mM EDTA/10 mM tris; pH 8.0 for 30 min at 60°C followed by a thorough wash in 1 x SSPE (180 mM NaCl/2 mM EDTA/10 mM NaH_2PO_4; pH 7.4).

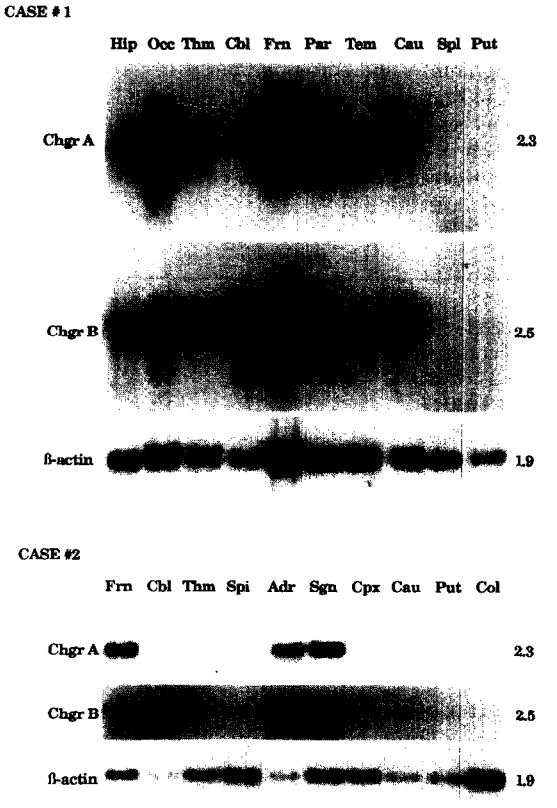

Fig. 1 Northern hybridization analysis of mRNA from human brain regions and peripheral tissues. Approximately 10 μg of poly (A)$^+$ RNA was prepared from each tissue sample: Hip, hippocampus; Occ, occipital pole; Thm, thalamus; Cbl, cerebellum; Frm, frontal lobe; Par, parietal cortex; Tem, temporal tip; Cau, caudate nucleus; Spl, spleen; Put, putamen; Spi, spinal cord; Adr, adrenal gland; Sgn, sympathetic ganglion; Cpx, choroid plexus; Col, colon. The RNA blots of case 1 (upper) and case 2 (lower) were successively hybridized, stripped and reprobed with cDNA (chromogranin A) or oligonucleotide probes derived from chromogranin A (51-mer), chromogranin B (39-mer) and β-actin (37-mer). Exposure time was approximately 4 days for the chromogranins A and B probes and 45 min for the β-actin probe at −70°C with intensifying screens. The size of the chromogranins A and B and β-actin mRNAs (kb) are indicated on the right.

Results and Discussion

The regional distribution of CGA and CGB mRNA transcripts in human brain by Northern blotting is shown in Figure 1. Single mRNA species of 2.3 kb and 2.5 kb hybridized to CGA and CGB probes, respectively. CGA and CGB mRNA exhibited an uneven distribution with cerebral cortical areas (frontal lobe, occipital pole, parietal cortex and temporal tip) and dorsal root ganglia displaying greater levels than that observed in the thalamus (pulvinar), basal ganglia (caudate, putamen), and spinal cord. CGB mRNA was as abundant in the hippocampus (dentate) as cerebral cortex. A similar enrichment of CGA mRNA in cerebral cortex versus hippocampus has been observed in rat brain (42). In addition, CGA mRNA levels are enriched in cerebral cortex and caudate nucleus when compared to hippocampus and cerebellum in bovine brain (unpublished data). The greatest regional difference in expression of CGA and CGB mRNA in human brain was in the cerebellum (vermis) where no visualization of CGA mRNA was observed even after overexposure of the film. The mRNA obtained from human cerebellum was however, partly degraded. Yet, cerebellum was the richest source for CGB when the hybridization signals are compared to β-actin (Table). CGB immunoreactivity is highest in rat cerebellum particularly in Purkinje neurons (15, 29). CGB mRNA, however, has a more even distribution in rat brain which may be indicative of species variation (41). Low amounts of CGA and CGB mRNA were also detected in the colon while both the spleen and choroid plexus were negative for both mRNA species. CGA and CGB mRNA levels in human adrenal medulla are considerably greater than that observed in cerebral cortex based upon relative

Table Chromogranin A and chromogranin B mRNA expression in various human brain regions. The ratio of chromogranin A and chromogranin B mRNA to β-actin mRNA in brain areas from case 1 and case 2 was determined by spectrophotometric analysis of hybridization signals on X-ray films. The values are normalized to levels observed in the frontal cortex for each case. ND = not detectable.

	Chromogranin A	*Chromogranin B*
Case 1		
Frontal cortex	1.00	1.00
Parietal cortex	1.37	0.84
Occipital cortex	1.25	0.87
Temporal cortex	0.68	0.49
Hippocampus	0.41	1.20
Cerebellum	ND	2.33
Thalamus	0.34	0.52
Caudate	0.29	0.39
Case 2		
Frontal cortex	1.00	1.00
Cerebellum	ND	3.67
Sympathetic ganglion	0.56	1.69

expression of β-actin mRNA (Fig. 2). Differences in the abundance of CGA and CGB mRNAs in the adrenal gland of case 2 (Fig. 1) and case 3 (Fig. 2) may be due to differing amounts of adrenal cortical tissue present. A comparison of poly $(A)^+$ RNA from human cerebral grey and white matter indicated an enrichment of both CGA and CGB mRNAs in neuron-enriched cortex rather than glia-enriched white matter (Fig. 2). In situ hybridization histochemistry revealed the presence of silver grains over pyramidal neuron cell bodies of human cerebral cortex for both CGA and CGB probes (Fig. 3). Specificity of the in situ signal was assessed by hybridization in the present of a 100-fold excess of unlabeled probes. In addition, the specificity of the respective oligonucleotides for their appropriate mRNA species was determined by Northern analysis (Fig. 2). Recently, immunocytochemical detection of CGA-like material in large and small pyramidal neurons of the human cerebral cortex has been demonstrated (31). CGB mRNA has also been detected in pyramidal neurons of the cerebral cortex in rat (41). The pyramidal neurons of the cerebral cortex project extensive collaterals locally and to other cerebrocortical regions and send projection fibers to a variety of structures including the thalamic nuclei, the basal ganglia, many parts of the brain stem, and the spinal cord (47). The presence of CGA and CGB immunoreactivity in the cerebral cortex and these subcortical structures in several species (15, 20, 28, 30-33) may be contained, in part, in cerebral cortical pyramidal nerve endings.

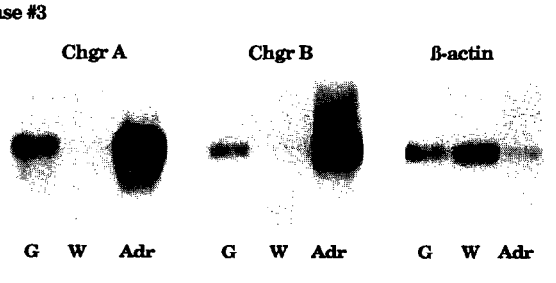

Fig. 2 Northern hybridization analysis of mRNA from human cerebral cortical grey matter, white matter and adrenal medulla. Approximately 5 μg of poly $(A)^+$ RNA was prepared from each tissue sample and the RNA blots were successively hybridized, stripped and reprobed with oligonucleotide probes derived from chromogranin A (24-mer), chromogranin B (39-mer) and β-actin (37-mer). Blot hybdridization and washing stringency for CGA and CGB signals were similar to those used for in situ experiments.

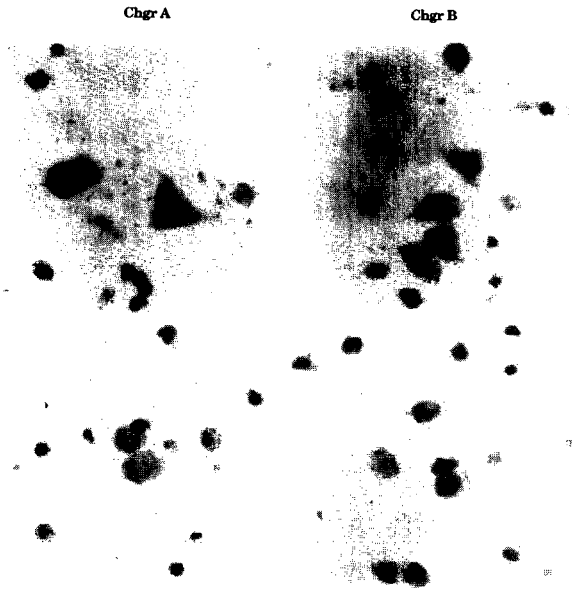

Fig. 3 In situ hybridization histochemistry of chromogranins A and B mRNA-containing cells in sections of human cerebral cortex. Large and small pyramidal-shaped neuronal cell bodies were positive for both chromogranins A and B mRNA (upper panel) while similiar cells were unlabeled in the presence of 100-fold excess of the respective unlabeled probe (lower panel).

We have examined the distribution of CGA and CGB mRNA in various human brain regions and in individual cells of cerebral cortex. The uneven distribution of CGA and CGB mRNA in the brain suggests that expression is restricted to specific neuronal subsets with the central nervous system. The expression of CGA and CGB mRNA in predominantly human cerebral grey matter, and particularly in pyramidal neurons of the cerebral cortex, supports a role for their gene products in central neuronal function.

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