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Neuroscience Vol. 49, No. 3, pp. 497–528, 1992
Printed in Great Britain

0306-4522/92 $5.00 + 0.00
Pergamon Press Ltd

COMMENTARY

THE CHROMOGRANINS A AND B: THE FIRST 25 YEARS AND FUTURE PERSPECTIVES

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1. INTRODUCTION

Twenty-five years ago, in 1967, Blaschko, Comline, Schneider, Silver and Smith[27] named the soluble proteins of bovine chromaffin granules chromogranins, and in the same year Schneider, Smith and Winkler[285] coined the term chromogranin A (CgA) for their major component (see Fig. 1). When this Journal, *Neuroscience*, was started in 1976, the first commentary[362] in volume 1 dealt with the early investigations on the chromogranins. It now appears timely to have a commentary derived from two questions. (i) What is finally established after 25 years? (ii) What has still to be done? Early studies have already revealed that the chromogranins were a

*To whom correspondence should be addressed.
Abbreviations: CgA, chromogranin A; CgB, chromogranin B; PSP, parathyroid secretory protein; RFLP, restriction fragment length polymorphism; SDS, sodium dodecyl sulfate.

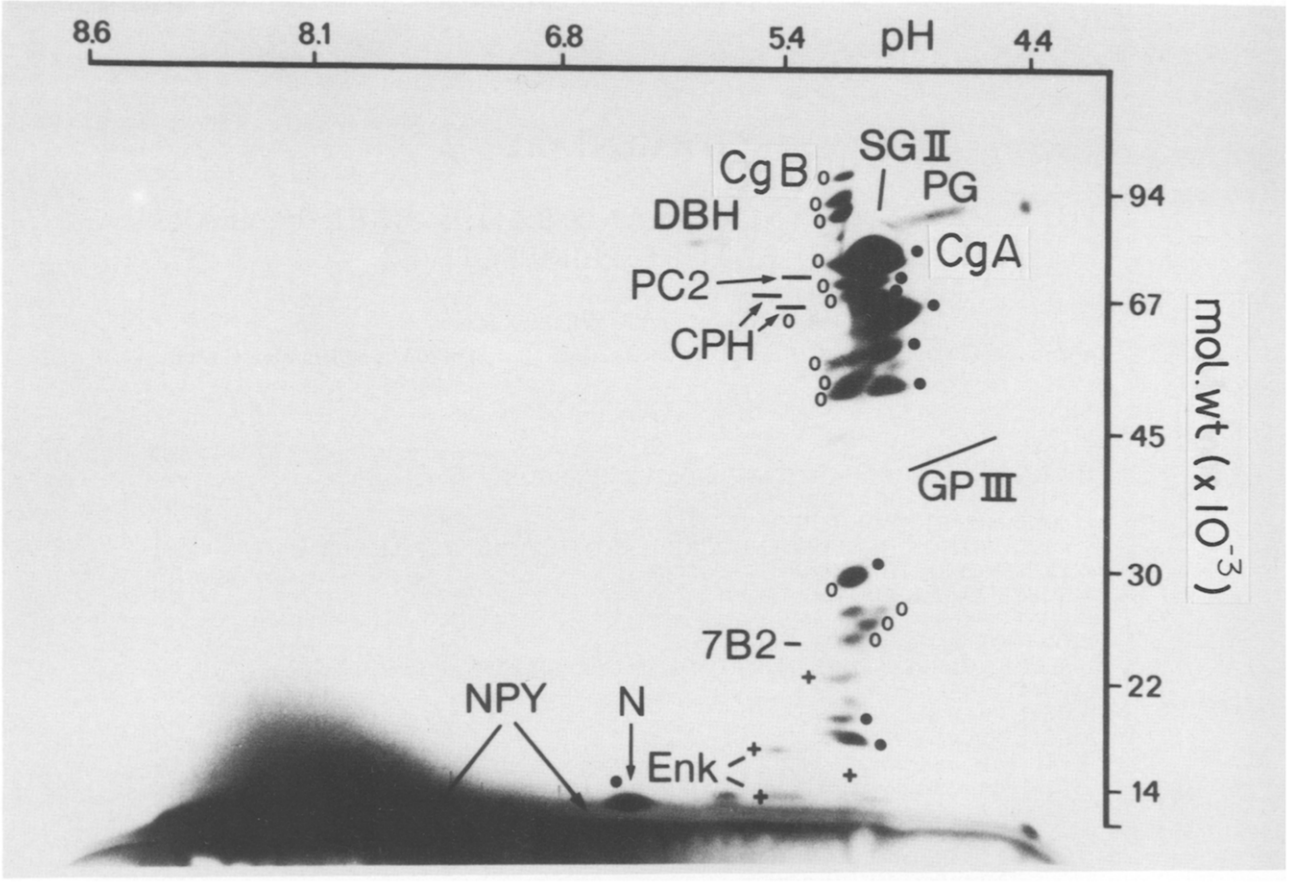
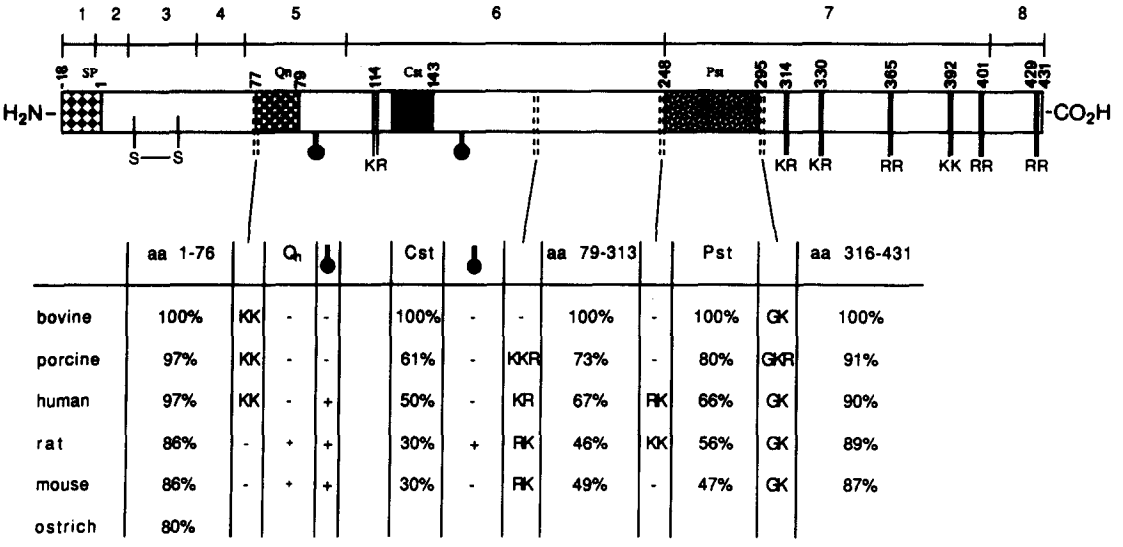


Fig. 1. Chromogranins in bovine chromaffin granules. The soluble proteins of bovine chromaffin granules were separated by two-dimensional electrophoresis.[8] For CgA (●), CgB (○) and enkephalins (Enk: +) the immunologically cross-reacting spots are marked. For CgA and CgB the largest molecules represent the respective proproteins. The spot (marked N) represents the N-terminal peptide, derived from CgA.[14,373] Further spots are: NPY, neuropeptide Y; PG, proteoglycan form of CgA; DBH, dopamine β-hydroxylase;[364] SGII, secretogranin II; 7B2: see Ref. 223. For the following minor components of the soluble lysate their position is marked; however, spots cannot be visualized with Coomassie Blue, but only after enrichment with lectin affinity chromatography (CPH, carboxypeptidase;[193] GPIII, glycoprotein III;[89] PC2, endoprotease[183a]).

complex mixture of proteins[362] (see Fig. 1). This is due to the presence of a large number of distinct proteins, but is also caused by the fact that these proteins are processed within the chromaffin granules by endogenous proteases (see below). Thus in addition to CgA several breakdown products of this proprotein are present[149] (see Fig. 1).

In 1984[79,366] we described a second group of acidic proteins, i.e. the chromogranins B (CgB) which could be immunologically differentiated from CgA. These proteins were subsequently also called secretogranin I;[274] however, since they are related to CgA (see below) in some respects this name is less appropriate (see nomenclature agreement).[69] In addition (see Fig. 1) chromaffin granules contain secretogranin II[81,274] (originally discovered in the pituitary gland).[276] This protein has also been called chromogranin C[81] but a nomenclature proposal agreed on the name secretogranin II.[69] Furthermore, another protein isolated from pituitary, 7B2,[222] is also present in chromaffin granules[223] and so are numerous neuropeptides and their precursors. Higher concentrations are found for the enkephalins and neuropeptide Y.[364,371] Finally the secretory proteins include dopamine β-hydroxylase, glycoprotein III,[364] carboxypeptidase H[93,193] and endoproteases of the kex2 type.[44,290,183a] The recent proposal[154] to call the acidic proteins of secretory organelles "granins" would at present add a further, quite unnecessary name, since the original name chromogranins already covered the same proteins. Further nomenclature proposals should await a better understanding of the function of all these proteins.

In the present commentary we will concentrate on the two closely related proteins, CgA and CgB, and will not deal with the other secretory proteins. Several reviews on this topic have been published.[57,82,132,143,261,306,315,355,362,364,365,371]



	aa 1-76		Q_n		Cst			aa 79-313		Pst		aa 316-431
bovine	100%	KK	-	-	100%	-	-	100%	-	100%	GK	100%
porcine	97%	KK	-	-	61%	-	KKR	73%	-	80%	GKR	91%
human	97%	KK	-	+	50%	-	KR	67%	RK	66%	GK	90%
rat	86%	-	+	+	30%	+	RK	46%	KK	56%	GK	89%
mouse	86%	-	+	+	30%	-	RK	49%	-	47%	GK	87%
ostrich	80%											

Fig. 2. CgA: amino acid sequences and gene structure. The top part of this figure presents the gene structure[162,374] with the exons of the CgA gene numbered from 1 to 8. Below, a schematic consensus sequence is shown. The numbers on top indicate amino acid positions of bovine CgA.[1,20,160] In addition, the sequences for rat,[158,163,256] human,[138,186] pig[161] and mouse[374] CgA and for a peptide from ostrich[199] are given. Certain peptides are indicated: SP, signal peptides; Q_n, polyglutamyl region; Cst, chromostatin; Pst, pancreastatin. Glycosylation sites are marked by black dots. Dibasic cleavage sites are marked by two lines for those conserved in the five species (the single-letter amino acid code is given for these sites). Cleavage sites not conserved are marked by double broken lines. The species comparison demonstrates which features of the sequence are conserved. A high degree of conservation is found in the N- (aa, amino acid 1-76) and C-terminals (aa, amino acid 316-431) whereas in the middle part of the molecule, where the defined peptides are located, the conservation is rather limited.

2. ISOLATION AND QUANTITATIVE DETERMINATION

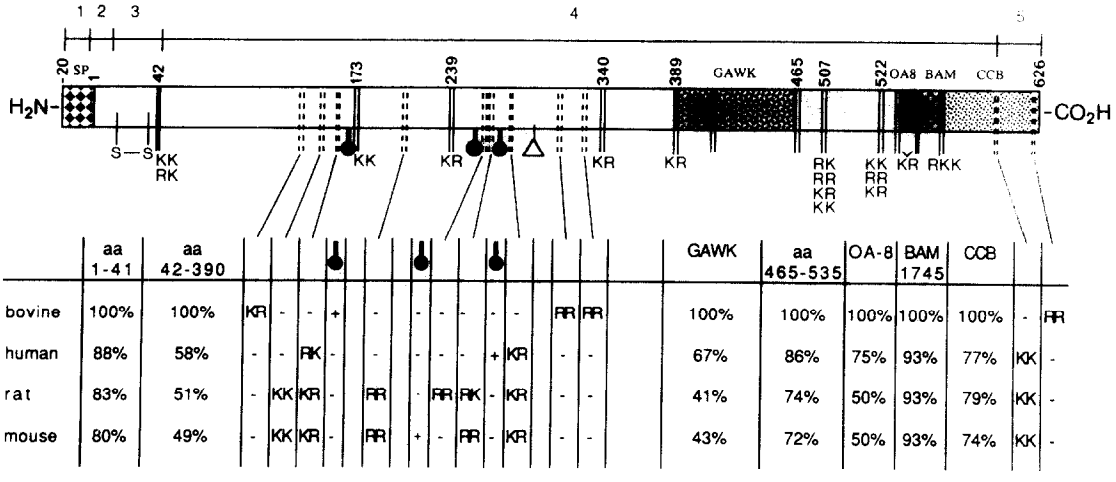
For the isolation of chromogranins several methods have employed chromatography on molecular sieve, ionic exchange or butyl Sepharose columns.[49,134,180,317,318,323] More recent purification schemes are based on high pressure liquid chromatography with molecular sieve, ionic exchange and reverse phase columns,[86,123,296] chromatography on calmodulin columns,[375] immunoaffinity chromatography[359] and preparative one- or two-dimensional electrophoresis.[251,252,274] The heat stability[274] of these proteins is very useful for preventing proteolysis during isolation and also provides a very efficient step of purification since many other proteins can simply be removed by boiling tissue.[286] Since clones for both CgA and CgB are available, pure chromogranins can now also be produced by recombinant techniques.[144]

Radioimmunoassays[70,232,247,253] and enzyme assays[64,180] have been developed with antisera for CgA, but also for the CgA-derived polypeptide pancreastatin[31,53,99,178,255,284,345] and the GAWK peptides derived from CgB.[279,294] These assays are very sensitive (100 pg for radioimmunoassay, 1 pg for enzyme assay); however, it is not clear which peptide moieties are actually measured, since the antisera react both with intact CgA and its breakdown products irrespective of whether they were originally raised against CgA or peptides derived from it. With quantitative immunoblotting[79,350] one can measure the relative concentrations of CgA or its larger breakdown products but one misses smaller peptides (< 10,000 mol. wt) formed from them. An ideal assay would depend on an antiserum against a synthetic peptide (flanked by basic pairs in the CgA sequence) used in a radioimmunoassay before and after digestion of all larger CgA molecules to smaller peptides (cf. enkephalin assays).[337] For the chromogranins this approach has apparently only been used once.[345]

3. PHYSICO-CHEMICAL PROPERTIES

In the following we will only deal with some general features related to the primary amino acid sequence of these proteins. Further properties acquired by post-translational modification (e.g. glycosylation) and the calcium binding ability of CgA will be discussed below.

The primary amino acid sequences of these proteins have been determined for several species (see Figs 2 and 3). For bovine CgA the mRNA has a length of 2100 bases, for CgB 2600. This message codes for mature proteins of 431 amino acids (48,000 mol. wt) for bovine CgA and 626 amino acids (71,500 mol. wt) for CgB. As already recognized previously from amino acid analyses (see Ref. 362) common features of these proteins are the high glutamic acid (25%) and proline (about 10%) content yielding an acidic pI of about 4.5–5.0.[8,362] The homology between CgA and CgB is rather limited, in agreement with the obvious lack of immunological cross-reaction;[79,274]



	aa 1-41	aa 42-390												GAWK	aa 465-535	OA-8	BAM 1745	CCB		
bovine	100%	100%	KR	-	-	+	-	-	-	-	-	-	RR	RR	100%	100%	100%	100%	100%	-	RR
human	88%	58%	-	-	RK	-	-	-	-	-	+	KR	-	-	67%	86%	75%	93%	77%	KK	-
rat	83%	51%	-	KK	KR	-	RR	-	RR	RK	-	KR	-	-	41%	74%	50%	93%	79%	KK	-
mouse	80%	49%	-	KK	KR	-	RR	+	-	RR	-	KR	-	-	43%	72%	50%	93%	74%	KK	-

Fig. 3. CgB: amino acid sequences and gene structure. The data are expressed exactly as in Fig. 2. The sequences for bovine,[17] human,[21] rat[92] and mouse[208,263] CgB are given. The various peptides (GAWK, OA8, BAM1745 and CCB) are discussed in the text. A tyrosine sulfation site is indicated by a triangle. As already mentioned for CgA and also for CgB, the N- and C-terminal ends are the most highly conserved parts of the molecule.

however, there is a potential disulfide loop present in both molecules and in this N-terminal region (coded by exon 3 of both proteins) a homology of 42% is found. In the C-terminal end a homology of 44% (exons 5 and 8: see Figs 2 and 3) is present (see Fig. 2). Further properties of these molecules are discussed in the subsequent sections.

Previous estimates of the molecular weight of bovine CgA by analytical ultracentrifugation[184,317] indicated a value of about 80,000. On the other hand, in the presence of guanidine chloride a molecular weight of 53,000 was obtained[184a] and for parathyroid CgA a molecular weight of 51,900 was determined in a sedimentation equilibrium study.[108] The reasons for these discrepancies are obscure. It is easier to explain the discrepancies between the molecular size found in sodium dodecylsulfate (SDS) electrophoresis (about 75,000) vs the real molecular mass of 48,000. This abnormal electrophoretic behavior is probably due to the acidic and hydrophilic nature of this protein causing reduced SDS binding, since a protein with a similar high glutamic acid content of 25%, DARP-32, exhibits an analogous anomaly between migration in SDS and molecular mass.[357] Similarly, bovine CgB moves in SDS electrophoresis like a protein of 100,000 mol. wt[79] but actually has a molecular mass of 71,461.[17]

The molecular size of CgA as determined by gel-filtration or by analytical ultracentrifugation depends very much on the ionic strength of the solvent. Based on these findings, Smith and Winkler[317] suggested that CgA has a configuration approaching that of a random coil polypeptide. This concept was confirmed by further *in vitro* studies[184] and also by nuclear magnetic resonance experiments on intact chromaffin granules[54,298] indicating that this property of CgA was not due to denaturing conditions during isolation. A detailed recent study employing circular dichroism measurements found that "native" CgA had a dominant random coil (>60%) but also some alpha-helix structure (25–40%) with no beta-sheets.[375] Such a model is also in agreement with predictions based on the primary structure of CgA.[114] Data on the secondary structure of CgB have apparently not been obtained; however, due to the similar amino acid composition "random coil" properties are likely to be present.

CgA is a hydrophilic protein without any hydrophobic stretches (see Ref. 114). Despite these properties the question of whether this molecule exists also in a membrane bound form has remained a controversial one (for a discussion of early work see Ref. 362). More recently Settleman[297] claimed that CgA is the major integral membrane protein of chromaffin granules which could not be removed by high or low salt washes; however, the most efficient procedure, i.e. washing in 0.1 M Na_2 CO_3[150] was apparently not used. In immunelectron microscopy of the adrenal medulla, positive immunostaining is confined to the content of chromaffin granules whereas the membrane separated from the content in these micrographs is clearly unlabeled.[10,370] On the other hand, for dopamine β-hydroxylase, which is present in the membrane, a positive immunostaining of the membrane was indeed obtained.[10] During isolation of membranes *in vitro*, small amounts of CgA might become enclosed in small vesicles formed during the lysis procedure. This might explain the difficulty in removing these traces by washing procedures. An analysis of isolated membranes by immunoelectron microscopy might finally settle this question. The hydrophilic nature of the chromogranins and the random coil properties are probably responsible for the heat stability of these proteins.[274,286]

4. ORGANIZATION OF THE GENES

A Southern blot analysis of genomic DNA digested with various restriction enzymes revealed that both CgA[1,158,186,230,240,256] and CgB[263] are present as single genes in the haploid genome. However, it has also been claimed that there are genomic differences in the amino acid sequences of CgA which may have arisen from a duplicated CgA gene.[345] The gene for human CgA was assigned to chromosome 14q32[230,240] and that of mouse CgB to chromosome 2.[171] In the human genome a two-allele restriction fragment length polymorphism (RFLP) was reported for CgA. Nine out of 10 restriction enzymes did not detect a RFLP; however, Bgl II digests of genomic DNA from 56 Caucasians did identify two alleles of 24.0 and 8.0 kb with frequencies of 0.34 and 0.66, respectively, and a constant band of 6 kb.[230]

The bovine and mouse CgA gene comprise eight exons separated by seven introns (Fig. 2) which span approximately 13.6 kb[162] and 11 kb.[374] The mouse CgB gene consists of five exons and four introns[263] and the coding region spans 12.1 kb (Fig. 3). A comparison of the mouse CgA gene with the mouse CgB gene reveals homologies in the structural organization of three exons. These three exons code for the N-terminal and C-terminal domains which share significant sequence homologies between both proteins. Exon 2 of mouse CgA and CgB encodes the last three amino acids of the signal peptide plus the first 13 amino acids of the N-terminus of mature CgA and CgB; exon 3 encodes for the next 33 amino acids containing the disulfide loop motif present in both CgA and CgB. Exon 8 of CgA and exon 5 of CgB code for the 27 and 25 C-terminal amino acids of both proteins. The variable middle domain of CgA and CgB that differs substantially between species and shares no significant homology between CgA and CgB is reflected also by a different genomic organization.

The region upstream of the Cg promoter contains one cyclic AMP response element in both CgA and CgB. With the exception of two Sph 1 sites for CgA and one Sph 1 site for CgB no further consensus matches for other known transcription factors were found.[162,263,374] The elements responsible for tissue-specific expression in multiple neuroendocrine cells await identification; for CgA the region containing this information was shown to reside within 1.2 kb upstream of the coding region.[374]

5. DISTRIBUTION IN NEUROENDOCRINE AND IMMUNE SYSTEM

Originally CgA was isolated from the chromaffin granules[134,317,318] of adrenal medulla. It was also found in the large dense-core vesicles of sympathetic nerve[12,15,56] and in the brainstem rich in adrenergic neurons.[147] Based on these studies it was assumed that CgA was an "adrenergic" protein confined to sympathetic neurons and to the adrenal medulla. In 1982 we discovered[50] that CgA and secretory protein I (also called parathyroid secretory protein, PSP, Ref. 323) independently characterized (see Ref. 47) in the parathyroid gland were apparently identical proteins. Lloyd and Wilson[216] had raised a monoclonal antibody against a protein present in pheochromocytoma tissue which reacted with a large number of endocrine tissues and their tumors and the responsible antigen was then identified as CgA.[358] Within a short time several groups firmly established that CgA had a widespread distribution in endocrine tissues[46,84,248,358] (for further references see Table 1). When CgB was characterized in adrenal medulla in 1984[79,366] it was clear from the beginning that CgB shared this property with CgA[84,274] (for further references see Table 1).

The occurrence of immunoreactive CgA and CgB, of peptides derived from them and of their mRNAs in most endocrine tissues is well established and we will only comment on a few points (for references for the presence of CgA and CgB in tumor tissue see Refs 210, 217, 348, and an extensive review, 355).

Chromogranin immunoreactivity as defined by immunohistochemistry exhibits great variations depending on the fixation method[116,203,237,244,273,277] and dilution of antisera.[118] This capricious behavior makes it rather difficult to draw definite conclusions on the presence or absence of chromogranins in various cell types and therefore explains discrepancies found in the literature. For Table 1 we have not included negative results when positive immunostaining was reported by other groups, since a positive result would appear more reliable. In any case, for many tissues independent methods (immunohistochemistry, immunoblotting, *in situ* hybridization and radioimmunoassay) provide us with concordant results.

Immunohistology does not reveal the molecular properties of the detected antigens, but for several tissues immunoblotting (see Table 1) has established that these endocrine tissues contain CgA and CgB of the same size as in the adrenal medulla but also a variable amount (see below) of breakdown products.[84,122,159,210,244,277] Furthermore, for two tissues (pituitary and endocrine pancreas) the cDNA clone for CgA was found to be practically identical to the adrenal one.[1,158]

It is interesting to consider the relative size of the CgA stores in neuroendocrine tissues.[327] Thus in human beings the largest store of CgA is found in the adrenal medulla; the pituitary contains 25% of this amount (which appears surprisingly high), the pancreas and stomach plus intestine 5% each, whereas all other endocrine glands represent less than 1%. In addition to the classical endocrine tissues, CgA has also been found in scattered neuroendocrine cells in several organs like the breast, the lung or the prostate (see Table 1). Its presence in the storage granules of the myoendocrine cells of the heart storing the atrial

Table 1. Distribution of chromogranins and related peptides in neuroendocrine tissues

	Human	Bovine	Rat	Other
Anterior pituitary				
Unspecified cells	IB: A,[122] B[122] RIA: A,[327] GAWK[25] IH: A,[214,216,249,282,358] B,[122,282] GAWK[22a] EM: A[131] NB: A[138,139] IS: A,[211,212] B[211,212]	IB: A,[14,84,180,202,210,274,319] B:[84,106] RIA: A,[14,180,245,249,251] IH: A,[195,248] B[195] NB: A[1,160] IS: A[302]	IB: A,[5,88,190,350] βG[159] B[88] RIA: Pst,[53] GAWK[279] IH: A,[84] Pst,[53] βG,[159] B[274] NB: A,[87,88,163] B[87,92] IS: A,[87] B[92]	IB: A,[319,277] B[277] RIA: Pst,[31,178,284] GAWK[279] IH: Pst[191,267,284] EM: Pst[191]
TSH cells	IH: A,[33,215] B,[33] GAWK[22] EM: GAWK[25]	EM: A,[16] B[16]	IH: A[46]	IH: A,[273,277] Pst,[31,178,191] B[273]
LH/FSH cells	IH: A,[33,215] B[33] EM: GAWK[25]	IH: A[249] EM: A,[16] B[16]	IH: A[46,88,344] EM: A[344]	IH: A,[273,277] Pst,[31,178,191] B[273,277]
GH cells	IH: A,[33] B,[33] GAWK[22] EM: GAWK[22]	IH: A[249]		
Multihormonal cells		EM: A,[16] B[16]		
Intermediate pituitary		RIA: A[245,249] IH: A[195,248] IS: A[302]	IH: βG,[159] B[88]	IH: B[277]
Posterior pituitary		RIA: A[245,249]	IH: βG[159]	RIA: Pst[178] IH: Pst[178]
Pancreas				
Unspecified cells	IB: A[122] RIA: A,[259,327] Pst,[284] GAWK[25,294] IH: A,[214,216,270,358] Pst,[249,270] B[122] IS: A,[212] B[212]	IB: A,[14,68,378] B[378] RIA: A[245,249] IH: A,[68,249] Pst,[345] B[378]	IB: A[350] RIA: Pst[53] IH: Pst,[53] B[84]	IB: Pst[31,284] IH: A,[270] Pst[191,270,284]
Insulin cells	IH: A,[33,116,213,282] B[33] EM: A[131]	IH: A[67,116,117,248,378] EM: A[67]	IB: βG[159] IH: βG[159]	IH: A,[39,34,116,117,118] Pst,[31,267] B[39] EM: A[41] Pst[191,267]
Glucagon cells	IH: A,[33,116,117,213,273,282] Pst,[284] B[273,282] EM: A,[131,174,339] B[25] GAWK[25]	IH: A,[67,116,378] B[273] EM: A[67]	IH: A,[116] βG[159]	IH: A,[34,116,273] Pst,[31] B[34,273] EM: A,[39] Pst[191]
Somatostatin cells	IH: Pst[284]	IH: A[67,116,378] EM: A[67]	IH: A[46]	IH: A,[39,116,117,118] Pst,[31,267] B[118] EM: A,[39] Pst[191,267]
PP cells	IH: A,[33,273,282] B[33,282]	IH: A[67,116]	IH: A[46]	IH: A,[34,39,116,117,118,273] Pst,[31] B[118] EM: A[39,41] IH: A[140]
Esophagus				
Stomach				
Unspecified cells	RIA: A,[327] Pst,[284] GAWK[25] IH: A,[270,356,358] Pst,[270] B[356] GAWK[25] EM: A[131]	IH: Pst[345]	RIA: Pst[53] IH: A,[270] βG,[159] Pst[53,270]	RIA: Pst[31] IH: A,[270] Pst[169,270]

Enterochromaffin	IH: A,[33,42,72,214,282] B[25,282] GAWK[25]	IH: A,[42] B[84]	IH: A,[117] B[84]	IH: A,[40,42] Pst[31,191] EM: A,[41] Pst[191]
Somatostatin	IH: A[42,72,356]		IH: A[46]	IH: A,[40,42] Pst[191] EM: Pst[191]
Substance P			IH: A[46]	
EC-like cell	IH: A[33,42,72,273]		IH: A[273]	IH: A[40,42,273] EM: A[41]
Gastrin cell	IH: A,[33,72,273,282,356] B,[25,33] GAWK[25] EM: A[339]	IH: A,[179,273] B[273]	IH: A,[117,273] B[273]	IH: A,[34,40,117,273] Pst,[31,191] B[34,40,117,273] EM: A,[41] Pst,[191] B[41]
Glucagon cell	EM: A[339]	IH: A[179]		
PP cell				IH: A,[34] B[34]
Small intestine				
Unspecified cells	RIA: A,[326] GAWK[25] IH: A[42,249,262,282,356,358] B:[282,356] EM: A[131]	IB: A[14] RIA: A[249] IH: A,[42,248,249] Pst[345]	IB: B[274] RIA: Pst[53] IH: B,[274] βG[159] NB: B[92]	RIA: Pst[31,284] IH: A,[42,300] Pst[267,284,300]
Enterochromaffin	IH: A,[33,72] B[33]	IH: A,[195] B[195,273]	IH: A,[273] B[273]	IH: A,[34,40,273] Pst,[31,191] B[34,273]
Gastrin cell	IH: A,[33,72,356] B[33]	IH: A[179]		IH: Pst[31,191]
Peptide YY cells	IH: A[33,42]	IH: A[42]		IH: A[40,42]
Motilin cells	IH: A[72]	IH: A[179]		IH: A,[42] Pst[31]
Neurotensin cells	IH: A[33,42,72] EM: A[339]			IH: A,[40,42] Pst[31]
Secretin cell	IH: A,[33,42,72,338] B,[25] GAWK[25] EM: A[338]	IH: A[42]		IH: A,[34,40,42] Pst[31,191]
Substance P		IH: A[179]		
Somatostatin cells	IH: A[72,356]			IH: Pst[31]
CCK cell	IH: A[72]	IH: A[42]		IH: A,[40,42] Pst[31]
Enteroglucagon	IH: A[33,72]			IH: Pst[31,191]
GIP cell	IH: A[42,72]	IH: A[42]		IH: A,[40,42] Pst[31]
PP cell				IH: B[34]
Cells in lamina propria	IH: A[265]		IH: A[265]	
Large bowel				
Unspecified cells	RIA: GAWK[25] IH: A[359,270]		RIA: Pst[53] IH: βG[159]	RIA: Pst[31,284] IH: A,[270] Pst[270]
Enterochromaffin	IH: A,[72] B,[25] GAWK[25] EM: A,[339] GAWK[25]			IH: A,[34,40] Pst,[31] B[34]
Enteroglucagon	IH: A[72]			IH: Pst[31]
Peptide YY	IH: A[72]			
Somatostatin	IH: A[72]			IH: Pst[31]
Parathyroid	IH: A[122] RIA: A[326] IH: A,[33,216,249,273,282,358] B[33] EM: A[131] IS: A,[211] B[211]	IB: A[195,202] RIA: A[180] IH: A,[195,244,248,249,273] B[84] EM: A[9] NB: A[1,160] IS: A[302]		IB: A[107] IH: A[273]

—continues overleaf

Table 1—*continued*

	Human	Bovine	Rat	Other
Thyroid C-cells	RIA: A[326] IH: A,[197,216,249,273,282,358] B[33,282]	IB: A[244] RIA: A[249] IH: A,[195,244,248,249] B[195]	RIA: Pst[53] IH: Pst[53]	IB: A,[347] B[347] RIA: Pst[31] IH: A,[273] Pst[31,169]
Merkel cells	IH: A[127] EM: Pst[126]			IH: A[104,127] EM: A,[128,127,125] Pst[126,125,169]
Neuroendocrine cells of				
Breast	IH: A[37]			
Heart (ANF cells)			IB: A,[321] B[321] IH: A,[321] B[321]	
Lung, trachea	IH: A[197,358]			IH: A[197]
Urether, bladder				IH: A[124,340]
Prostate				EM: A[55]
Uterus				IH: A[341]
Lymphoreticular tissue		IB: A[244] IH: A[244]	IH: A[145]	
Central nervous system				
Neurons	RIA: A,[326] GAWK[279] IH: A,[236,235,237] B[282]	IB: A[14,180,319] RIA: A[180,249,251] IH: A[244] NB: A[160] IS: A[302]	IB: A,[190,350] B[274,350] RIA: Pst,[53] GAWK[279] IH: A,[190] B[274] NB: A,[92,219] B[92,219] IS: A,[219] B[92,219]	IB: A[319] RIA: Pst,[178,284] GAWK[279] IH: A,[319] Pst[169,178]
Glia	IH: A[225]			
Spinal cord	IH: B[282]	IH: A[244]	IH: A[29]	IH: A,[319] Pst[178] RIA: Pst[178]
Ganglia and peripheral nerves	RIA: A[326] IH: A[141]	IB: A,[84,121,242,289] B[84,121] RIA: A[251,249] IH: A,[195] B[195]	IH: A[29,46,141,342] B[84]	IH: A,[141,176] Pst[169]
Retina	IH: A[244]	IB: A[244] IH: A[244]		IH: Pst[169]
Liquor	RIA: A,[301] GAWK[279]			
Serum	RIA: A,[52,64,151,172,325,326,327,246,247] GAWK[294]	RIA: A[180,249,251]		RIA: Pst[3]
Urine	RIA: A,[151] Pst[100,329,330]			

The table gives the distribution for the three species as defined, whereas all other species are included in the "other" column. A, chromogranin A; B, chromogranin B; Pst, pancreastatin; βG, beta-granin; IB, immunoblot; RIA, radioimmunoassay; IH, immunohistochemistry; EM, immunoelectron microscopy; NB, Northern blot; IS, *in situ* hybridization histochemistry; TSH, thyroid stimulating hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; GH, growth hormone; PP, pancreatic polypeptide; EC, enterochromaffin; CCK, cholecystokinin; GIP, gastric inhibitory peptide; ANF, atrial natriuretic factor.

natriuretic factor[321] raises the question whether it is also found in the renin granules of the kidney. One might also ask whether the erythropoietin cells of the same organ contain CgA. Thus CgA presents a general neuroendocrine marker and that has made it useful for a histopathological classification of neuroendocrine tumors.[244,355] However, recent publications for the first time also reported an immunostaining for CgA in exocrine cells, i.e. alveolar type II cells of rat lung[173] and for CgA and CgB in duct cells of the submandibular gland whose nature was, however, not well defined.[204,205,244]

As far as the immune system is concerned, one study reported the presence of scarcely distributed CgA-positive cells in spleen, lymph node, thymus and fetal liver.[145] By Western blotting small amounts of immunoreactive CgA were also found in these organs. Unfortunately this interesting observation has not been followed up. Are these special cells natural killer cells which are known to store and secrete enzymes and peptides by exocytosis?[324]

The relative concentration of CgA and CgB varies from tissue to tissue; however, in general, both proteins are found to be co-stored, but there is also evidence for a dissociation of their storage. Examples are the high concentration of CgA in the parathyroid gland (see Table 1) but the presence of only small amounts of CgB[210,212] if any[33,122,195,273] or the presence of CgA in ovine and bovine intermediate pituitary but the absence of CgB[195,277] or the differential staining of some enterochromaffin cells for A, but not for B in the stomach;[33,282] however, a staining of at least some of these cells for the GAWK protein derived from CgB has also been reported.[25] Analogous results, i.e. a differential localization of CgA and CgB, have been obtained for brain (see below).

6. PRESENCE IN THE NERVOUS SYSTEM

The presence of CgA in the central nervous system was already established in early studies.[147,244,251,319] A detailed immunohistochemical mapping of ovine brain and spinal cord[319] revealed a widespread distribution of the antigen. The immunoreactivity partly overlapped with those of several neuropeptides and other markers like tyrosine hydroxylase and dopamine β-hydroxylase. However, the conclusion reached was that CgA is present in neurons using many different transmitters. This protein, originally thought to be "adrenergic" is also found in cholinergic neurons like the motoneurons of the spinal cord.[319] In agreement, CgA was also found in cholinergic nerve terminals in the muscles of the diaphragm.[342] For human brain, Munoz and collaborators demonstrated that if methodical difficulties are overcome, a widespread distribution of CgA can be found.[235-237] Interestingly, a high proportion of senile plaques in Alzheimer's disease,[32,196,233,234,349] and also of Pick's bodies in another brain disease leading to dementia, consistently immunostain for CgA.[349] Furthermore, the CgA/synaptophysin ratio in such diseased brains is high.[196,349]

In porcine brain, CgA and a peptide derived from it, i.e. pancreastatin, have an identical, widespread distribution.[178] CgA immunoreactivity has also been found in bovine[244] and porcine[169] retina. Quantitative analyses of CgA by radioimmunoassay in bovine brain[180,251] and by immunoblotting in rat brain[350] revealed that the concentration of CgA was only about 0.01–0.2% of that in the respective adrenal medulla. The claim[190] that the rat olfactory bulb as measured by immunoblots contains CgA in amounts comparable to that of adrenal medulla and the pituitary is likely to be wrong since at least in ox the pituitary gland has only 3% of the CgA concentration found in adrenal medulla.[180,251]

Bovine and rat brain contain CgA mRNA which has been measured by Northern blotting.[160,190,219,350] With tissue hybridization CgA mRNA was found in the caudate nucleus of bovine brain.[302] A detailed mapping analysis of rat brain[219] revealed the highest concentration in the pyramidal cell layer of the hippocampus, in the subiculum and the septum, with lower concentrations in many other brain areas, in agreement with the immunohistochemical data. For CgB a first comprehensive study measured the distribution of GAWK, a peptide derived from CgB, in the brain of several species, by radioimmunoassay.[279] In human brain the highest concentrations were found in the hypothalamus, hippocampus, amygdala, nucleus accumbens, the basal ganglia and the cerebellum; much lower concentrations were present in rat and guinea-pig brain. Immunoblotting measuring the proprotein CgB in rat brain revealed the highest concentration in the rat cerebellum, followed by the hypothalamus and amygdala.[350] Only very limited immunohistochemical data are available for human brain with a positive staining found in the Purkinje cells of the cerebellum[282] and for rat brain.[274] Two studies[92,219] used *in situ* hybridization to study the distribution of CgB mRNA in rat brain. In agreement with the immunochemical data, a wide distribution was reported with high concentrations in parts of the cortex, pyramidal cell layer of the hippocampus, in the dorsal raphe, pontine nucleus and several other regions. A comparison of the distribution of the mRNA of CgA and CgB[219] revealed that some nuclei apparently synthesized only CgA but not CgB, e.g. the subiculum, whereas the dorsal raphe and the granule cell layer of the cerebellum were only positive for the CgB-message. Immunoblotting indicated that CgA and CgB found in brain are identical to the adrenal proteins in size and migration behavior (ovine CgA;[319] rat CgA and CgB).[274,350] However, a relatively higher concentration of the proteoglycan form of CgA was present when compared with adrenal medulla and especially with endocrine tissue like the anterior pituitary.[350]

7. SUBCELLULAR LOCALIZATION

Two methods are particularly useful to establish the subcellular localization of a component, i.e. subcellular fractionation and immunohistochemistry at the ultrastructural level. With the first method the specific localization of CgA in bovine[79,251,278] and human[252] and of CgB in bovine[79] chromaffin granules has been established. The same approach was used for localizing CgA in the storage vesicles of anterior pituitary[245] and of betagranin, a peptide derived from CgA, in the insulin granules of pancreas[157] and of CgA[56,242,289] and CgB in the large dense-core vesicles of splenic nerve.[121,289] One of these studies[242] also revealed that small dense-core vesicles of adrenergic nerves were devoid of CgA. What is the concentration of CgA in these vesicles relative to other peptide constituents? In bovine chromaffin granules the CgA family represents the major component, although the values obtained rather differ (70% as calculated from densitometric scans and the degree of degradation[82] vs 46% as determined by radioimmunoassay).[14] We have calculated that in a single bovine chromaffin granule there are about 5000 molecules of the proprotein CgA present.[371] A quite similar value (2763 molecules) was found by quantitative immunelectron microscopy.[224] In the anterior pituitary granules, CgA is only a minor (4.5%) component[14] and this is also true for the endocrine pancreas where the insulin/CgA ratio is about 2000.[68] Even considering that in this tissue, most of the CgA is processed (see below) this would make CgA a minor component in insulin granules (taking 90% breakdown into account: only 3 mg total CgA/100 mg insulin). The observation[251] that after subcellular fractionation of the bovine brain the cytosol contained the majority (69%) of CgA apparently suggested that in this tissue CgA is not localized within the secretory compartment. However, immunelectron-microscopy[319] revealed that CgA reactivity is confined to Golgi stacks and large dense-core vesicles, but absent from translucent synaptic vesicles and from the cytosol. Most likely during homogenization of brain a considerable part of the Golgi stacks and large dense-core vesicles release their content including CgA.

In 1974 we published the first data on the immunelectron microscopic localization of CgA in adrenal medulla.[370] Immunoreactivity was confined to the content of the vesicles; there was no staining of their membranes. Several studies[10,41,191,224,320] have confirmed this finding and extended it to CgB[41,320] and to other endocrine tissues (Table 1). By double immunolabeling or labeling of serial sections not only the presence of CgA and CgB in vesicles but also their co-localization with transmitters or neuropeptides within the same vesicles was established. Combinations of co-localization were: CgA/CgB/secretogranin II/neuropeptide Y,[320] CgA/parathyroid hormone,[9] CgA/insulin,[67] CgA/glucagon,[67] CgA/atrial natriuretic factor,[321] CgA/calcitonin,[304] thyroid stimulating hormone/CgA/CgB/secretogranin II,[16] and luteinizing hormone/CgA/CgB/secretogranin II.[16,344]

Within the same cell the respective hormones and the chromogranins are not always stored together in the same vesicles. This was first demonstrated for secretogranin II, prolactin and growth hormone for bovine somatomammotroph cells[129] and has now been extended to CgA and CgB.[16] In the somatomammotroph cells, two types of granules are present, i.e. small (150–300 nm) and larger (400–550 nm) ones. Only the small ones apparently contained CgA, CgB (and also secretogranin II, thyroid stimulating hormone and luteinizing hormone), whereas only the large ones contained growth hormone and prolactin. Apparently, in this special type of cell with multihormone storage, quite distinct sorting takes place. An analogous result was obtained for gonadotrophs of the anterior pituitary, in which large-sized granules reacted for follicle stimulating hormone, luteinizing hormone and CgA, whereas smaller ones were only positive for luteinizing hormone and secretogranin II.[344] It will be interesting to see whether detailed studies on other cells will reveal further subpopulations of granules exhibiting a differential storage of the various hormones and chromogranins.

Immunelectron microscopy has also revealed some uneven labeling for CgA within the vesicles. This was particularly marked for the glucagon-storing vesicles of endocrine pancreas where CgA or pancreastatin labeling was preferentially localized on a less electron-dense halo surrounding a dense core[67,131,191,339] whereas for insulin granules a peripheral immunostaining was reported only by one group[67] but not by others.[131,191] A labeling of the peripheral part of the vesicles has also been reported for Merkel cells[127] and for secretin-storing cells of the intestine,[338] and for CgB in small granules of the somatomammotroph cells,[16] and in the granules of gastrin cells[41] but apparently not for all the other secretory granules where CgA and CgB have been localized by immunostaining (see Table 1).

8. PHYLOGENY

The initial studies on CgA were performed with bovine tissues.[139,317,318] However, it was soon recognized that analogous components to CgA as shown by electrophoresis or by immunoreaction were present in horse, pig and sheep adrenals[133,264,372] and in human adrenals and pheochromocytoma.[322] CgA was then isolated and characterized from human[252,359] and also rat material.[86,274] In immunohistochemical studies a cross-reaction with antigens in several species including pig, cat, guinea-pig, dog and rabbit was observed.[42,118,273] For CgB in the first study[79] the occurrence of similar (electrophoresis, immunoreaction) components in several species (pig, horse,

cat, guinea-pig and rat) was recognized and this was soon extended to human material.[106,122]

The cloning of CgA and CgB from several species provides now the molecular basis for these immunological results. In some parts of the molecules (especially the N- and C-terminals) there is a high degree of homology between the individual mammalian species in agreement with the immunological cross-reaction (see Fig. 2). Unfortunately, only clones from mammalian species have been analysed. However, it is already clear that analogous proteins are present much earlier in phylogeny. Rieker *et al.*[272] found immunoreactive components comparable in size to CgA and CgB in chicken, frog, fish and lobster. In chicken ultimobranchial gland,[175] in toad urinary bladder[55] and in several fish species, CgA immunoreactivity (with antimammalian CgA) could also be demonstrated by immunohistology and radioimmunoassay.[58,333] In the ostrich pituitary a large peptide (see Fig. 2) with an 80% homology to the N-terminal of bovine CgA has been described[199] and in *Drosophila* heads an immunoreactive band for CgA could be detected by immunoblotting (Kapelari and Winkler, unpublished observation). A detailed immunohistochemical study on CgA and pancreastatin in the gastro-entero-pancreatic system revealed positive reaction apart from mammalian species in birds, reptiles and fish but not in deuterostomian invertebrates.[270]

A molecular cloning of chromogranins from non-mammalian species would seem extremely useful for defining regions with a high degree of conservation. Peptides in these regions would be good candidates for a relevant function which has evolved during phylogeny. Finally, even in the protozoan, *Paramecium tetraurelia*, peptides immunologically cross-reacting with antisera against CgA and synthetic peptides derived from it have been found.[260] However, in contrast to all other species only small peptides of 20,000 mol. wt were present. On the other hand, the mRNA hybridizing with a CgA probe suggests a much larger molecular weight at least for an unprocessed proprotein. Since the CgA antisera immunostained components which represented the major soluble proteins of the trichocysts of *Paramecium* an artefact is very difficult to exclude. Therefore these data should be confirmed by an independent approach, e.g. sequencing of some of these peptides.

As compiled by Fischer-Colbrie *et al.*,[82] the relative amounts of CgA vs CgB in the different species vary. In ox adrenals, CgA is by far the dominating component, whereas in rat and human material CgB is prominent.[86,122] Thus the CgA/CgB ratio in ox is 9, whereas in human material it is about 0.8.[106]

9. ONTOGENY

Several immunohistochemical studies investigated the ontogenic development of CgA. In rat fetuses, CgA immunoreactivity appeared at day 18 of the gestation period and at 15 days of postnatal age was similar to the adult staining pattern.[181] In human fetuses a positive reaction for CgA[231] and CgA-mRNA[138] was found in adrenal primordia at six to eight weeks of the gestational period, slightly proceeded by immunostaining for tyrosine hydroxylase. At nine weeks, positive staining was confined to "large cells" considered to be progenitors of chromaffin cells.[231] In the endocrine cells of the human intestinal tract, CgA immunoreactivity appeared together with peptides typical to these cells, e.g. somatostatin, at eight weeks of gestation[71] and also in the ultimobranchial gland of the chicken[175] and in the carotid body[176] there was a concomitant appearance with other peptides and with tyrosine hydroxylase. For CgB the appearance of the mRNA has been studied in developing rat brain.[92] It was first detected at embryonic day 14 and then increases till three weeks postnatally, when it starts to drop again to a level close to that found at birth.

A detailed study by immunoblotting of the development of CgA and CgB in rat adrenal medulla revealed two additional interesting features.[287] Both chromogranins were detected on the 17th prenatal day. Their levels increased together with that of the catecholamines. However, the relative concentration of CgA vs CgB showed a significant change. At the end of the gestational period the ratio of CgA to CgB increases strongly at exactly the same time when the adrenaline/noradrenaline ratio becomes elevated. This latter effect is known to be due to the induction of methylation caused by the increase in cortisone production at this gestational time point.[45] For CgA we have shown[83,303] that its biosynthesis is increased by corticosteroids. The observation of an increase of CgA biosynthesis together with that of adrenaline establishes the ontogenic significance of this concept. A second finding, described by Schober *et al.*,[287] concerns the proteoglycan form of CgA. Whereas fetal adrenal contains a relatively high concentration of this CgA form, in adult animals it is much less prominent. Since rat brain also contains a prominent proteoglycan form of CgA[350] this result was interpreted as a sign of the developmental relationship between early chromaffin cells and neurons.

10. SECRETION, PRESENCE IN SERUM AND CEREBROSPINAL FLUID AND FINAL FATE

Secretion of catecholamines from the adrenal medulla and chromaffin cell culture is accompanied by the release of all soluble constituents of chromaffin granules[13,363] including CgA[14,70,185,285,308,346] and CgB.[79] This is also true for noradrenaline release from the sympathetic nerve.[316] From the parathyroid gland, CgA is released with the parathormone[48] and betagranin, derived from CgA, is co-released with insulin from endocrine pancreas[157] and so is another CgA-derived peptide, pancreastatin.[3,255] This latter peptide

is also secreted from a human pancreastatin-producing cell line.[99] GAWK, a peptide derived from CgB, is released when rat anterior pituitary or bovine chromaffin cells are depolarized.[164,279] CgB is also released from PC12 cells[274] and a medullary thyroid carcinoma cell.[164]

Since secretion from all endocrine tissues and neurons occurs by exocytosis, it is obvious that in all these tissues chromogranins must be co-secreted with the respective hormone. After secretion from endocrine organs, CgA reaches the bloodstream via the capillary or the lymph. For the adrenal medulla it has been shown that, in contrast to the catecholamines, most of CgA is first directed into the lymph vessels.[38] In human serum, O'Connor and collaborators found by radioimmunoassay about 30–50 ng/ml CgA in normal controls,[250,253] whereas in patients with endocrine tumors these levels were significantly elevated.[250] Differential stimulation of various endocrine organs revealed that only stimulation of the adrenal gland led to measurable increases in plasma CgA levels.[327] On the other hand, in adrenalectomized patients, CgA levels in serum are in the normal range.[325] Furthermore, blockade of the sympathetic system by a ganglion blocker and non-selective blockade of neuroendocrine secretion by somatostatin led to a decline of the basal levels by 25 and 48%, respectively.[325] Thus basal CgA levels in plasma are mainly derived from these sources and not from the adrenal medulla (see also Ref. 52). The half-life of CgA in plasma is about 18 min.[247] Plasma levels of pancreastatin-like immunoreactive material, mainly representing high molecular weight peptides with only small amounts of free pancreastatin have also been determined.[3,31]

For GAWK (20–38), a peptide derived from CgB, a level of 150 pmol/l was found for human plasma.[294] Also, these levels are elevated in patients with neuroendocrine tumors. This concentration corresponds to 10 ng/ml of the proprotein CgB which is in the same range as the values for CgA. This seems reasonable considering that equal amounts of CgA and CgB are present at least in human adrenal medulla.

Human cerebrospinal fluid contains 170 ng/ml CgA[301] which is higher than the serum values (40 ng). This finding indicates either significant release of CgA from brain or a longer half-life of CgA in the cerebrospinal fluid. For GAWK (20–38) a value of 1.1 pmol/ml has been reported (85 ng CgB/ml).[279]

The studies discussed above were based on radioimmunoassays which do not define the molecular size of the measured molecules. However, in a recent study with immunoblotting the presence of the unprocessed proprotein CgA could be demonstrated in bovine serum.[352] Apparently, neuroendocrine tissues release unprocessed CgA which is not quickly degraded in serum. O'Connor and collaborators[151,253,379] reported that patients with renal failure had high CgA levels in serum. This indicated that the kidney might be essential for the removal of plasma CgA. Kidney tissue contains CgA, but no CgA-mRNA.[352] Immunelectron microscopy revealed that CgA immunoreactivity is found in proximal tubule cells. Within the cells it was present in small vesicles with or in close proximity to the brush border and closer to the nucleus in typical lysosomal structures. These results make it likely that CgA reaches kidney tubule cells by glomerular filtration and is taken up into the endocytotic lysosomal pathway followed by degradation. On the other hand, smaller peptides derived from CgA may reach the urine. At least, pancreastatin-like immunoreactivity has been detected in it.[330]

11. CELL-FREE SYNTHESIS

After *in vitro* translation of adrenal medullary mRNA, two translation products of very similar molecular weight (apparent molecular weight in SDS electrophoresis of about 70,000) can be immunoprecipitated with antisera against bovine CgA.[74,183] In analogous findings, four products of very similar size were reported for parathyroid mRNA.[220,221] When the *in vitro* translation for adrenal medulla was performed in the presence of dog pancreas microsomes only one immunoprecipitable translation product is found.[74] The two or more spots seen after *in vitro* translation could be due to different lengths of the signal peptides, but they may represent *in vitro* artefacts, since there is no evidence that signal peptides of different length are present in the bovine CgA sequence.[20,160] In any case, in agreement with the fact that there is apparently only one gene for CgA, only one *in vitro* translated product is found after the removal of the signal peptide. The claim[295] that several translation products are produced in such a system is therefore disproven. For CgB, cell-free systems yielded one translation product in bovine adrenal medulla.[74] For rat adrenal (PC12 cells) two products of very similar size are apparently produced.[274]

12. CELLULAR SYNTHESIS

12.1. *Rate of synthesis*

Early studies on bovine adrenal medulla had established that after labeling with tritiated amino acids the chromogranins were the major labeled products. Only 30 min after a radioactive pulse could the secretion of labeled CgA be induced by stimulation of the adrenal gland.[200,367,369] Apparently, the synthesis rate of the secretory protein CgA was much higher than that of the membrane proteins which led to the suggestion that the membranes of chromaffin granules are re-used for several secretory cycles from the Golgi region to the plasma membrane.[361,369] These data were recently extended in a careful study[360] comparing the synthesis rates of CgA, enkephalins and dopamine β-hydroxylase in bovine chromaffin cell cultures. Whereas CgA and enkephalin had about

the same rate of synthesis, that of dopamine β-hydroxylase was lower by a factor of 10. The most likely explanation is that this enzyme which is partly membrane bound[368] can be replenished at a lower rate since the membranes are re-used.

Further studies investigated the rate of amino acid incorporation in the presence of various secretagogues. In one series of experiments a direct correlation of this synthesis to the rate of secretion, as induced by several secretagogues, was found.[307] In contrast, Wilson *et al.*[360] did not discover any changes in CgA synthesis under very similar conditions. There was also no increase in total (cells and medium) CgA levels after stimulation of chromaffin cells in culture by nicotine treatment.[70] Apparently, there are some discrepancies and we will discuss this topic further in the section on the regulation of biosynthesis.

12.2. *Post-translational modifications*

Pulse-chase labeling of chromaffin cells of bovine adrenal medulla followed by two-dimensional electrophoresis revealed that the early labeled CgA and CgB spots behaved identically to the *in vitro* translation products formed in the presence of microsomes.[74] However, at later intervals significant changes in size and pI occurred which were especially marked for CgB. These modifications were obviously due to the post-translational processes which we will now discuss.

12.2.1. *Glycosylation.* Bovine CgA of adrenal medulla is a glycoprotein (5.4% carbohydrate) with galactose, *N*-acetylgalactosamine and sialic acid as major components.[85,105,317] The sugars are mainly present as O-glycosidically linked tri- and tetrasaccharides composed of *N*-acetylgalactosamine, galactose and sialic acid.[182] The primary amino acid sequence does not contain potential sites for N-glycosylation in this species; such sites are present in human, rat and mouse sequences (see Fig. 2), but it is not known whether N-glycosidically linked sugars are actually added in these species. For CgA of the parathyroid gland a very similar sugar composition has been reported;[50] however, with one exception, i.e. a much lower content of galactosamine. It seems unlikely that this low value is correct since the tri- and tetrasaccharides require about equal amounts of galactose and galactosamine. It would therefore seem premature to suggest that CgA outside the adrenal medulla is differently glycosylated, but further studies should settle this question.

The sugar composition of rat chromogranins has been investigated but a separation of CgA and CgB was not performed at that time.[280] Bovine CgB has a sugar composition similar to that of CgA but with more fucose and mannose (see Ref. 85; CgB in this early paper was called A1). In contrast to CgA it reacts with *Pisum sativum* lectin, but like CgA it binds peanut agglutinin after neuraminidase treatment.[7] Sites for N-glycosylation are present in the amino acid sequences of ovine, mouse and human CgB. The presence of fucose and mannose in bovine CgB may indicate that this molecule is actually N-glycosylated. In other tissues the glycosylation of CgB has apparently not been investigated.

12.2.2. *Phosphorylation.* Settleman *et al.*[296] have established that adrenal CgA is a phosphoprotein containing five phosphoserine residues per molecule. In agreement, labeling of chromaffin granules with [^{32}P]phosphate leads to incorporation of this isotope into CgA[51,274] mainly into serine and to a small extent into threonine.[274] An analogous finding has been reported for CgB.[51,274] For other tissues it is only established that CgA of parathyroid gland is also phosphorylated.[24]

12.2.3. *Sulfation.* Early studies have shown that [^{35}S]sulfate labeling of the adrenal medulla leads to the incorporation of this isotope into proteoglycans (see Ref. 182 for earlier references) and into chromogranins.[18] Detailed recent studies have elucidated these phenomena in more detail. Lee and Huttner[201] demonstrated in PC12 cells the sulfation of secretory proteins which were later identified as CgB.[73,274] Most of the sulfate was found to be linked to tyrosine, but some was also present in O-linked oligosaccharides.[274] Since such sulfation mechanisms are widespread post-translational modifications[152] there is no reason to assume that CgB in other tissues is not tyrosine sulfated. Adrenal CgA also incorporates [^{35}S]sulfate during biosynthesis.[18,73,274] Most if not all of this sulfate appears to be bound to carbohydrates.[274] For CgA of porcine parathyroid gland it was originally reported that it contained sulfate linked to tyrosine;[189] however, in a more recent study both bovine and porcine parathyroid CgA were found to be sulfated on oligosaccharides.[109]

Chromaffin granules also contain proteoglycans in which the glycosaminoglycan component consists of dermatan sulfate, chondroitin 4- and 6-sulfate and heparan sulfate.[11,26,105,182] When bovine chromaffin cells are labeled with [^{35}S]sulfate and analysed by two-dimensional electrophoresis a spot moving slower and to a more acidic pH than CgA (see Fig. 1) becomes labeled.[73,274] This spot was identified as a proteoglycan form of CgA by its sensibility to chondroitinase treatment[73] and by its immunostaining for CgA.[183,274] This proteoglycan form of CgA represents only a minor component of the total proteoglycans in bovine chromaffin granules and only about 1–2% of CgA is present in this form.[112] Proteoglycan CgA contains probably only a single chondroitin sulfate or dermatan sulfate chain of approximately 15,000 mol. wt.[112] In rat PC12 chromaffin cells CgA represents a significant portion of the larger proteoglycans. There is no evidence that bovine CgB is present in a proteoglycan form.[112] In the parathyroid gland a proteoglycan form of CgA was only found in bovine, but not in porcine tissue.[109] On the other hand, in rat tissues the relative amounts of proteoglycan CgA varied from tissue to tissue, being low or absent in anterior pituitary, with

intermediate amounts in the adrenal and a higher concentration in brain.[350] During ontogeny of the rat adrenal medulla the relative amount of the proteoglycan form of CgA starts from a high level and then declines.[287]

12.2.4. *Pyroglutamylation.* This post-translational modification of peptides leads to the formation of pyroglutamyl residues from N-terminal glutamic acid[36] and the responsible enzyme, glutamic cyclase, is also present in adrenal medulla. A first analysis of bovine chromaffin granules for the presence of pyroglutamyl peptides revealed a 14-amino acid peptide derived from CgB.[90] It will be interesting to see whether this post-translational modification is an essential prerequisite for a possible function of this peptide.

12.2.5. *Carboxymethylation.* CgA is a carboxymethylated protein.[243] Since carboxymethylase activity in adrenal medulla is cytosolic,[63] the modification of the CgA molecules as suggested[102,243] would have to occur co-translationally which, however, is difficult to imagine.

12.2.6. *α-Amidation.* Pancreastatin, a peptide derived from CgA has been shown to possess a C-terminal glycine amide structure in pig,[331] bovine[241] and human[100,284,293] tissues.

12.2.7. *Formation of disulfide bonds.* Based on reduction studies it has been proposed[21] that the two N-terminal cysteines are present *in vivo* in a disulfide-bonded loop structure. This probably requires the action of a disulfide isomerase.

13. PROTEOLYTIC PROCESSING

In 1974 we observed[149] that in bovine chromaffin granules there were several smaller proteins immunologically cross-reacting with CgA.[183,251,296] Based on these findings we suggested[362] "that these smaller proteins are formed in chromaffin granules *in vivo* due to the presence of proteases. One is of course reminded of the fact that in insulin-containing secretory granules, proteases are present which are essential for forming insulin from proinsulin". By now this concept is well established not only for CgA but also for additional components of chromaffin granules, e.g. the enkephalins[337] and CgB.[79] Several defined small peptides proteolytically derived from CgA and CgB have been shown to occur in neuroendocrine secretory granules: pancreastatin[331] and betagranin[156] from CgA; and GAWK and CCB,[22a] BAM 1745[90] and OA-8[207] from CgB.

13.1. *Rate and degree of proteolysis*

Apparently, the processing of the chromogranins in different neuroendocrine vesicles does not occur at the same rate. Thus in adrenal medulla a relatively slow processing is obvious. In pulse-label experiments newly synthesized CgA appeared unprocessed after two[74] and even after 18 h,[14,308] whereas for CgB a limited proteolysis occurred earlier[74] with some large processed peptides being present after 2 h. On the other hand, in endocrine pancreas (insulinoma cells) a significant breakdown of CgA was already seen within much shorter times. Thus the half-time of conversion of CgA to betagranin (representing the N-terminal end of CgA) was only about 30 min.[155]

To what degree the chromogranins are processed in various tissues depends both on the rate of processing and on the average life-span of the vesicles before they release their content, since even a low rate of processing may lead to considerable breakdown if vesicles do not secrete their content. In adrenal medulla the half-life of the secretory content (catecholamines) of chromaffin granules has been estimated to be close to seven days.[336] Despite this long half-life, CgA in bovine adrenal medulla is only processed on average (in total adrenal medulla) to about 50% whereas for CgB a value of 85% has been reported.[14,82] Obviously the rate of processing might be very low (in agreement with the pulse-labeling data discussed above), but one cannot exclude that processing does not occur throughout the whole life-span of chromaffin granules. For bovine neuroendocrine tissues it is only in the anterior pituitary that the degree of CgA processing is apparently less than in adrenal medulla,[14,82] whereas in the intestinal mucosa (enterochromaffin cells) and especially in the endocrine pancreas, proteolytical degradation of CgA is extensive.[345] This conclusion was established by a radioimmunoassay with antisera against synthetic peptides and measurements before and after trypsin digestion. The ratio of free peptide to total (after trypsin) peptide was 0.11 in adrenal medulla, 0.52–0.76 for intestinal mucosa and 1.09 for pancreas. Similar results were obtained for other species: in the porcine adrenal, CgA is little processed, whereas in the pancreas considerable breakdown has occurred as measured by pancreastatin radioimmunoassays and gel permeation chromatography,[284] while in hypothalamus and especially in anterior pituitary, processing is limited.[178]

For rat tissues the degree of breakdown increases from the adrenal medulla to the pituitary and finally to the endocrine pancreas.[159] In these studies, antibodies against betagranin and the intact CgA molecule were used to probe the degree of proteolysis. In another study[53] only an antibody against pancreastatin was employed for this purpose. In this case most immunoreactive material eluted close to the void volume when extracts of adrenal, pituitary, brain but also pancreas were subjected to gel permeation chromatography. Apparently, depending on what antisera are used the results may differ; however, the general conclusion seems valid that the degree of proteolytic processing of CgA in adrenal medulla and anterior pituitary is low; in other tissues especially the endocrine pancreas it is high. For CgB a study using antibodies against the GAWK peptides indicated a more extensive proteolysis in pituitary when compared with adrenals.[165]

Proteolytic processing of CgA may also occur after their release from the neuroendocrine cells.[346] In primary cultures of bovine chromaffin cells such a processing was observed in two studies;[308,346] but not in another one.[14] In any case it is not known whether such processing can also occur *in vivo*. The first evidence that the rate of proteolytic processing of CgA can be upregulated was recently obtained for chromaffin cells treated with reserpine.[346a]

13.2. *Pathways of proteolysis*

One of the best-defined cleavage points in the processing of propeptides is at dibasic sites and in fact as shown in Figs 2 and 3, several of such cleavage sites are found in the CgA and CgB sequences and several of them are phylogenetically conserved at least in mammalian species. A detailed study using microsequencing and region-specific antibodies established actual processing at such sites for bovine CgA at residue 79 (preceded by KK) and 116 (by KR) and for human CgA at the second dibasic (KR) site counting from the N-terminus[14] (for the latter cleavage see also Ref. 328). For CgA of the latter species (isolated from carcinoid tumors) there was also processing at position 210 (preceded by KR) and at position 340 (by KR).[283] For the endocrine pancreas cleavage at dibasic site 131 (KR) in rat CgA has been established leading to the release of the N-terminal peptide called betagranin.[155] An analogous peptide (22,000 mol. wt) seems to be present in chromaffin granules.[136]

For the well-characterized peptide, pancreastatin, which has been originally isolated from porcine pancreas[331] but is also found in human[293,328] and bovine tissue,[241] processing at the C-terminal end requires cleavage (see Fig. 2) at a Glys-Lys site and on the N-terminal site cleavage at a monobasic amino acid at least for the porcine[331] and the bovine peptide.[241] A peptide found in bovine adrenal medulla[30] which is now known to be derived from CgA, also requires processing at monobasic site 16 (lysine) for its formation. In contrast, it is noteworthy that human CgA-derived peptides require for their formation cleavage at Asp-Pro and Trp.[283] For CgB, processing at dibasic sites is well established, since several peptides detected in various tissues depend on such a cleavage, i.e. human GAWK (420–493) and CCB (597–657),[22] bovine BAM-1745 (547–560),[90]; OA 21,[207] ovine OA 8[207] and OA 60.[226]

Proteolytic processing of large proteins like the chromogranins could occur in successive steps either from the C- or N-terminal end or also independently from both sites. For CgA this latter process is apparently operating in bovine chromaffin granules.[14,373] In fact, a preferential cleavage site is the dibasic site at the N-terminal end which leads to the formation of a smaller peptide (1–77) representing a significant spot in 2D-electrophoresis (see Fig. 1) and a larger size residue of the CgA molecule.[14,373] It seems likely, although it has not been definitely demonstrated, that in rat pancreas the formation of betagranin can also occur by first removing the N-terminal end of CgA from the intact molecule by cleavage at a dibasic site which represents in this species the first cleavage site from the N-terminal end. For CgB of several species, processing of the intact molecule starting either from the N- or C-terminal site has also been established for several neuroendocrine tissues.[106]

We have already pointed out above that in the various endocrine tissues the degree of proteolytic processing of chromogranins varies. This may lead to an accumulation of peptides of different sizes within the various tissues as, for example, shown in a detailed study on bovine tissues[14] or in endocrine tumor cells lines.[59] These authors concluded that the "pattern of processing was qualitatively similar across tissues". However, we cannot yet exclude that in one of these tissues, CgA is specifically processed to a particular peptide (see Ref. 53a). For CgB significant differences between proteolysis in adrenal medulla vs pituitary have been found[106,165] with the appearance of a unique 40,000 mol. wt C-terminal fragment in the anterior pituitary.[106]

13.3. *Proteases involved in processing*

The first study on secretory granule enzymes responsible for the processing of CgA were performed by Hutton *et al.*[156] They found in insulin granules of the endocrine pancreas a calcium-activated enzyme which together with a carboxypeptidase H produced betagranin from CgA. In bovine chromaffin granules we described an analogous enzyme activity which produced a small peptide (22,000 mol. wt) from CgA probably also presenting betagranin.[192] In the mean time two kex2-related proteases have been cloned for which an involvement in prohormone processing has been established.[23,290,314,332] The mRNA of both PC1 (high concentration) and PC2 (low concentration) have been found in rat adrenal medulla[290] and both PC1 and PC2 sequences have been shown to be present in glycoprotein H of bovine chromaffin granule membrane.[44] By using antisera against synthetic peptides derived from the primary amino acid sequence we could immunostain glycoprotein H for PC2, but the PC1-reactive spot corresponded to a larger (85,000 mol. wt) glycoprotein.[183a] Thus it seems quite likely that the enzymes processing CgA in insulin and chromaffin granules as discussed above are in fact PC1 and PC2. These enzymes are capable of processing prohormones at pairs of basic residues and we have already pointed out that such cleavage occurs for both CgA and CgB. The involvement of those enzymes in CgA processing does not exclude the action of other enzymes which have been described previously in chromaffin granules (see Ref. 364 for references). Thus CgA can apparently also be processed by a trypsin-like enzyme,[192] first described by Lindberg *et al.*[299] This enzyme was reported to have

a molecular weight of 31,000.[299] Furthermore, a thiol protease of 33,000 mol. wt splitting at dibasic sites has also been found in chromaffin granules.[188] A possible action of this enzyme on chromogranins has not been established. As already pointed out, CgA processing does not only occur at dibasic sites, thus proteases acting at monobasic sites or in other positions might also be involved. An endopeptidase with such a specificity (monobasic sites) has recently been purified from pituitary.[62] When all these proteases are defined in molecular terms it will be interesting to see whether a concerted action of them is necessary to produce all the peptides formed from chromogranins and whether different activities of these enzymes in various tissues will explain the varying degrees of processing and possibly various ways of peptide formation.

Finally, the unusual possibility has to be considered, that the enzyme acetylcholinesterase may be involved in CgA breakdown. It has been claimed that highly purified acetylcholinesterase has proteolytic activity[311] and can also process CgA.[313] However, this concept is still controversial[312] and evidence against such proteolytic properties has also been presented.[43] Whether acetylcholinesterase is present in chromaffin granules and therefore in a position to attack CgA has also been controversial (see Ref. 364); however, at least this topic now seems settled, since a subspecies of this enzyme appears to be specifically localized in chromaffin granules.[28]

14. REGULATION OF BIOSYNTHESIS

The chromogranins are constituents of the secretory content of neuroendocrine vesicles. In principle the biosynthesis of the chromogranins could be regulated *en bloc* together with other secretory components like the neuropeptides or in differential patterns allowing modifications of the secretory cocktail. We here present evidence that the latter mechanism is operating.

14.1. *Regulation of synthesis by hormones*

14.1.1. *Chromogranin A—a peptide regulated by steroids.* In 1987 we provided the first evidence that the biosynthesis of CgA in adrenal medulla was specifically regulated by corticosteroids.[303] After hypophysectomy in rats the CgA but not the CgB content of adrenal medulla declined markedly, apparently due to the atrophy of the adrenal cortex leading to a lack of corticosteroids. This concept was confirmed when it was demonstrated that after hypophysectomy the lowered mRNA levels of CgA (but not that of CgB) in adrenal medulla could be restored to control levels by cortisone treatment.[83] The specificity of the action of cortisone could be seen by its lack of effect on the CgB message. Similarly, in PC12 cells and in bovine chromaffin cells in culture, cortisone treatment specifically increases the levels of the CgA message.[162,266] For the anterior pituitary an analogous influence of cortisone could also be demonstrated;[88,115] on the other hand, in brain dexamethasone treatment or adrenalectomy did not change CgA levels or that of its mRNA.[350] In the corticotrophic cell line, AtT-20, cortisone treatment increases the CgA mRNA levels to 250% whereas those for POMC peptides declines (to 40%)[343] and an analogous regulation was observed for the CgA message in pituitary tumor cell cultures[319a] and in an insulinoma cell line.[162] It has not been established by what mechanism cortisone induces the biosynthesis of CgA. The induction by cortisone of the CgA message in the pituitary occurred relatively late, i.e. with a time-lag of 24 h after the injection, interpreted as the action of cortisone being indirect via protein synthesis.[88] However, a direct effect of the cortisone receptor on the gene cannot be excluded, although gene analysis did not reveal a perfect match to a glucocorticoid responsive element in the promoter region.[162,374]

Another example of steroids regulating CgA synthesis has been found in the parathyroid cell cultures. 1,25-Dihydroxycalciferol-treatment induced a several-fold increase of the CgA mRNA[232,277a] via an effect on CgA gene transcription, whereas CgA mRNA stability was not affected. Finally oestrogen steroids are also involved in the regulation of CgA synthesis in the pituitary gland. In female rat pituitaries, CgA drops with maturation of the animals.[6] After ovarectomy CgA and its mRNA rises and is suppressed again by oestrogen treatment.[4,5] In male rats oestrogen treatment markedly lowered the pituitary mRNA for CgA.[87] Furthermore, male rats had higher CgA mRNA levels in the pituitary than female rats which are of course under the constant influence of oestrogens, whereas CgB message was equal for both male and female. Thus the biosynthesis of CgA in the pituitary is suppressed by endogenous or exogenous oestrogen. These changes take apparent place in the gonadotrophic cells as indicated by immunohistochemistry.[87] However, for these oestrogen effects it has not yet been demonstrated that they are due to a direct action on these cells. They may also be indirect via hypothalamic regulation. In adrenal chromaffin cells, oestrogen is without influence on CgA mRNA levels.[4,87]

14.1.2. *Regulation of chromogranin B.* When GH_4C_1 cells (a rat pituitary tumor cell line) are treated with humoral factors (E_2, insulin and epidermal growth factor, EGF) the number of secretory granules increases several-fold. This leads to increased levels of prolactin, but also of CgB and secretogranin II.[281] In GH_3B_6 pituitary cell lines, CgB-mRNA is reduced by dexamethasone, whereas oestrogen increased it.[198]

14.2. *Regulation of synthesis by stimulation of cells*

When chromaffin cells of the rat adrenal medulla are stimulated reflexly *in vivo* by insulin-induced hypoglycemia, the levels of enkephalins,[177,303] but also

of several other neuropeptides, i.e. calcitonin gene-related peptide, neuropeptide Y, neurotensin and substance P,[78,194] increase several-fold. On the other hand, those of CgA and CgB remain unchanged[303] clearly demonstrating an apparent dissociation of the biosynthesis of chromogranins and neuropeptides. This conclusion was confirmed at the mRNA level.[83] Whereas in these experiments with insulin-induced hypoglycemia the biosynthesis of chromogranins was apparently unchanged, treatment of rats with reserpine induced such effects.[142] A high dose of reserpine leads to a prolonged stimulation of the adrenal medulla. This treatment induced a significant increase of the levels of CgB and its mRNA (600%), whereas for CgA mRNA a smaller increase (230%) was observed. No definite explanation can be offered as to why indirect stimulation of the adrenal by insulin or by reserpine affects chromogranin biosynthesis differently; however, it has been suggested[142] that the time of stimulation may be important with only a prolonged stimulation as provided by reserpine leading to such biosynthesis changes. For the chromogranin biosynthesis in brain the first data are already available. Thus in rats in which seizures have been induced by the injection of kainic acid, changes in the mRNA levels for CgA[350] and for CgB[219a] have been observed. Apparently, in this model intensive stimulation of certain neurons in brain does not only change the levels of neuropeptide Y but also those of the chromogranins.

For bovine chromaffin cells in culture, results on the biosynthesis of CgA are controversial. Two groups of authors measured the incorporation of radioactively labeled amino acids into CgA. Whereas one group[307] reported an increased incorporation after depolarization (nicotine, K^+), another one found no change[360] for CgA, but the expected change for the enkephalins. Possibly, *in vitro* factors like the length or intensity of stimulation (see above) may lead to different results. In any case, in pancreatic endocrine islets, amino acid incorporation into both insulin and CgA is increased several-fold in the presence of glucose which was interpreted as an effect at the translational level;[119] however, in all these studies, *in vitro* mRNA levels of CgA were not yet determined.

14.3. *Regulation by second messengers*

In cultured chromaffin cells, amino acid incorporation into CgA is increased in the presence of phorbolesters (short incubation), but not by forskolin.[307] At the mRNA level, forskolin has been shown to induce an insignificant rise after 36–48 h,[80] but after more than two days of treatment a doubling of the mRNA levels was apparent.[162] This result is consistent with the finding of a cAMP-responsive element in the CgA-gene.[162,374] Varying effects of phorbolester on mRNA levels have been reported in different cells. In a calcitonin-producing cell line, phorbolester but not forskolin treatment for 48 h led to a doubling of the CgA mRNA level.[238,239] On the other hand, in bovine chromaffin cells, treatment for two days with the same agent significantly decreased the CgA message[80,162] and an analogous finding has been obtained for human neuroblastoma cells.[351] Since phorbolesters, depending on the time of incubation, can activate or down-regulate protein kinase C it is not clear by what mechanisms the CgA gene is regulated. Gene analysis has not revealed an established AP-I site for CgA.[162,374]

In conclusion, the chromogranin genes can apparently be activated in a specific way. Thus the biosynthesis of the secretory content of neuroendocrine vesicles is not regulated *en bloc* (see also Ref. 103a). Much further work is required before we will understand the detailed mechanisms at the cellular and genetic level which regulate the biosynthesis of this secretory cocktail. Certainly, such sophisticated mechanisms argue for specific functions of these regulated peptides, but are we yet in a position to understand them?

15. FUNCTION

15.1. *Involvement in catecholamine storage*

When CgA was still considered to be a specific component of chromaffin granules which also contain a high concentration of catecholamines, their possible involvement in the storage of these molecules was a much discussed topic. As outlined above, the chromogranins are now known to be present in many neuroendocrine granules which store only small amounts if any of biogenic amines. This finding indicates that chromogranins cannot be proteins with an exclusive function for amine storage, but it does not exclude such a function in some vesicles. As discussed in detail previously[371] (see also Ref. 132) the chromogranins cannot be involved in a stoichiometric binding of catecholamines or ATP and there is also, as shown by nuclear magnetic resonance studies, no stable storage complex made up of chromogranins, nucleotides and catecholamines. However, a certain interaction between all these molecules apparently takes place (see Ref. 371) and could help to reduce the high osmolar pressure inside chromaffin granules,[137] although catecholamine/ATP complexes fulfil this purpose.[187] Direct evidence for an interaction of nucleotides with purified CgA has recently been obtained with a fluorescence probe.[376] It was concluded that adenosine bases play an important role in this interaction and if this interaction also occurs *in vivo* it might provide a basis for some involvement in catecholamine storage. From solutions containing CgA, catecholamines and ATP in stoichiometric concentrations comparable to the granule content, an opaque precipitate can be sedimented which also indicates some interaction.[94] Finally, calcium, which interacts with the chromogranins (see below), increases the binding of catecholamines to the chromogranins.[339a,354]

15.2. *Enzyme inhibition*

CgA was shown to inhibit the proteolytic cleavage of prohormones by trypsin or serine proteases.[291] It would be interesting to see whether such inhibition can also be observed with the newly defined endoproteases PC1 and PC2. However, to prove whether chromogranins have a role as regulators of post-translational processing of prohormones[291] would require more direct experiments, e.g. by introducing the chromogranin genes into secretory cells originally lacking it and subsequently defining their effects on proteolytic cleavage rates.

15.3. *Binding of calcium and possible consequences for granule formation*

In 1977 we reported[361] the following unpublished observation and some speculations based on it: "In this context it is interesting to note that *in vitro* calcium can precipitate the acidic proteins of the granule content, i.e. the chromogranins (Winkler, unpublished observations), which is reminiscent of the fact that calcium precipitates casein, an acidic protein of the milk secretion granules.[75] Furthermore, the condensation process, at least in exocrine pancreas, is apparently not energy dependent.[170] This would be consistent with the involvement of a calcium uptake which does not require energy as found for chromaffin granules. It therefore seems justified to speculate that during "the condensation process" calcium enters newly formed chromaffin granules before the other small molecules, i.e. ATP and catecholamines are accumulated. However, there is no direct evidence to support such a sequence of events." In the meantime, detailed studies on the relationship of calcium and chromogranins have appeared. Gratzl *et al.*[268,269] have established that CgA can bind considerable amounts of calcium (152 nmol/mg protein with a dissociation constant of 54 μM). This property explains why in intact chromaffin granules despite the high concentration of total calcium (40 mM) the free concentration is low being about 20 μM.[35] Not surprisingly, parathyroid secretory protein I which is in fact CgA was also found to bind calcium.[108,202] In the presence of this cation, CgA binding to membranes was also enhanced.[108,202] CgB is apparently also able to bind calcium.[111] The binding of calcium to CgA induces pH-dependent conformational changes.[375,377] In these studies, CgA was found to bind up to 1150 nmol of Ca^{2+}/mg protein (dissociation constant 2.7–4 mM).

What are the functional implications of this calcium-binding by chromogranins? Several groups suggested[108,110,367,371] that the aggregation of CgA by calcium may be involved in the condensation process in the early phase of granule formation in agreement with the speculation quoted above. However, more complicated schemes for the function of chromogranins (and also secretogranin II) have been proposed: thus it has been suggested that these acidic proteins "function as helper proteins in the packaging of various hormones".[274] Although it has been demonstrated[275] that antibodies against CgB when co-expressed in secretory cells became co-packed with CgB in the regulated pathway this does not provide direct evidence for co-packing of hormones and chromogranins which do not interact specifically like antigen/antibodies. Furthermore, the fact that the concentration of chromogranins/secretogranins in secretory granules other than chromaffin granules is low makes it difficult to envisage a stoichiometric "helper process". The suggestion that the tyrosine sulfation of these proteins[274] may act as a sorting mechanism has not been supported by subsequent studies.[148] In a more detailed modification of these proposals it has been suggested that chromogranins are partly membrane bound in the trans-Golgi network and that these molecules together with calcium, aggregate with further chromogranins forming together with other secretory peptides the "granule core" for vesicles which subsequently pinch off.[153,262a] This scheme assumes that sorting occurs concomitant with condensation as proposed previously;[334] however, evidence to the contrary, i.e. sorting precedes condensation has also been obtained.[335] One can only re-emphasize that many neurosecretory granules contain only small amounts of chromogranins, thus a general hypothesis which concentrates on these minor components apparently neglects the role of the major granule constituents, i.e. the respective hormones, for the granule formation process.

15.4. *Precursors of functional peptides*

Two features of chromogranins discussed above are consistent with a function of chromogranins as precursors of active peptides: chromogranins, like prohormones or proneuropeptides, are proteolytically processed *in vivo* and their biosynthesis can be regulated by humoral factors or by stimulation of the cells storing them. Direct support for this concept was provided when pancreastatin, a 49-amino acid peptide derived from CgA, was isolated from porcine pancreas and found to have a defined function in inhibiting the stimulated secretion of insulin from endocrine pancreas.[66,331] In the mean time pancreastatin peptides were isolated from other species including human[101,283,293] and bovine[241] material and pancreastatin immunoreactivity was found to be widespread in the neuroendocrine system (see Table 1), although this does not prove the presence of the free peptides in all these organs since the antisera against pancreastatin can also react with CgA. However, in addition to the pancreas the free peptide has also been found in the pituitary gland[241,284] and chromatographic analysis suggests its presence also in porcine thyroid gland and duodenum.[31]

Many subsequent studies, but not all of them (see below and Ref. 288), have confirmed the original finding, that the 49-amino acid porcine pancreastatin

and also its 16-amino acid C-terminal peptide can inhibit glucose-stimulated insulin release from perfused rat pancreas or isolated acini[66,331] and from a pancreas tumor cell line.[218] Apparently, porcine pancreastatin is not only effective in the rat pancreas,[66,113,167,258,305,331] but also in mouse,[2,209] whereas in canine pancreas an effect[271] or no effects[254] have been reported.

For porcine pancreas "no effect at all on endocrine secretion of insulin, glucagon and somatostatin" was found.[146] Inhibiting effects on rat pancreas were also exhibited by human pancreastatin[95,292] and not surprisingly also by rat pancreastatin and its 26-residue C-terminal peptide.[96,228,257] For glucagon-mediated insulin release, pancreastatin was reported either as inhibitory[258] or as stimulatory.[168] For glucagon release itself, increases caused by pancreastatin were reported in two studies[66,271] whereas another one, even with the homologous pancreastatin in rat, was unable to find any effect.[257] Effects of pancreastatin on exocrine pancreatic (e.g. amylase release) secretion have also been reported;[96,227] however, on isolated acini no effect could be seen[65,98,229] and it has been suggested that pancreastatin acts on the secretion by inhibiting vagal nerve activity.[229] On the other hand, for guinea-pig, isolated acini pancreastatin inhibited cholecystokinin-induced amylase release.[166] Several further tissues have been tested for effects of pancreastatin. In rabbit isolated parietal cells of the stomach, pancreastatin had a direct inhibitory effect on secretion through interference with multiple second messenger pathways;[206] on the other hand, in dogs, gastric acid secretion was increased.[130] Secretion is also inhibited by this peptide in the parathyroid gland.[76,77] Interestingly, antibodies against pancreastatin potentiate the secretion from these cells at different calcium concentrations, apparently by neutralizing CgA peptides released into the medium from these cells.[76] This appears to be the first evidence that CgA-related peptides when secreted have a "physiological" function.

Some actions on the central nervous system are also already indicated. Peripherally administered pancreastatin modulates memory processing in tests on mice[91] and intracranical microinfusion[120] leads to changes in blood glucose and free fatty acids.

A second peptide derived from CgA, i.e. chromostatin, has been shown to be an effective inhibitor of catecholamine secretion from chromaffin cells.[103,309] In a first study[309] it was established that CgA inhibits catecholamine secretion induced by nicotine, but not that by high K^+, only after pre-incubation with the cells or after trypsin treatment indicating an involvement of a breakdown product. In a second study CgA was processed by proteases and the peptides inhibiting catecholamine secretion were analysed. A shorter peptide present in these sequences was synthesized and found to be a potent inhibitor of catecholamine secretion induced both by carbamoylcholine or by depolarizing K^+ concentration. The peptide, i.e. chromostatin, inhibited secretagogue-induced calcium influx.[103,103b] It has not been demonstrated that chromostatin is actually formed *in vivo* or whether a larger molecule has this function on catecholamine release. Chromostatin is not flanked by basic pairs of amino acids (Fig. 1); thus the actual processing of this or larger peptides has still to be elucidated.

Finally, some actions of further peptides derived from CgA are likely. The N-terminal peptide (1–40) stimulated the secretion of CGRP and inhibited the secretion from calcitonin and of parathyroid hormone-related protein from a cell line.[60,61,65a] CgA, or a peptide formed from it during incubation, inhibits the secretion of POMC from the AtT–20 mouse cell line. Furthermore, CgA antisera increased basal secretion indicating a physiological inhibitory feedback of CgA secreted from these cells as discussed above.[343]

Two recent findings raise interesting new possibilities for the function of chromogranins. CgA, or peptides derived from it, were shown to have neuronotrophic activity for embryonic chick dorsal root ganglia.[353] With the availability of synthetic peptides it should be possible to define this observation in more detail. In endocrine pancreas, pancreastatin inhibited beta-cell DNA synthesis and lowered the content of spermidine in these cells which has been implicated in beta-cell replication.[310] An interaction with other peptides has also been indicated, since the N-terminal segment (1–46) of CgA significantly suppressed contraction of vascular smooth muscle caused by endothelin.[135]

We can conclude that there seems to be a good indication that peptides derived from CgA are functional. Thus the CgA molecule, which is proteolytically processed *in vivo* and whose biosynthesis is regulated (see above) seems to belong to the ever-growing group of pro-peptides like those giving rise to neuropeptides. In apparent contrast to many of the neuropeptides, the phylogenetic conservation of the functional peptides (pancreastatin and especially chromostatin) is rather limited (see Fig. 2). If these peptides have specific receptors, then it is difficult to see how the lack of conservation of the peptide can be correlated in phylogeny with such a specific receptor. Furthermore, we will still need more data on the actual formation of these peptides (especially chromostatin) in the various tissues *in vivo*.

The indications that these peptides have trophic functions, or influence cell replication or can interact with other peptides should stimulate more research. Finally, regulatory functions, e.g. on synapse formation during development and on the regulation of postsynaptic receptors, should be considered.

16. CONCLUSIONS

Twenty-five years of research on the chromogranins have led to many established facts on their physico-chemical and molecular properties and on their widespread distribution in the neuroendocrine

system. The function of these proteins is most likely to be as precursors for active peptides. In this context, future research to yield the most rewarding results should answer the following questions. Which functional peptides are actually formed *in vivo*? Which proteases are involved? Is the function of the peptides, at present only defined by their exogenous addition, also of physiological significance? Are functional peptides (and their yet undefined receptors) conserved during phylogeny going beyond the mammalian system? Do changes in the biosynthesis of these proteins control the functions of peptides formed from them? Is the proteolytic processing of the chromogranins regulated?

Acknowledgements—The original research of the authors quoted in this paper was supported by the Dr Legerlotz-Stiftung and by the Fonds zur Förderung der wissenschaftlichen Forschung (Austria). We are grateful to Mrs M. Viehweider for typing the manuscript and to Mr Ch. Trawöger for preparing the photographs.

REFERENCES

1. Ahn T. G., Cohn D. V., Gorr S. U., Ornstein D. L., Kashdan M. A. and Levine M. A. (1987) Primary structure of bovine pituitary secretory protein I (chromogranin A) deduced from the cDNA sequence. *Proc. natn. Acad. Sci. U.S.A.* **84,** 5043–5047.
2. Ahrén B., Lindskog S., Tatemoto K. and Efendic S. (1988) Pancreastatin inhibits insulin secretion and stimulates glucagon secretion in mice. *Diabetes* **37,** 281–285.
3. Ahrén B., Östenson C.-G., Mårtensson H. and Efendic S. (1991) Pancreatic release of pancreastatin in the pig. *Pancreas* **6,** 324–329.
4. Anouar Y., Benie T., Demonti M., Counis R. and Duval J. (1991) Estradiol negatively regulates secretogranin II and chromogranin A messenger ribonucleic acid levels in the female rat pituitary but not in the adrenal. *Endocrinology* **129,** 2393–2399.
5. Anouar Y., Demonti M., Galindo E., Benié T., Aunis D. and Duval J. (1991) Regulatory effects of estrogens on the pituitary content of secretogranin II and chromogranin A. *J. Neuroendocr.* **3,** 233–235.
6. Anouar Y. and Duval J. (1991) Differential expression of secretogranin II and chromogranin A genes in the female rat pituitary through sexual maturation and estrous cycle. *Endocrinology* **128,** 1374–1380.
7. Apps D. K., Phillips J. H. and Purves F. C. (1985) Glycoproteins of the chromaffin-granule matrix: use of lectin blotting to distinguish several separate classes. *Neuroscience* **16,** 477–487.
8. Apps D. K., Pryde J. G. and Phillips J. H. (1980) Cytochrome b-561 is identical with chromomembrin B, a major polypeptide of chromaffin granule membranes. *Neuroscience* **5,** 2279–2287.
9. Arps H., Ditel M., Lauritzen B., Elting J. J., Niendorf A. and Cohn D. V. (1987) Co-localization of parathyroid hormone and secretory protein-I in bovine parathyroid glands: a double immunocytochemical study at the electron microscopical level. *Bone Min.* **2,** 175–183.
10. Aunis D., Hesketh J. E. and Devilliers G. (1980) Immunohistochemical and immunocytochemical localization of myosin, chromogranin A and dopamine-β-hydroxylase in nerve cells in culture and in adrenal glands. *J. Neurocytol.* **9,** 255–274.
11. Banerjee S. and Margolis R. U. (1982) Peptide mapping studies of the chromogranins and of two chromaffin granule proteoglycans. *J. Neurochem.* **39,** 1700–1703.
12. Banks P., Helle K. B. and Mayor D. (1968) Evidence for the presence of a chromogranin-like protein in bovine splenic nerve granules. *Molec. Pharmac.* **5,** 210–212.
13. Banks P. and Helle K. (1965) The release of protein from the stimulated adrenal medulla. *Biochem. J.* **97,** 40c–41c.
14. Barbosa J. A., Gill B. M., Takiyyuddin M. A. and O'Connor D. T. (1991) Chromogranin A: posttranslational modifications in secretory granules. *Endocrinology* **128,** 174–190.
15. Bartlett S. F., Lagercrantz H. and Smith A. D. (1976) Gel electrophoresis of soluble and insoluble proteins of noradrenergic vesicles from ox splenic nerve: a comparison with proteins of adrenal chromaffin granules. *Neuroscience* **1,** 339–344.
16. Bassetti M., Huttner W. B., Zanini A. and Rosa P. (1990) Co-localization of secretogranins/chromogranins with thyrotropin and luteinizing hormone in secretory granules of cow anterior pituitary. *J. Histochem. Cytochem.* **38,** 1353–1363.
17. Bauer J. W. and Fischer-Colbrie R. (1991) Primary structure of bovine chromogranin B deduced from cDNA sequence. *Biochim. biophys. Acta* **1089,** 124–126.
18. Baumgartner H., Gibb J. W., Hörtnagl H., Snider S. R. and Winkler H. (1974) Labelling of chromaffin granules in adrenal medulla with ^{35}S-sulfate. *Molec. Pharmac.* **10,** 678–685.
19. Bellmann R., Widmann R., Olenik C., Meyer D. K., Maas D., Marksteiner J. and Sperk G. (1991) Enhanced rate of expression and biosynthesis of neuropeptide Y after kainic acid-induced seizures. *J. Neurochem.* **56,** 525–530.
20. Benedum U. M., Baeuerle P. A., Konecki D. S., Frank R., Powell J., Mallet J. and Huttner W. B. (1986) The primary structure of bovine chromogranin A: a representative of a class of acidic secretory proteins common to a variety of peptidergic cells. *Eur. molec. Biol. Org. J.* **5,** 1495–1502.
21. Benedum U. M., Lamouroux A., Konecki D. S., Rosa P., Hille A., Baeuerle P. A., Frank R., Lottspeich F., Mallet J. and Huttner W. B. (1987) The primary structure of human secretogranin I (chromogranin B): comparison with chromogranin A reveals homologous terminal domains and a large intervening variable region. *Eur. molec. Biol. Org. J.* **6,** 1203–1211.
22. Benjannet S., Leduc R., Adrouche N., Falgueyret J. P., Marcinkiewicz M., Seidah N. G., Mbikay M., Lazure C. and Chretien M. (1987) Chromogranin B (secretogranin I), a putative precursor of two novel pituitary peptides through processing at paired basic residues. *Fedn Eur. biochem. Socs Lett.* **224,** 142–148.

22a. Benjannet S., Leduc R., Lazure C., Seidah N. G., Marcinkiewicz M. and Chrétien M. (1985) GAWK, a novel human pituitary polypeptide: isolation, immunocytochemical localization and complete amino acid sequence. *Biochem. biophys. Res. Commun.* **126,** 602–609.

23. Benjannet S., Rondeau N., Day R., Chrétien M. and Seidah N. G. (1991) PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. *Proc. natn. Acad. Sci. U.S.A.* **88,** 3564–3568.
24. Bhargava G., Russel J. and Sherwood L. M. (1983) Phosphorylation of parathyroid secretory protein I. *Proc. natn. Acad. Sci. U.S.A.* **80,** 878–881.
25. Bishop A. E., Sekiya K., Salahuddin M. J., Carlei F., Rindi G., Fahey M., Steel J. H., Hedges M., Domoto T., Fischer-Colbrie R., Winkler H., Krausz T., Ghatei M. A., Bloom S. R. and Polak J. M. (1989) The distribution of GAWK-like immunoreactivity in neuroendocrine cells of the human gut, pancreas, adrenal and pituitary glands and its co-localisation with chromogranin B. *Histochemistry* **90,** 475–483.
26. Blaschke E., Bergqvist U. and Uvnäs B. (1976) Identification of the mucopolysaccharides in catecholamine-containing subcellular particle fractions from various rat, cat and ox tissues. *Acta physiol. scand.* **97,** 110–120.
27. Blaschko H., Comline R. S., Schneider F. H., Silver M. and Smith A. D. (1967) Secretion of a chromaffin granule protein, chromogranin, from the adrenal gland after splanchnic stimulation. *Nature* **215,** 58–59.
28. Bon S., Bader M.-F., Aunis D., Massoulie J. and Henry J.-P. (1990) Subcellular distribution of acetylcholinesterase forms in chromaffin cells. *Eur. J. Biochem.* **190,** 221–232.
29. Bööj S., Goldstein M., Fischer-Colbrie R. and Dahlström A. (1989) Calcitonin gene-related peptide and chromogranin A: presence and intra-axonal transport in lumbar motor neurons in the rat, a comparison with synaptic vesicle antigens in immunohistochemical studies. *Neuroscience* **30,** 479–501.
30. Brandau D. T., Ray P., Stern A. S. and Lewis R. V. (1985) New bovine adrenal medullary peptide and its precursor. *Int. J. Pept. Prot. Res.* **25,** 238–241.
31. Bretherton-Watt D., Ghatei M. A., Bishop A. E., Facer P., Fahey M., Hedges M., Williams G., Valentino K. L., Tatemoto K., Roth K., Polak J. M. and Bloom S. R. (1988) Pancreastatin distribution and plasma levels in the pig. *Peptides* **9,** 1005–1014.
32. Brion J.-P., Couck A. M., Bruce M., Anderton B. and Flament-Durand J. (1991) Synaptophysin and chromogranin A immunoreactivities in senile plaques of Alzheimer's disease. *Brain Res.* **539,** 143–150.
33. Buffa R., Gini A., Pelagi M., Siccardi A. G., Bisiani C., Zanini A. and Solcia E. (1989) Immunoreactivity of hormonally-characterized human endocrine cells against 3 novel anti-human chromogranin B (B11 and B13) and chromogranin A (A11) monoclonal antibodies. *Arch. histol. Cytol.* **52,** 99–105.
34. Buffa R., Marè P., Gini A. and Salvadore M. (1988) Chromogranins A and B and secretogranin II in hormonally identified endocrine cells of the gut and the pancreas. *Basic appl. Histochem.* **32,** 471–484.
35. Bulenda D. and Gratzl M. (1985) Matrix free Ca^{2+} in isolated chromaffin vesicles. *Biochemistry* **24,** 7760–7765.
36. Busby J. W. H., Quackenbush G. E., Humm J., Youngblood W. W. and Kizer J. S. (1987) An enzyme(s) that converts glutaminyl-peptides into pyroglutamyl-peptides: presence in pituitary, brain, adrenal medulla, and lymphocytes. *J. biol. Chem.* **262,** 8532–8536.
37. Bussolatti G., Gugliotta P., Sapino M., Eusebi V. and Lloyd R. V. (1985) Chromogranin-reactive endocrine cells in argyrophilic carcinomas ('carcinoids') and normal tissue of the breast. *Am. J. Path.* **120,** 186–192.
38. Carmichael S. W., Stoddard S. L., O'Connor D. T., Yaksh T. L. and Tyce G. M. (1990) The secretion of catecholamines, chromogranin A and neuropeptide Y from the adrenal medulla of the cat via the adrenolumbar vein and thoracic duct: different anatomic routes based on size. *Neuroscience* **34,** 433–440.
39. Cetin Y. and Grube D. (1990) Immunoreactivities for chromogranin A and chromogranin B, and Secretogranin II in the guinea-pig endocrine pancreas. *Histochemistry* **94,** 479–484.
40. Cetin Y. and Grube D. (1991) Immunoreactivities for chromogranin A and B, and secretogranin II in the guinea pig entero-endocrine system: cellular distributions and intercellular heterogeneities. *Cell Tiss. Res.* **264,** 231–241.
41. Cetin Y. and Grube D. (1991) Topology of chromogranins in secretory granules of endocrine cells. *Histochemistry* **96,** 301–310.
42. Cetin Y., Müller-Köppel L., Aunis D., Bader M. F. and Grube D. (1989) Chromogranin A (CgA) in the gastro-entero-pancreatic (GEP) endocrine system. II. CgA in mammalian entero-endocrine cells. *Histochemistry* **92,** 265–275.
43. Checler F. and Vincent J.-P. (1989) Peptidasic activities associated with acetylcholinesterase are due to contaminating enzymes. *J. Neurochem.* **53,** 924–928.
44. Christie D. L., Batchelor D. C. and Plamer D. J. (1991) Identification of kex2-related proteases in chromaffin granules by partial amino acid sequence analysis. *J. biol. Chem.* **266,** 15,679–15,683.
45. Ciaranello R. D. (1978) Regulation of phenylethanolamine *N*-methyltransferase synthesis and degradation. *Molec. Pharmac.* **14,** 478–489.
46. Cohn D. V., Elting J. J., Frick M. and Elde R. (1984) Selective localization of the parathyroid secretory protein-I/adrenal medulla chromogranin A protein family in a wide variety of endocrine cells of the rat. *Endocrinology* **114,** 1963–1974.
47. Cohn D. V. and MacGregor R. R. (1981) The biosynthesis, intracellular processing, and secretion of parathormone. *Endocr. Rev.* **2,** 1–26.
48. Cohn D. V., Morrissey J. J., Shofstall R. E. and Chu L. L. H. (1982) Cosecretion of secretory protein-I and parathormone by dispersed bovine parathyroid cells. *Endocrinology* **110,** 625–630.
49. Cohn D. V., Morrissey J. J., Hamilton J. W., Shofstall R. E., Smardo F. and Chu L. L. H. (1981) Isolation and partial characterization of secretory protein I from bovine parathyroid glands. *Biochemistry* **20,** 4135–4140.
50. Cohn D. V., Zangerle R., Fischer-Colbrie R., Chu L. L. H., Elting J. J., Hamilton J. W. and Winkler H. (1982) Similarity of secretory protein I from parathyroid gland to chromogranin A from adrenal medulla. *Proc. natn. Acad. Sci. U.S.A.* **79,** 6056–6059.
51. Coté A., Doucet J.-P. and Trifaró J.-M. (1986) Phosphorylation and dephosphorylation of chromaffin cell proteins in response to stimulation. *Neuroscience* **19,** 629–645.
52. Cryer P. E., Wortsman J., Shah S. D., Nowak R. M. and Deftos L. J. (1991) Plasma chromogranin A as a marker of sympathochromaffin activity in humans. *Am. J. Physiol.* **260,** E243–E246.
53. Curry W. J., Johnston C. F., Shaw C. and Buchanan K. D. (1990) Distribution and partial characterisation of immunoreactivity to the putative C-terminus of rat pancreastatin. *Regul. Pept.* **30,** 207–219.

53a. Curry W. J., Johnston C. F., Hutton J. C., Arden S. D., Rutherford N. G., Shaw C. and Buchanan K. D. (1991) The tissue distribution of rat chromogranin A-derived peptides: evidence for differential tissue processing from sequence specific antisera. *Histochemistry* **96,** 531–538.
54. Daniels A. J., Williams R. J. P. and Wright P. E. (1978) The character of the stored molecules in chromaffin granules of the adrenal medulla: a nuclear magnetic resonance study. *Neuroscience* **3,** 573–585.
55. Davis W. L., Schmid K. O., Huettner J. W., Farmer G. R., Jacoby B. H. and Goodman D. B. P. (1990) Immunolocalization of secretory protein-I or chromogranin A in amphibian urinary bladder granular cell granules. *Cell Biol. int. Rep.* **14,** 601–612.
56. De Potter W. P., Smith A. D. and De Schaepdryver A. F. (1970) Subcellular fractionation of splenic nerve: ATP, chromogranin A and dopamine β-hydroxylase in noradrenergic vesicles. *Tiss. Cell* **2,** 529–546.
57. Deftos L. J. (1991) Chromogranin A: its role in endocrine function and as an endocrine and neuroendocrine tumor marker. *Endocr. Rev.* **12,** 181–187.
58. Deftos L. J., Bjornsson B. T., Burton D. W., O'Connor D. T. and Copp D. H. (1987) Chromogranin A is present in and released by fish endocrine tissue. *Life Sci.* **40,** 2133–2136.
59. Deftos L. J., Gazdar A. F., Hogue-Angeletti R., Mullen P. S. and Burton D. W. (1990) Distinct patterns of chromogranin-A-related species can be demonstrated in endocrine cells. *Bone Min.* **9,** 169–178.
60. Deftos L. J., Hogue-Angeletti R., Chalberg C. and Tu S. (1990) A chromogranin A-derived peptide differentially regulates the secretion of calcitonin gene products. *J. Bone Min. Res.* **5,** 989–991.
61. Deftos L. J., Hogue-Angeletti R., Chalberg C. and Tu S. (1989) PTHrP secretion is stimulated by CT and inhibited by CgA peptides. *Endocrinology* **125,** 563–565.
62. Devi L., Gupta P. and Fricker L. D. (1991) Subcellular localization, partial purification, and characterization of a dynorphin processing endopeptidase from bovine pituitary. *J. Neurosci.* **56,** 320–329.
63. Diliberto J. E. J., Viveros O. H. and Axelrod J. (1976) Subcellular distribution of protein carboxymethylase and its endogenous substrates in the adrenal medulla: possible role in excitation–secretion coupling. *Proc. natn. Acad. Sci. U.S.A.* **73,** 4050–4055.
64. Dillen L., De Block J., Van Lear L. and De Potter W. (1989) Enzyme-linked immunosorbent assay for chromogranin A. *Clin. Chem.* **35,** 1934–1938.
65. Doi R., Inoue K., Hosotani R., Higashide S., Takaori K., Funakoshi S., Yajima H., Rayford P. L. and Tobe T. (1991) Effects of synthetic human pancreastatin on pancreatic secretion and blood flow in rats and dogs. *Peptides* **12,** 499–502.
65a. Drees B. M., Rouse J. and Johnson J. (1991) Bovine parathyroid glands secrete a 26-kDa N-terminal fragment of chromagranin-A which inhibits parathyroid cell secretion. *Endocrinology* **129,** 3381–3387.
66. Efendic A., Tatemoto K., Mutt V., Quan C., Chang D. and Östenson C.-G. (1987) Pancreastatin and islet hormone release. *Proc. natn. Acad. Sci. U.S.A.* **84,** 7257–7260.
67. Ehrhard M., Grube D., Bader M.-F., Aunis D. and Gratzl M. (1986) Chromogranin A in the pancreatic islet: cellular and subcellular distribution. *J. Histochem. Cytochem.* **34,** 1673–1682.
68. Ehrhart M., Jörns A., Grube D. and Gratzl M. (1988) Cellular distribution and amount of chromogranin A in bovine endocrine pancreas. *J. Histochem. Cytochem.* **36,** 467–472.
69. Eiden L. E., Huttner W. B., Mallet J., O'Connor D. T., Winkler H. and Zanini A. (1987) A nomenclature proposal for the chromogranin/secretogranin proteins. *Neuroscience* **21,** 1019–1021.
70. Eiden L. E., Iacangelo A., Hsu C.-M., Hotchkiss A. J., Bader M. F. and Aunis D. (1987) Chromogranin A synthesis and secretion in chromaffin cells. *J. Neurochem.* **49,** 65–74.
71. Facer P., Bishop A. E., Cole G. A., Aitchison M., Kendall C. H., Aswegen van G., Penketh R. J., Rodek C. H., McKeever P. and Polak J. M. (1989) Developmental profile of chromogranin, hormonal peptides, and 5-hydroxytryptamine in gastrointestinal endocrine cells. *Gastroenterology* **97,** 48–57.
72. Facer P., Bishop A. E., Lloyd R. V., Wilson B. S., Hennessy R. J. and Polak J. M. (1985) Chromogranin A: a newly recognized marker for endocrine cells of the human gastrointestinal tract. *Gastroenterology* **89,** 1366–1373.
73. Falkensammer G., Fischer-Colbrie R. and Winkler H. (1985) Biogenesis of chromaffin granules: incorporation of sulfate into chromogranin B and into a proteoglycan. *J. Neurochem.* **45,** 1475–1480.
74. Falkensammer G., Fischer-Colbrie R., Richter K. and Winkler H. (1985) Cell-free and cellular synthesis of chromogranin A and B of bovine adrenal medulla. *Neuroscience* **14,** 735–746.
75. Farell J. M. (1973) Models for casein micelle formation. *J. Dairy Sci.* **56,** 1195–1205.
76. Fasciotto B. H., Gorr S. U., Bourdeau A. M. and Cohn D. V. (1990) Autocrine regulation of parathyroid secretion: inhibition of secretion by chromogranin-A (secretory protein-I) and potentiation of secretion by chromogranin-A and pancreastatin antibodies. *Endocrinology* **127,** 1329–1335.
77. Fasciotto B. H., Gorr S. U., DeFranco D. J., Levine M. A. and Cohn D. V. (1989) Pancreastatin, a presumed product of chromogranin-A (secretory protein-I) processing, inhibits secretion from porcine parathyroid cells in culture. *Endocrinology* **125,** 1617–1622.
78. Fischer-Colbrie R., Eskay R. L., Eiden L. E. and Maas D. (1992) Long-term trans-synaptic regulation of galanin, neurotensin and substance P in the rat adrenal medulla *in vivo*: correlation with second-messenger control of neuropeptide biosynthesis in isolated chromaffin cells. *J. Neurochem.* (in press).
79. Fischer-Colbrie R. and Frischenschlager I. (1985) Immunological characterization of secretory proteins of chromaffin granules: chromogranins A, chromogranins B and enkephalin-containing peptides. *J. Neurochem.* **44,** 1854–1861.
80. Fischer-Colbrie R., Gutierrez J., Hsu C. M., Iacangelo A. and Eiden L. E. (1990) Sequence analysis, tissue distribution and regulation by cell depolarization, and 2nd messengers of bovine secretogranin II (chromogranin C) messenger RNA. *J. biol. Chem.* **265,** 9208–9213.
81. Fischer-Colbrie R., Hagn C., Kilpatrick L. and Winkler H. (1986) Chromogranin C: a third component of the acidic proteins in chromaffin granules. *J. Neurochem.* **47,** 318–321.
82. Fischer-Colbrie R., Hagn C. and Schober M. (1987) Chromogranins A, B and C: widespread constituents of secretory vesicles. *Ann. N.Y. Acad. Sci.* **493,** 120–134.
83. Fischer-Colbrie R., Iacangalo A. and Eiden L. E. (1988) Neural and humoral factors separately regulate neuropeptide Y, enkephalin, and chromogranin A and B mRNA levels in rat adrenal medulla. *Proc. natn. Acad. Sci. U.S.A.* **85,** 3240–3244.

84. Fischer-Colbrie R., Lassmann H., Hagn C. and Winkler H. (1985) Immunological studies on the distribution of chromogranin A and B in endocrine and nervous tissues. *Neuroscience* **16,** 547–555.
85. Fischer-Colbrie R., Schachinger M., Zangerle R. and Winkler H. (1982) Dopamine β-hydroxylase and other glycoproteins from the soluble content and the membranes of adrenal chromaffin granules: isolation and carbohydrate analysis. *J. Neurochem.* **38,** 725–732.
86. Fischer-Colbrie R. and Schober M. (1987) Isolation and characterization of chromogranins A, B and C from bovine chromaffin granules and a rat pheochromocytoma. *J. Neurochem.* **48,** 262–270.
87. Fischer-Colbrie R., Schmid K. W., Mahata S. K., Mahata M., Laslop A. and Bauer J. W. (1992) Sex-related differences in chromogranin A, chromogranin B and secretogranin II gene expression in rat pituitary. *J. Neuroendocr.* **4,** 125–130.
88. Fischer-Colbrie R., Wohlfarter T., Schmid K. W., Grino M. and Winkler H. (1989) Dexamethasone induces an increased biosynthesis of chromogranin A in rat pituitary gland. *J. Endocr.* **121,** 487–494.
89. Fischer-Colbrie R., Zangerle R., Frischenschlager I. and Winkler H. (1984) Isolation and immunological characterization of a glycoprotein from adrenal chromaffin granules. *J. Neurochem.* **42,** 1008–1016.
90. Flanagan T., Taylor L., Poulter L., Viveros O. H. and Diliberto E. J. (1990) A novel 1745-dalton pyroglutamyl peptide derived from chromogranin B is in the bovine adrenomedullary chromaffin vesicle. *Cell. molec. Neurobiol.* **10,** 507–523.
91. Flood J. F. and Morley J. E. (1988) Effects of systemic pancreastatin on memory retention. *Peptides* **9,** 1077–1080.
92. Forss-Petter S., Danielson P., Battenberg E., Bloom F. and Sutcliffe J. G. (1989) Nucleotide sequence and cellular distribution of rat chromogranin B (secretogranin I) mRNA in the neuroendocrine system. *J. molec. Neurosci.* **1,** 63–75.
93. Fricker L. D. (1988) Carboxypeptidase E. *A. Rev. Physiol.* **50,** 309–321.
94. Fujino Y. and Fujii T. (1991) A macro-molecular complex of bovine adrenal chromogranin A, adrenaline and ATP formed *in vitro*. *Biomed. Res.* **12,** 125–130.
95. Funakoshi A., Jimi A., Yasunami Y., Tateishi K., Funakoshi S., Tamamura H. and Yajima H. (1989) Bioactivity of human pancreastatin and its localization in pancreas. *Biochem. biophys. Res. Commun.* **159,** 913–918.
96. Funakoshi A., Miyasaka K., Nakamura R., Kitani K., Funakoshi S., Tamamura H., Fujii N. and Yajima H. (1988) Bioactivity of synthetic human pancreastatin on exocrine pancreas. *Biochem. biophys. Res. Commun.* **156,** 1237–1242.
97. Funakoshi A., Miyasaka K., Kitani K., Tamamura H., Funakoshi S. and Yajima H. (1989) Bioactivity of synthetic C-terminal fragment of rat pancreastatin on endocrine pancreas. *Biochem. biophys. Res. Commun.* **158,** 844–849.
98. Funakoshi A., Miyasaka K., Nakamura R., Kitani K. and Tatemoto K. (1989) Inhibitory effect of pancreastatin on pancreatic exocrine secretion in the conscious rat. *Regul. Pept.* **25,** 157–166.
99. Funakoshi A., Tateishi K., Tsuru M. and Jimi A. (1991) Acetylcholine regulates pancreastatin secretion from the human pancreastatin-producing cell line (QGP-1N). *Clin. Endocr. Metab.* **73,** 151–155.
100. Funakoshi A., Tateishi K., Shinozaki H., Matsumoto M. and Wakasugi H. (1990) Elevated plasma levels of pancreastatin (PST) in patients with non-insulin-dependent diabetes mellitus (NIDDM). *Regul. Pept.* **30,** 159–164.
101. Funakoshi S., Tamamura H., Ohta M., Yoshizawa K., Funakoshi A., Miyasaka K., Tateishi K., Tatemoto K., Nakano I., Yajima H. and Fujii N. (1989) Isolation and characterization of a tumor-derived human pancreastatin-related protein. *Biochem. biophys. Res. Commun.* **164,** 141–148.
102. Gagnon C., Veeraragavan K. and Coulombe R. (1988) Protein-carboxyl methylation in adrenal medullary cells. *Cell. molec. Neurobiol.* **8,** 95–103.
103. Galindo E., Rill A., Bader M.-F. and Aunis D. (1991) Chromostatin, a 20-amino acid peptide derived from chromogranin A, inhibits chromaffin cell secretion. *Proc. natn. Acad. Sci. U.S.A.* **88,** 1426–1430.
103a. Galindo E., Bader M.-F. and Aunis D. (1991) Regulation of chromogranin A and chromogranin B (secretogranin I) synthesis in bovine cultured chromaffin cells. *J. Neuroendocr.* **3,** 669–677.
103b. Galindo E., Mendez M., Calvo S., Gonzalez-Garcia C., Cena V., Hubert P., Bader M.-F. and Aunis D. (1992) Chromostatin receptors control calcium channel activity in adrenal chromaffin cells. *J. biol. Chem.* **267,** 407–412.
104. Gauweiler B., Weihe E., Hartschuh W. and Yanaihara N. (1988) Presence and coexistence of chromogranin A and multiple neuropeptides in Merkel cells of mammalian oral mucosa. *Neurosci. Lett.* **89,** 121–126.
105. Geissler D., Martinek A., Margolis R. U., Margolis R. K., Skrivanek J. A., Ledeen R., König P. and Winkler H. (1977) Composition and biogenesis of complex carbohydrates of ox adrenal chromaffin granules. *Neuroscience* **2,** 685–693.
106. Gill B. M., Barbosa J. A., Dinh T. Q., Garrod S. and O'Connor D. T. (1991) Chromogranin B: isolation from pheochromocytoma, N-terminal sequence, tissue distribution and secretory vesicle processing. *Regul. Pept.* **33,** 223–235.
107. Gorr S.-V., Dean W. L., Kumarasamy R. and Cohn D. V. (1987) Calcium binding properties of parathyroid secretory protein-I. In *Calcium Regulation and Bone Metabolism: Basic and Clinical Aspects* (eds Cohn D. V., Martin T. J. and Meunier P. J.), Vol. 9, pp. 49–55. Elsevier, Amsterdam.
108. Gorr S.-U., Dean W. L., Radley T. L. and Cohn D. V. (1988) Calcium-binding and aggregation properties of parathyroid secretory protein-I (chromogranin A). *Bone Min.* **4,** 17–25.
109. Gorr S. U., Hamilton J. W. and Cohn D. V. (1991) Sulfated secreted forms of bovine and porcine parathyroid chromogranin A (secretory protein-I). *J. biol. Chem.* **266,** 5780–5784.
110. Gorr S.-U., Kumarasamy R., Dean W. L. and Cohn D. V. (1987) New suggestions for the physiological role of secretory protein-I. *Bone Min.* **2,** 251–255.
111. Gorr S. U., Shioi J. and Cohn D. V. (1989) Interaction of calcium with porcine adrenal chromogranin A (secretory protein-I) and chromogranin B (secretogranin I). *Am. J. Physiol.* **257,** E247–E254.
112. Gowda D. C., Hogue-Angeletti R., Margolis R. K. and Margolis R. U. (1990) Chromaffin granule and PC12 cell chondroitin sulfate proteoglycans and their relation to chromogranin A. *Archs Biochem. Biophys.* **281,** 219–224.
113. Greeley G. H. Jr, Thompson J. C., Ishizuka J., Cooper C. W., Levine M. A., Gorr S. U. and Cohn D. V. (1989) Inhibition of glucose-stimulated insulin release in the perfused rat pancreas by parathyroid secretory protein-I (chromogranin-A). *Endocrinology* **124,** 1235–1238.
114. Grimes M., Iacangelo A., Eiden L. E., Godfrey B. and Herbert E. (1987) Chromogranin A: the primary structure deduced from cDNA clones reveals the presence of pairs of basic amino acids. *Ann. N.Y. Acad. Sci.* **493,** 351–378.
115. Grino M., Wohlfarter T., Fischer-Colbrie R. and Eiden L. E. (1989) Chromogranin A messenger RNA expression in the rat anterior pituitary is permissively regulated by the adrenal gland. *Neuroendocrinology* **49,** 107–110.

116. Grube D., Aunis D., Bader F., Cetin Y., Jörns A. and Yoshie S. (1986) Chromogranin A (CGA) in the gastro-entero-pancreatic (GEP) endocrine system. I. CGA in the mammalian endocrine pancreas. *Histochemistry* **85,** 441–452.
117. Grube D., Bargsten G., Cetin Y. and Yoshie S. (1989) Chromogranins in mammalian GEP endocrine cells: their distribution and interrelations with co-stored amines and peptides. *Arch. Histol. Cytol.* **52,** 91–98.
118. Grube D. and Yoshie S. (1989) Immunohistochemistry of chromogranin A and B, and secretogranin II in the canine endocrine pancreas. *Arch. Histol. Cytol.* **52,** 287–298.
119. Guest P. C., Rhodes C. J. and Hutton J. C. (1989) Regulation of the biosynthesis of insulin-secretory-granule proteins. *Biochem. J.* **257,** 431–437.
120. Gunion M. W., Rosenthal M. J., Tatemoto K. and Morley J. E. (1989) Intracranial microinfusion of pancreastatin elevates blood glucose, free fatty acids, and corticosterone in rats. *Brain Res.* **485,** 251–257.
121. Hagn C., Klein R. L., Fischer-Colbrie R., Douglas B. H. II and Winkler H. (1986) An immunological characterization of five common antigens of chromaffin granules and of large dense-cored vesicles of sympathetic nerve. *Neurosci. Lett.* **67,** 295–300.
122. Hagn C., Schmid K. W., Fischer-Colbrie R. and Winkler H. (1986) Chromogranin A, B and C in human adrenal medulla and endocrine tissues. *Lab. Invest.* **55,** 405–411.
123. Hamilton J. W., Chu L. L. H., Rouse J. B., Reddig K. and MacGregor R. R. (1986) Structural characterization of adrenal chromogranin A and parathyroid secretory protein-I as homologs. *Archs Biochem. Biophys.* **244,** 16–26.
124. Hanyu S., Iwanaga T., Kano K. and Fujita T. (1987) Distribution of serotonin-immunoreactive paraneurons in the lower urinary tract of dogs. *Am. J. Anat.* **180,** 349–350.
125. Hartschuh W., Weihe E. and Egner U. (1989) Chromogranin A in the mammalian Merkel cell: cellular and subcellular distribution. *J. invest. Dermat.* **93,** 641–648.
126. Hartschuh W., Weihe E. and Egner U. (1990) Electron microscopic immunogold cytochemistry reveals chromogranin A confined to secretory granules of porcine Merkel cells. *Neurosci. Lett.* **116,** 245–249.
127. Hartschuh W., Weihe E. and Yanaihara N. (1989) Immunohistochemical analysis of chromogranin A and multiple peptides in the mammalian Merkel cell: further evidence for its paraneuronal function. *Arch. Histol. Cytol.* **52,** 423–431.
128. Hartschuh W. and Weihe E. (1989) Pancreastatin-like immunoreactivity in epidermal Merkel cells of pig and man. *Neurosci. Lett.* **98,** 258–263.
129. Hashimoto S., Fumagalli G., Zanini A. and Meldolesi J. (1987) Sorting of three secretory proteins to distinct secretory granules in acidophilic cells of cow anterior pituitary. *J. Cell Biol.* **105,** 1579–1586.
130. Hashimoto T., Kogire M., Lluis F., Gomez G., Tatemoto K., Greeley G. H. and Thompson J. C. (1990) Stimulatory effect of pancreastatin on gastric acid secretion in conscious dogs. *Gastroenterology* **99,** 61–65.
131. Hearn S. A. (1987) Electron microscopic localization of chromogranin A in osmium-fixed neuroendocrine cells with a protein A-gold technique. *J. Histochem. Cytochem.* **35,** 795–801.
132. Helle K. B. (1990) Chromogranins: universal proteins in secretory organelles from *Paramecium* to man. *Neurochem. Int.* **17,** 165–175.
133. Helle K. B. (1966) Comparative studies on the soluble protein fractions of bovine, equine, porcine and ovine adrenal chromaffin granules. *Biochem. J.* **100,** 6C–7C.
134. Helle K. B. (1966) Some chemical and physical properties of the soluble protein fraction of bovine adrenal chromaffin granules. *Molec. Pharmac.* **2,** 298–310.
135. Helle K. B., Aardal S., Reed R. K. and Elsayed S. (1990) N-terminal fragment of bovine chromogranin A inhibits the contractile response to endothelin-1 in human venous and arterial isolated segments. *J. Physiol.* **430,** 40P.
136. Helle K. B., Reed R. K., Ehrhart M., Aunis D. and Hogue Angeletti R. (1990) Chromogranin A: osmotically active fragments and their susceptibility to proteolysis during lysis of the bovine chromaffin granules. *Acta physiol. scand.* **138,** 565–574.
137. Helle K. B., Reed R. K., Pihl K. E. and Serck-Hanssen G. (1985) Osmotic properties of the chromogranins and relation to osmotic pressure in catecholamine storage granules. *Acta physiol. scand.* **123,** 21–33.
138. Helman L. J., Ahn T. G., Levine M. A., Allison A., Cohen P. S., Cooper M. J., Cohn D. V. and Israel M. A. (1988) Molecular cloning and primary structure of human chromogranin A (secretory protein I) cDNA. *J. biol. Chem.* **263,** 11,559–11,563.
139. Helman L. J., Gazdar A. F., Park J. G., Cohen P. S., Cotelingam J. D. and Israel M. A. (1988) Chromogranin A expression in normal and malignant human tissues. *J. clin. Invest.* **82,** 686–690.
140. Heym C. and Kummer W. (1989) Morphology and immunocytochemistry of two endocrine cell types in the guinea-pig esophageal epithelium. *Cell Tiss. Res.* **256,** 635–643.
141. Heym C. and Kummer W. (1988) Chromogranins and related peptides. In *Regulatory Peptides in Paraganglia.* Gustav Fischer, New York.
142. Höfle G., Weiler R., Fischer-Colbrie R., Humpel C., Laslop A., Wohlfarter T., Hogue-Angeletti R., Saria A., Fleming P. J. and Winkler H. (1991) Stimulation of rat adrenal medulla can induce differential changes in the peptide and messenger RNA levels of chromogranins, neuropeptides and other constituents of chromaffin granules. *Regul. Pept.* **32,** 321–331.
143. Hogue-Angeletti R. (1986) Chromogranins and neuroendocrine secretion. *Lab. Invest.* **55,** 387–389.
144. Hogue-Angeletti R. (1991) Expression of chromogranin A in *E. coli. 6th International Symposium on Chromaffin Cell Biology* **F-11,** 53.
145. Hogue-Angeletti R. and Hickey W. F. (1985) A neuroendocrine marker in tissues of the immune system. *Science* **230,** 89–90.
146. Holst J. J., Östenson C. G., Harling H. and Messell T. (1990) Porcine pancreastatin has no effect on endocrine secretion from the pig pancreas. *Diabetologia* **33,** 403–406.
147. Hopwood D. (1968) An immunohistochemical study of the adrenal medulla of the ox. A comparison of antibodies against whole ox chromaffin granules and ox chromogranin A. *Histochemie* **13,** 323–330.
148. Hortin G. L. and Graham J. P. (1988) Sulfation of tyrosine residues does not influence secretion of alpha2-antiplasmin or C4. *Biochem. biophys. Res. Commun.* **151,** 417–421.
149. Hörtnagl H., Lochs H. and Winkler H. (1974) Immunological studies on the acidic chromogranins and on dopamine β-hydroxylase (E.C. 1.14.2.1) of bovine chromaffin granules. *J. Neurochem.* **22,** 197–199.

150. Howell K. E. and Palade G. E. (1982) Hepatic golgi fractions resolved into membrane and content subfractions. *J. Cell Biol.* **92,** 822–832.
151. Hsiao R. J., Mezger M. S. and O'Connor D. T. (1990) Chromogranin A in uremia: progressive retention of immunoreactive fragments. *Kidney Int.* **37,** 955–964.
152. Huttner W. B. (1988) Tyrosine sulfation and the secretory pathway. *A. Rev. Physiol.* **50,** 363–376.
153. Huttner W. B., Barr F., Chanat E., Gerdes H.-H., Pimplikar S., Regnier-Vigouroux A., Rosa P., Stinchcombe J. and Tooze S. (1991) Biogenesis of neuroendocrine secretory vesicles. *6th International Symposium on Chromaffin Cell Biology* **S4-2,** 28.
154. Huttner W. B., Gerdes H. H. and Rosa P. (1991) The granin (chromogranin/secretogranin) family. *Trends biochem. Sci.* **16,** 27–30.
155. Hutton J. C., Davidson H. W., Grimaldi K. A. and Peshavaria M. (1987) Biosynthesis of betagranin in pancreatic beta-cells. Identification of a chromogranin A-like precursor and its parallel processing with proinsulin. *Biochem. J.* **244,** 449–456.
156. Hutton J. C., Davidson H. W. and Peshavaria M. (1987) Proteolytic processing of chromogranin A in purified insulin granules. Formation of a 20 kDa N-terminal fragment (betagranin) by the concerted action of a Ca^{2+}-dependent endopeptidase and carboxypeptidase H (EC 3.4.17.10). *Biochem. J.* **244,** 457–464.
157. Hutton J. C., Hansen F. and Peshavaria M. (1985) β-Granins: 21 kDa co-secreted peptides of the insulin granule closely related to adrenal medullary chromogranin. *Fedn. Eur. biochem. Socs Lett.* **188,** 336–340.
158. Hutton J. C., Nielsen E. and Kastern W. (1988) The molecular cloning of the chromogranin A-like precursor of β-granin and pancreastatin from the endocrine pancreas. *Fedn. Eur. biochem. Socs Lett.* **236,** 269–274.
159. Hutton J. C., Peshavaria M., Johnston C. V., Ravazzola M. and Orci L. (1988) Immunolocalization of betagranin: a chromogranin A-related protein of the pancreatic B-cell. *Endocrinology* **122,** 1014–1020.
160. Iacangelo A., Affolter H. U., Eiden L. E., Herbert E. and Grimes M. (1986) Bovine chromogranin A: its sequence and the distribution of its messenger RNA in endocrine tissues. *Nature* **323,** 82–86.
161. Iacangelo A., Fischer-Colbrie R., Koller K. J., Brownstein M. J. and Eiden L. E. (1988) The sequence of porcine chromogranin A messenger RNA demonstrates chromogranin A can serve as the precursor for the biologically active hormone, pancreastatin. *Endocrinology* **122,** 2339–2341.
162. Iacangelo A., Grimes M. and Eiden L. E. (1991) The bovine chromogranin A gene: structural basis for hormone regulation and generation of biologically active peptides. *Molec. Endocrinol.* **5,** 1651–1660.
163. Iacangelo A., Okayama H. and Eiden L. E. (1988) Primary structure of rat chromogranin A and distribution of its mRNA. *Fedn. Eur. biochem. Socs Lett.* **227,** 115–121.
164. Iguchi H., Kameya T., Kadoya K. and Shimaoka K. (1990) Secretion of GAWK, a presumed product of chromogranin B processing, by a human medullary thyroid carcinoma cell line: effect of phorbol ester, forskolin and calcium-ionophore. *J. Neuroendocr.* **2,** 505–508.
165. Iguchi H., Natori S., Kato K.-I., Nawata H. and Chrétien M. (1988) Different processing of chromogranin B into GAWK-immunoreactive fragments in the bovine adrenal medulla and pituitary gland. *Life Sci.* **43,** 1945–1952.
166. Ishizuka J., Asada I., Poston G. J., Lluis F., Tatemoto K., Greeley G. H. and Thompson J. C. (1989) Effect of pancreastatin on pancreatic endocrine and exocrine secretion. *Pancreas* **4,** 277–281.
167. Ishizuka J., Singh P., Greeley J. G. H., Townsend C. M. Jr, Cooper C. W., Tatemoto K. and Thompson J. C. (1988) A comparison of the insulinotropic and the insulin-inhibitory actions of gut peptides on newborn and adult rat islet cells. *Pancreas* **3,** 77–82.
168. Ishizuka J., Tatemoto K., Cohn D. V., Thompson J. C. and Greeley G. H. (1991) Effects of pancreastatin and chromogranin A on insulin release stimulated by various insulinotropic agents. *Regul. Pept.* **34,** 25–32.
169. Iwanaga T., Ishiguro H., Shimizu F. and Fujita T. (1989) Chromogranin A/pancreastatin-related molecules are richly contained in enteric neurons but not in perivascular sympathetic neurons. *Biomed. Res.* **10,** 435–442.
170. Jamieson J. D. and Palade G. E. (1971) Condensing vacuole conversion and zymogen granule discharge in pancreatic exocrine cells: metabolic studies. *J. Cell Biol.* **48,** 503–522.
171. Jenkins N. A., Mattei M. G., Gilbert D. J., Linard C. G., Mbikay M., Chrétien M. and Copeland N. G. (1991) Assignment of secretogranin I locus to mouse chromosome 2 by *in situ* hybridization and interspecific backcross analysis. *Genomics* **11,** 479–480.
172. Kadmon D., Thompson T. C., Lynch G. R. and Scardino P. T. (1991) Elevated plasma chromogranin-A concentrations in prostatic carcinoma. *J. Urol.* **146,** 358–361.
173. Kalina M. and Grimelius L. (1991) Chromogranins or chromogranin-like proteins are present in lamellar bodies and pulmonary surfactant of rat alveolar type II cells. *J. Histochem. Cytochem.* **39,** 213–220.
174. Kalina M., Lukinius A., Grimelius L., Höög A. and Falkmer S. (1991) Ultrastructural localization of synaptophysin to the secretory granules of normal glucagon and insulin cells in human islets of Langerhans. *Ultrastruct. Path.* **15,** 215–219.
175. Kameda Y. (1991) Immunocytochemical localization and development of multiple kinds of neuropeptides and neuroendocrine proteins in the chick ultimobranchial gland. *J. comp. Neurol.* **304,** 373–386.
176. Kameda Y., Amano T. and Tagawa T. (1990) Distribution and ontogeny of chromogranin A and tyrosine hydroxylase in the carotid body and glomus cells located in the wall of the common carotid artery and its branches in the chicken. *Histochemistry* **94,** 609–616.
177. Kanamatsu T., Unsworth C. D., Diliberto J. E. J., Viveros O. H. and Hong J. S. (1986) Reflex splanchnic nerve stimulation increases levels of proenkephalin A mRNA and proenkephalin A-related peptides in the rat adrenal medulla. *Proc. natn. Acad. Sci. U.S.A.* **83,** 9245–9249.
178. Kar S., Bretherton-Watt D., Gibson S. J., Steel J. H., Gentleman S. M., Roberts G. W., Valentino K., Tatemoto K., Ghatei M. A., Bloom S. R. and Polak J. M. (1989) Novel peptide pancreastatin: its occurrence and codistribution with chromogranin A in the central nervous system of the pig. *J. comp. Neurol.* **288,** 627–639.
179. Kawakita S., Takatsuki K., Tsukada M., Yoneda M., Takano T., Kawakubo A. and Nagura H. (1990) Localization of chromogranin A immunoreactivity in bovine gastrointestinal endocrine cells with special reference to Grimelius silver stain. *Endocr. Jap.* **37,** 299–308.

180. Kawakubo A., Takatsuki K., Yoneda M., Kurokawa M., Suzuki A., Semba R. and Kato K. (1989) Highly sensitive enzyme immunoassay for bovine chromogranin A: application for studies of regional distribution in bovine central nervous system. *J. molec. Neurosci.* **1,** 215–223.
181. Kent C. and Coupland R. E. (1989) Localisation of chromogranin A and chromogranin B, met-enkephalin-Arg6-Gly7-Leu8 and PGP9.5-Like immunoreactivity in the developing and adult rat adrenal medulla and extra-adrenal chromaffin tissue. *J. Anat.* **166,** 213–225.
182. Kiang W. L., Krusius T., Finne J., Margolis R. U. and Margolis R. K. (1982) Glycoproteins and proteoglycans of the chromaffin granule matrix. *J. biol. Chem.* **257,** 1651–1659.
183. Kilpatrick L., Gavine F., Apps D. and Phillips J. (1983) Biosynthetic relationship between the major matrix proteins of adrenal chromaffin granules. *Fedn. Eur. biochem. Socs Lett.* **164,** 383–388.
183a. Kirchmair R., Gee P., Hogue-Angeletti R., Laslop A., Fischer-Colbrie R. and Winkler H. (1992) Immunological characterization of the endoproteases PC1 and PC2 in adrenal chromaffin granules and in the pituitary gland. *Fedn. Eur. biochem. Socs. Lett.* **297,** 302–305.
184. Kirshner A. G. and Kirshner N. (1969) A specific soluble protein from the catecholamine storage vesicles of bovine adrenal medulla. *Biochim. biophys. Acta* **181,** 219–225.
184a. Kirshner N. (1974) Molecular organization of the chromaffin vesicles of the adrenal medulla. In *Cytopharmacology of Secretion* (eds Ceccarelli B., Medlolesi J. and Clementi F.), pp. 265–272. Raven Press, New York.
185. Kirshner N., Sage H. J., Smith W. J. and Kirshner A. G. (1966) Release of catecholamines and specific protein from adrenal glands. *Science* **154,** 529–531.
186. Konecki D. S., Benedum U. M., Gerdes H.-H. and Huttner W. B. (1987) The primary structure of human chromogranin A and pancreastatin. *J. biol. Chem.* **262,** 17,026–17,030.
187. Kopell W. N. and Westhead E. W. (1982) Osmotic pressure of solutions of ATP and catecholamines relating to storage in chromaffin granules. *J. biol. Chem.* **257,** 5707–5710.
188. Krieger T. J. and Hook V. Y. (1991) Purification and characterization of a novel thiol protease involved in processing the enkephalin precursor. *J. biol. Chem.* **266,** 8376–8383.
189. Kumarasamy R. and Cohn D. V. (1986) Sulfation of porcine parathyroid secretory protein I. *J. biol. Chem.* **261,** 16,473–16,477.
190. Lahr G., Heiss C., Mayerhofer A., Schilling K., Parmer R. J., O'Connor D. T. and Gratzl M. (1990) Chromogranin A in the olfactory system of the rat. *Neuroscience* **39,** 605–611.
191. Lamberts R., Schmidt W. E. and Creutzfeldt W. (1990) Light and electron microscopical immunocytochemical localization of pancreastatin-like immunoreactivity in porcine tissues. *Histochemistry* **93,** 369–380.
192. Laslop A., Fischer-Colbrie R., Kirschke H., Hogue-Angeletti R. and Winkler H. (1990) Chromogranin A-processing proteinases in purified chromaffin granules: contaminants or endogenous enzymes? *Biochim. biophys. Acta* **1033,** 65–72.
193. Laslop A., Fischer-Colbrie R., Hook V., Obendorf D. and Winkler H. (1986) Identification of two glycoproteins of chromaffin granules as the carboxypeptidase H. *Neurosci. Lett.* **72,** 300–304.
194. Laslop A., Wohlfarter T., Fischer-Colbrie R., Steiner H. J., Humpel C., Saria A., Schmid K. W., Sperk G. and Winkler H. (1989) Insulin hypoglycemia increases the levels of neuropeptide Y and calcitonin gene-related peptide, but not of chromogranins A and B in rat chromaffin granules. *Regul. Pept.* **26,** 191–202.
195. Lassmann H., Hagn C., Fischer-Colbrie R. and Winkler H. (1986) Presence of chromogranin A, B and C in bovine endocrine and nervous tissues: a comparative immunohistochemical study. *Histochem. J.* **18,** 380–386.
196. Lassmann H., Weiler R., Fischer P., Bancher C., Jellinger K., Floor E., Danielczyk W., Seitelberger F. and Winkler H. (1992) Synaptic pathology in Alzheimer's disease. Immunological data for markers of synaptic and large dense core vesicles. *Neuroscience* **46,** 1–8.
197. Lauweryns J. M., van Ranst L., Lloyd R. V. and O'Connor D. T. (1987) Chromogranin in bronchopulmonary neuroendocrine cells. Immunocytochemical detection in human, monkey, and pig respiratory mucosa. *J. Histochem. Cytochem.* **35,** 113–118.
198. Laverriere J. N., Richard J. L., Morin A., Buisson N., Tixiervidal A., Huttner W. B. and Gourdji D. (1991) Secretogranin I (chromogranin B) messenger RNA accumulation is hormonally regulated in GH3B6 rat pituitary tumor cells. *Molec. cell. Endocr.* **80,** 41–51.
199. Lazure C., Paquet L., Litthauer D., Naudé R. J., Oelofsen W. and Chrétien M. (1990) The ostrich pituitary contains a major peptide homologous to mammalian chromogranin A(1-76). *Peptides* **11,** 79–87.
200. Ledbetter F. H., Kilpatrick D. and Sage H. L. (1978) Synthesis of chromogranins and dopamine β-hydroxylase by perfused bovine adrenal glands. *Am. J. Physiol.* **235,** E475–E486.
201. Lee R. W. H. and Huttner W. B. (1983) Tyrosine-O-sulfated proteins of PC12 pheochromocytoma cells and their sulfation by a tyrosylprotein sulfotransferase. *J. biol. Chem.* **258,** 11,326–11,334.
202. Leiser M. and Sherwood L. M. (1989) Calcium-binding proteins in the parathyroid gland. *J. biol. Chem.* **264,** 2792–2800.
203. Leone B. E., Taccagni G. L., Dell'Antonio G. and Cantaboni A. (1990) Chromogranin A as a marker of neuroendocrine histogenesis of tumours: an immunoelectron microscopic study with considerations about the influence of fixation and embedding media on immunolabelling. *Basic appl. Histochem.* **34,** 143–153.
204. Letic-Gavrilovic A., Abe K. and Mori M. (1990) Chromogranin B-like immunoreactivity in the mouse submandibular salivary gland during postnatal development. *Acta histochem.* **89,** 1–10.
205. Letic-Gavrilovic A. and Abe K. (1990) Localization of chromogranins, non-neuron-specific enolase, and different forms of somatostatins in the submandibular salivary glands of mice. *J. Dent. Res.* **69,** 1494–1499.
206. Lewis J. J., Zdon M. J., Adrian T. E. and Modlin I. M. (1988) Pancreastatin: a novel peptide inhibitor of parietal cell secretion. *Surgery* **104,** 1031–1036.
207. Lewis R. V., Micanovic R., Ray P., Blacher R. and Stern A. (1984) Adrenal medullary chromaffin granule peptides: two major ovine peptides and their precursor. *Archs Biochem. Biophys.* **230,** 154–157.
208. Linard C. G., Mbikay M., Seidah N. G. and Chrétien M. (1990) Primary structure of mouse chromogranin-B deduced from cDNA sequence. *Nucl. Acids Res.* **18,** 1298.
209. Lindskog S. and Ahren B. (1988) Galanin and pancreastatin inhibit stimulated insulin secretion in the mouse: comparison of effects. *Horm. Res.* **29,** 237–240.

210. Lloyd R. V., Cano M., Rosa P., Hille A. and Huttner W. B. (1988) Distribution of chromogranin A and secretogranin I (chromogranin B) in neuroendocrine cells and tumors. *Am. J. Path.* **130,** 296–304.
211. Lloyd R. V., Iacangelo A., Eiden L. E., Cano M., Jin L. and Grimes M. (1989) Chromogranin A and B messenger ribonucleic acids in pituitary and other normal and neoplastic human endocrine tissues. *Lab. Invest.* **60,** 548–556.
212. Lloyd R. V., Jin L. and Fields K. (1990) Detection of chromogranins A and B in endocrine tissues with radioactive and biotinylated oligonucleotide probes. *Am. J. Surg. Path.* **14,** 35–43.
213. Lloyd R. V., Mervak T., Schmidt K., Warner T. F. C. S. and Wilson B. S. (1984) Immunohistochemical detection of chromogranin and neuron-specific enolase in pancreatic endocrine neoplasms. *Am. J. Surg. Path.* **8,** 607–614.
214. Lloyd R. V., Sisson J. C., Shapiro B. and Verhofstad A. A. (1986) Immunohistochemical localization of epinephrine, norepinephrine, catecholamine-synthesizing enzymes, and chromogranin in neuroendocrine cells and tumors. *Am. J. Surg. Path.* **125,** 45–54.
215. Lloyd R. V., Wilson B. S., Kovacs K. and Ryan N. (1985) Immunohistochemical localization of chromogranin in human hypophyses and pituitary adenomas. *Arch. Path. Lab. Med.* **109,** 515–517.
216. Lloyd R. V. and Wilson B. S. (1983) Specific endocrine tissue marker defined by a monoclonal antibody. *Science* **222,** 628–630.
217. Lloyd R. V. (1987) Immunohistochemical localization of chromogranin in normal and neoplastic endocrine tissues. *Path. Ann.* **22,** 69–90.
218. Lorinet A.-M., Tatemoto K., Laburthe M. and Amiranoff B. (1989) Pancreastatin inhibits insulin release from Rin m 5F cells: reversion by pertussis toxin. *Eur. J. Pharmac.* **160,** 405–407.
219. Mahata S. K., Mahata M., Marksteiner J., Sperk G., Fischer-Colbrie R. and Winkler H. (1991) Distribution of mRNAs for chromogranins A and B and secretogranin II in rat brain. *Eur. J. Neurosci.* **3,** 895–904.
219a. Mahata S. K., Marksteiner J., Sperk G., Mahata M., Gruber B., Fischer-Colbrie R. and Winkler H. (1992) Temporal lobe epilepsy of the rat: differential expression of mRNAs of chromogranin B, secretogranin II, synaptin/synaptophysin and p65 in subfields of the hippocampus. *Molec. Brain Res.* (in press).
220. Majzoub J. A., Dee P. C. and Habener J. F. (1982) Cellular and cell-free processing of parathyroid secretory proteins. *J. biol. Chem.* **257,** 3581–3588.
221. Majzoub J. A., Kronenberg H. M., Potts J. T., Rich A. and Habener J. F. (1979) Identification and cell-free translation of mRNA coding for a precursor of parathyroid secretory protein. *J. biol. Chem.* **254,** 7449–7455.
222. Marcinkiewicz M., Benjannet S., Seidah N. G., Cantin M. and Chrétien M. (1985) Immunocytochemical localization of a novel pituitary protein (7B2) within the rat brain and hypophysis. *Histochem. Cytochem.* **33,** 1219–1226.
223. Marcinkiewicz M., Fischer-Colbrie R., Falgueyret J. P., Benjannet S., Seidah N. G., Lazure C., Winkler H. and Chrétien M. (1988) Two-dimensional immunoblotting analysis and immunocytochemical localization of the secretory polypeptide 7B2 in adrenal medulla. *Neurosci. Lett.* **95,** 81–87.
224. Matsumoto S., Tanaka K., Yamamoto A., Nakada H., Uchida M. and Tashiro Y. (1987) Immunoelectron microscopic localization of dopamine β-hydroxylase and chromogranin A in adrenomedullary chromaffin cells. *Struct. Funct.* **12,** 483–496.
225. McAuliffe W. G. and Hess A. (1990) Human chromogranin A-like immunoreactivity in the Bergmann glia of the rat brain. *Glia* **3,** 13–16.
226. Micanovic R., Ray P., Kruggel W. and Lewis R. V. (1985) Purification and sequence of a novel ovine adrenal medullary peptide and its precursor. *Neuroendocrinology* **41,** 197–200.
227. Miyasaka K., Funakoshi A., Nakamura R., Kitani K., Shimizu F. and Tatemoto K. (1989) Effects of porcine pancreastatin on postprandial pancreatic exocrine secretion and endocrine functions in the conscious rat. *Digestion* **43,** 204–211.
228. Miyasaka K., Funakoshi A., Kitani K., Tamamura H., Fujii N. and Funakoshi S. (1990) The importance of the C-terminal amide structure of rat pancreastatin to inhibit pancreatic exocrine secretion. *Fedn. Eur. biochem. Socs Lett.* **263,** 279–280.
229. Miyasaka K., Funakoshi A., Kitani K., Tamamura H., Funakoshi S. and Fujii N. (1990) Inhibitory effect of pancreastatin on pancreatic exocrine secretions. Pancreastatin inhibits central vagal nerve stimulation. *Gastroenterology* **99,** 1751–1756.
230. Modi W. S., Levine M. A., Seuanez H. N., Dean M. and O'Brien S. J. (1989) The human chromogranin A gene: chromosome assignment and RFLP analysis. *Am. J. Hum Genet.* **45,** 814–818.
231. Molenaar W. M., Lee V. M. Y. and Trojanowski J. Q. (1990) Early fetal acquisition of the chromaffin and neuronal immunophenotype by human adrenal medullary cells. An immunohistological study using monoclonal antibodies to chromogranin A, synaptophysin, tyrosine hydroxylase, and neuronal cytoskeletal proteins. *Expl Neurol.* **108,** 1–9.
232. Mouland A. J. and Hendy G. N. (1991) Regulation of synthesis and secretion of chromogranin-A by calcium and 1,25-dihydroxycholecalciferol in cultured bovine parathyroid cells. *Endocrinology* **128,** 441–449.
233. Munoz D. G. (1989) Chromogranin A immunoreactive peptides are major components of neocortical and limbic plaques in Alzheimer's disease. *J. Neuropath. exp. Neurol.* **48,** 378.
234. Munoz D. G. (1991) Chromogranin A-like immunoreactive neurites are major constituents of senile plaques. *Lab. Invest.* **64,** 826–832.
235. Munoz D. G. (1990) The distribution of chromogranin A like immunoreactivity in the human hippocampus coincides with the pattern of resistance to epilepsy-induced neuronal damage. *Ann. Neurol.* **27,** 266–275.
236. Munoz D. G. (1990) Monodendritic neurons: a cell type in the human cerebellar cortex identified by chromogranin A-like immunoreactivity. *Brain Res.* **528,** 335–338.
237. Munoz D. G., Kobylinski L., Henry D. D. and George D. H. (1990) Chromogranin A-like immunoreactivity in the human brain: distribution in bulbar medulla and cerebral cortex. *Neuroscience* **34,** 533–543.
238. Murray S. S., Burton D. W. and Deftos L. J. (1988) The coregulation of secretion and cytoplasmic ribonucleic acid of chromogranin A and calcitonin by phorbol ester in cells that produce both substances. *Endocrinology* **122,** 495–499.
239. Murray S. S., Burton D. W. and Deftos L. J. (1988) The effects of forskolin and calcium ionophore A23187 on secretion and cytoplasmatic RNA levels of chromogranin-A and calcitonin. *J. Bone Min. Res.* **3,** 447–452.
240. Murray S. S., Deaven L. L., Burton D. W., O'Connor D. T., Mellon P. L. and Deftos L. J. (1987) The gene for human chromogranin A (CgA) is located on chromosome 14. *Biochem. biophys. Res. Commun.* **142,** 141–146.

241. Nakano I., Funakoshi A., Miyasaka K., Ishida K., Makk G., Angwin P., Chang D. and Tatemoto K. (1989) Isolation and characterization of bovine pancreastatin. *Regul. Pept.* **25,** 207–213.
242. Neuman B., Wiedermann C. J., Fischer-Colbrie R., Schober M., Sperk G. and Winkler H. (1984) Biochemical and functional properties of large and small dense-cored vesicles in sympathetic nerves of rat and ox vas deferens. *Neuroscience* **13,** 921–931.
243. Nguyen M. H., Harbour D. and Gagnon C. (1987) Secretory proteins from adrenal medullary cells are carboxylmethylated *in vivo* and released under their methylated form by acetylcholine. *J. Neurochem.* **49,** 38–44.
244. Nolan J. A., Trojanowski J. and Hogue-Angeletti R. (1985) Neurons and neuroendocrine cells contain chromogranin: detection of the molecule in normal bovine tissues by immunochemical and immunohistochemical methods. *J. Histochem. Cytochem.* **33,** 791–798.
245. O'Connor D. T. (1983) Chromogranin: widespread immunoreactivity in polypeptide hormone producing tissues and in serum. *Regul. Pept.* **6,** 263–280.
246. O'Connor D. T. (1985) Plasma chromogranin A. Initial studies in human hypertension. *Hypertension* 7, *Suppl.* I, 176–179.
247. O'Connor D. T. and Bernstein K. N. (1984) Radioimmunoassay of chromogranin A in plasma as a measure of exocytotic sympathoadrenal activity in normal subjects and patients with pheochromocytoma. *New Engl. J. Med.* **311,** 764–770.
248. O'Connor D. T., Burton D. and Deftos L. J. (1983) Chromogranin A: immunohistology reveals its universal occurrence in normal polypeptide hormone producing endocrine glands. *Life Sci.* **33,** 1657–1663.
249. O'Connor D. T., Burton D. W., Parmer R. J. and Deftos L. J. (1984) Human chromogranin A: detection by immunohistochemistry in cells and diverse polypeptide hormone producing tumors. In *Endocrine Control of Bone and Calcium Metabolism* (eds Cohn D. V., Fujita T., Potts J. T. Jr and Talmage R. V.), pp. 187–190. Elsevier, Amsterdam.
250. O'Connor D. T. and Deftos L. J. (1986) Secretion of chromogranin A by peptide-producing endocrine neoplasms. *New Engl. J. Med.* **314,** 1145–1151.
251. O'Connor D. T. and Frigon R. P. (1984) Chromogranin A, the major catecholamine storage vesicle soluble protein. *J. biol. Chem.* **259,** 3237–3247.
252. O'Connor D. T., Frigon R. P. and Sokoloff R. L. (1984) Human chromogranin A. Purification and characterization from catecholamine storage vesicles of human pheochromocytoma. *Hypertension* **6,** 2–12.
253. O'Connor D. T., Pandian M. R., Carlton E., Cervenka J. H. and Hsiao R. J. (1989) Rapid radioimmunoassay of circulating chromogranin A: *in vitro* stability, exploration of the neuroendocrine character of neoplasia, and assessment of the effects of organ failure. *Clin. Chem.* **35,** 1631–1637.
254. Ohneda A., Koizumi F. and Ohneda M. (1989) Effect of porcine pancreastatin on endocrine function of canine pancreas. *Tohoku J. exp. Med.* **159,** 291–298.
255. Östenson C.-G., Efendic S. and Holst J. J. (1989) Pancreastatin-like immunoreactivity and insulin are released in parallel from the perfused porcine pancreas. *Endocrinology* **124,** 2986–2990.
256. Parmer R. J., Koop A. H., Handa M. T. and O'Connor D. T. (1989) Molecular cloning of chromogranin A from rat pheochromocytoma cells. *Hypertension* **14,** 435–444.
257. Peiró E., Degano P., Miralles P., Silvestre R. A. and Marco J. (1991) Homologous pancreastatin inhibits insulin secretion without affecting glucagon and somatostatin release in the perfused rat pancreas. *Regul. Pept.* **34,** 159–167.
258. Peiró E., Miralles P., Silvestre R. A., Villanueva M. L. and Marco J. (1989) Pancreastatin inhibits insulin secretion as induced by glucagon, vasoactive intestinal peptide, gastric inhibitory peptide, and 8-cholecystokinin in the perfused rat pancreas. *Metabolism* **38,** 679–682.
259. Perez M. A., Saul S. H. and Trojanowski J. Q. (1990) Neurofilament and chromogranin expression in normal and neoplastic neuroendocrine cells of the human gastrointestinal tract and pancreas. *Cancer* **65,** 1219–1227.
260. Peterson J. B., Nelson D. L., Ling E. and Hogue-Angeletti R. (1987) Chromogranin A-like proteins in the secretory granules of a protozoan, *Paramecium tetraurelia*. *J. biol. Chem.* **262,** 17,264–17,267.
261. Phillips J. H. (1982) Dynamic aspects of chromaffin granule structure. *Neuroscience* **7,** 1595–1609.
262. Pietroletti R., Bishop A. E., Carlei F., Bonamico M., Lloyd R. V., Wilson B. S., Ceccamea A., Lezoche E., Speranza V. and Polak J. M. (1986) Gut endocrine cell population in coeliac disease estimated by immunocytochemistry using a monoclonal antibody to chromogranin. *Gut* **27,** 838–843.
262a. Pimplikar S. W. and Huttner W. B. (1992) Chromogranin B (secretogranin I), a secretory protein of the regulated pathway, is also present in a tightly membrane-associated form in PC12 cells. *J. biol. Chem.* **267,** 4110–4118.
263. Pohl T. M., Phillips E., Song K. Y., Gerdes H.-H., Huttner W. B. and Rüther U. (1990) The organisation of the mouse chromogranin B (secretogranin I) gene. *Fedn. Eur. biochem. Socs Lett.* **262,** 219–224.
264. Porcelli G., Angelett M., Angeletti M. and Marini-Bettolo G. B. (1972) Chromatographic and electrophoretic behaviour of chromogranin obtained from bovine and equine adrenal medulla. *J. Chromat.* **66,** 162–166.
265. Qian J., Hickey W. F. and Hogue-Angeletti R. (1988) Neuroendocrine cells in intestinal lamina propria. Detection with antibodies to chromogranin A. *J. Neuroimmunol.* **17,** 159–165.
266. Rausch D. M., Iacangelo A. and Eiden L. E. (1988) Glucocorticoid- and nerve growth factor-induced changes in chromogranin A expression define two different neuronal phenotypes in PC12 cells. *Molec. Endocr.* **2,** 921–927.
267. Ravazzola M., Efendic S., Östenson C. G., Tatemoto K., Hutton J. C. and Orci L. (1988) Localization of pancreastatin immunoreactivity in porcine endocrine cells. *Endocrinology* **123,** 227–229.
268. Reiffen F. U. and Gratzl M. (1986) Ca^{2+} binding to chromaffin vesicle matrix proteins: effects of pH, Mg^{2+}, and ionic strength. *Biochemistry* **25,** 4402–4406.
269. Reiffen F. U. and Gratzl M. (1986) Chromogranins, widespread in endocrine and nervous tissue, bind Ca^{2+}. *Fedn. Eur. biochem. Socs Lett.* **195,** 327–330.
270. Reinecke M., Höög A., Östenson C.-G., Efendic S., Grimelius L. and Falkmer S. (1991) Phylogenetic aspects of pancreastatin- and chromogranin-like immunoreactive cells in the gastro-entero-pancreatic neuroendocrine system of vertebrates. *Gen. comp. Endocr.* **83,** 167–182.
271. Ribes G., Vecina F., Hillaire-Buys D. and Ahren B. (1990) Effects of pancreastatin on pancreatic hormone secretion in the dog. *Horm. Res.* **33,** 194–198.
272. Rieker S., Fischer-Colbrie R., Eiden L. E. and Winkler H. (1988) Phylogenetic distribution of peptides related to chromogranins A and B. *J. Neurochem.* **50,** 1066–1073.

273. Rindi G., Buffa R., Sessa F., Tortora O. and Solcia E. (1986) Chromogranin A, B and C immunoreactivities of mammalian endocrine cells. Distribution, distinction from costored hormones/prohormones and relationship with the argyrophil component of secretory granules. *Histochemistry* **85,** 19–28.
274. Rosa P., Hille A., Lee R. W. H., Zanini A., De Camilli P. and Huttner W. B. (1985) Secretogranins I and II: two tyrosine-sulfated secretory proteins common to a variety of cells secreting peptides by the regulated pathway. *J. Cell Biol.* **101,** 1999–2011.
275. Rosa P., Weiss U., Pepperkok R., Ansorge W., Niehrs C., Stelzer E. H. and Huttner W. B. (1989) An antibody against secretogranin I (chromogranin B) is packaged into secretory granules. *J. Cell Biol.* **109,** 17–34.
276. Rosa P. and Zanini A. (1983) Purification of a sulfated secretory protein from the adenohypophysis. Immunochemical evidence that similar macromolecules are present in other glands. *Eur. J. Cell Biol.* **31,** 94–98.
277. Rundle S., Somogyi P., Fischer-Colbrie R., Hagn C. and Winkler H. (1986) Chromogranin A, B and C: immunohistochemical localization in ovine pituitary and the relationship with hormone-containing cells. *Regul. Pept.* **16,** 217–233.
277a. Russell J., Lettieri D., Adler J. and Sherwood L. M. (1990) 1,25-Dihydroxyvitamin D_3 has opposite effects on the expression of parathyroid hormone genes. *Molec. Endocr.* **4,** 505–509.
278. Sage H. J., Smith W. J. and Kirshner N. (1967) Mechanism of secretion from the adrenal medulla. I. A microquantitative immunologic assay for bovine adrenal catecholamine storage vesicle protein and its application to studies of the secretory process. *Molec. Pharmac.* **3,** 81–89.
279. Salahuddin M. J., Sekiya K., Ghatei M. A. and Bloom S. R. (1989) Regional distribution of chromogranin B 420-493-like immunoreactivity in the pituitary gland and central nervous system of man, guinea-pig and rat. *Neuroscience* **30,** 231–240.
280. Salton S. R. J., Margolis R. U. and Margolis R. K. (1983) Release of chromaffin granule glycoproteins and proteoglycans from potassium-stimulated PC12 pheochromocytoma cells. *J. Neurochem.* **41,** 1165–1170.
281. Scammell J. G., Rosa P., Hille A. and Huttner W. B. (1990) Regulation of chromogranin B/secretogranin I and secretogranin II storage in GH4C1 cells. *J. Histochem. Cytochem.* **38,** 949–956.
282. Schmid K. W., Weiler R., Xu R. W., Hogue-Angeletti R., Fischer-Colbrie R. and Winkler H. (1989) An immunological study on chromogranin A and B in endocrine and nervous tissues. *Histochem. J.* **21,** 365–373.
283. Schmidt W. E., Siegel E. G., Kratzin H. and Creutzfeldt W. (1988) Isolation and primary structure of tumor-derived peptides related to human pancreastatin and chromogranin A. *Proc. natn. Acad. Sci. U.S.A.* **85,** 8231–8235.
284. Schmidt W. E., Siegel E. G., Lamberts R., Gallwitz B. and Creutzfeldt W. (1988) Pancreastatin: molecular and immunocytochemical characterization of a novel peptide in porcine and human tissues. *Endocrinology* **123,** 1395–1404.
285. Schneider F. H., Smith A. D. and Winkler H. (1967) Secretion from the adrenal medulla: biochemical evidence for exocytosis. *Br. J. Pharmac. Chemother.* **31,** 94–104.
286. Schober M., Fischer-Colbrie R., Schmid K. W., Bussolatti G., O'Connor D. T. and Winkler H. (1987) Comparison of chromogranins A, B, and secretogranin II in human adrenal medulla and pheochromocytoma. *Lab. Invest.* **57,** 385–391.
287. Schober M., Fischer-Colbrie R. and Winkler H. (1989) Ontogenesis of chromogranin A and B and catecholamines in rat adrenal medulla. *Brain Res.* **478,** 41–46.
288. Schönfeld V. J. and Müller M. K. (1991) The effect of pancreastatin on endocrine and exocrine pancreas. *Scand. J. Gastroent.* **26,** 993–999.
289. Schwarzenbrunner U., Schmidle T., Obendorf D., Scherman D., Hook V., Fischer-Colbrie R. and Winkler H. (1990) Sympathetic axons and nerve terminals: the protein composition of small and large dense-core and of a third type of vesicles. *Neuroscience* **37,** 819–827.
290. Seidah N. G., Gaspar J., Mion P., Marcinkiewicz M., Mbikay M. and Chrétien M. (1990) cDNA sequence of two distinct pituitary proteins homologous to Kex2 and furin gene products: tissue-specific mRNAs encoding candidates for pro-hormone processing proteinases. *DNA Cell Biol.* **9,** 415–424.
291. Seidah N. G., Hendy G. N., Hamelin J., Paquin J., Lazure C., Metters K. M., Rossier J. and Chrétien M. (1987) Chromogranin A can act as a reversible processing enzyme inhibitor. Evidence from the inhibition of the IRCM-serine protease 1 cleavage of pro-enkephalin and ACTH at pairs of basic amino acids. *Fedn. Eur. biochem. Socs Lett.* **211,** 144–150.
292. Seino Y., Tsuji K., Inagaki N., Okamoto Y., Takeda J., Taminato T. and Imura H. (1990) C-terminal peptide of the GIP precursor and pancreastatin fragments on insulin secretion from isolated rat islets. *Biomed. Res.* **11,** 283–286.
293. Sekiya K., Ghatei M. A., Minamino N., Bretherton-Watt D., Matsuo H. and Bloom S. R. (1988) Isolation of human pancreastatin fragment containing the active sequence from a glucagonoma. *Fedn. Eur. biochem. Socs Lett.* **228,** 153–156.
294. Sekiya K., Ghatei M. A., Salahuddin M. J., Bishop A. E., Hamid Q. A., Ibayashi H., Polak J. M. and Bloom S. R. (1989) Production of GAWK (chromogranin-B 420-493)-like immunoreactivity by endocrine tumors and its possible diagnostic value. *J. clin. Invest.* **83,** 1834–1842.
295. Serck-Hanssen G. and O'Connor D. T. (1984) Immunological identification and characterization of chromogranins coded by Poly(A) mRNA from bovine adrenal medulla and pituitary gland and human phaeochromocytoma. *J. biol. Chem.* **259,** 11,597–11,600.
296. Settleman J., Fonseca R., Nolan J. A. and Hogue-Angeletti R. (1985) Relationship of multiple forms of chromogranin. *J. biol. Chem.* **260,** 1645–1651.
297. Settleman J., Nolan J. A. and Hogue-Angeletti R. (1985) Chromogranin, an integral membrane protein. *J. biol. Chem.* **260,** 1641–1644.
298. Sharp R. R. and Richards E. P. (1977) Analysis of the carbon-13 and proton NMR spectra of bovine chromaffin granules. *Biochim. biophys. Acta* **497,** 14–28.
299. Shen F. S., Roberts S. F. and Lindberg I. (1989) A putative processing enzyme for proenkephalin in bovine adrenal chromaffin granule membranes. *J. biol. Chem.* **264,** 15,600–15,605.
300. Shimizu F., Ikei N., Iwanaga T. and Fujita T. (1987) An immunochemical and immunohistochemical study on pancreastatin-like immunoreactivity using synthetic peptides. *Biochem. Res.* **8,** 457–462.
301. Shults C. W., O'Connor D. T., Baird A., Hill R., Goetz C. G., Watts R. L., Klawans H. L., Carvey P. M., Bakay R. A., Gage F. H. and U H. S. (1991) Clinical improvement in parkinsonian patients undergoing adrenal to caudate

transplantation is not reflected by chromogranin A or basic fibroblast growth factor in ventricular fluid. *Expl Neurol.* **111,** 276–281.
302. Siegel R. E., Iacangelo A., Park J. and Eiden L. E. (1988) Chromogranin A biosynthetic cell populations in bovine endocrine and neuronal tissues; detection by *in situ* hybridization histochemistry. *Molec. Endocr.* **2,** 368–374.
303. Sietzen M., Schober M., Fischer-Colbrie R., Scherman D., Sperk G. and Winkler H. (1987) Rat adrenal medulla: levels of chromogranins, enkephalins, dopamine β-hydroxylase and of the amine transporter are changed by nervous activity and hypophysectomy. *Neuroscience* **22,** 131–139.
304. Silver M. M., Hearn S. A., Lines L. D. and Troster M. (1988) Calcitonin and chromogranin A localization in medullary carcinoma of the thyroid by immunoelectron microscopy. *J. Histochem. Cytochem.* **36,** 1031–1036.
305. Silvestre R. A., Peiro E., Miralles P., Villanueva M. L. and Marco J. (1988) Effects of pancreastatin on insulin, glucagon and somatostatin secretion by the perfused rat pancreas. *Life Sci.* **42,** 1361–1367.
306. Simon J.-P. and Aunis D. (1989) Biochemistry of the chromogranin A protein family. *Biochem. J.* **262,** 1–13.
307. Simon J. P., Bader M. F. and Aunis D. (1989) Effect of secretagogues on chromogranin A synthesis in bovine cultured chromaffin cells. Possible regulation by protein kinase C. *Biochem. J.* **260,** 915–922.
308. Simon J.-P., Bader M.-F. and Aunis D. (1989) Proteolytic processing of chromogranin A in cultured chromaffin cells. *Biochim. biophys. Acta* **1051,** 123–130.
309. Simon J.-P., Bader M. F. and Aunis D. (1988) Secretion from chromaffin cells is controlled by chromogranin A-derived peptides. *Proc. natn. Acad. Sci. U.S.A.* **85,** 1712–1716.
310. Sjöholm A., Funakoshi A., Efendic S., Östenson C.-G. and Hellerström C. (1991) Long term inhibitory effects of pancreastatin and diazepam binding inhibitor on pancreatic β-cell deoxyribonucleic acid replication, polyamine content, and insulin secretion. *Endocrinology* **128,** 3277–3282.
311. Small D. H. (1989) Acetylcholinesterases: zymogens of neuropeptide processing enzymes? *Neuroscience* **29,** 241–249.
312. Small D. H. (1990) Non-cholinergic actions of acetylcholinesterases: proteases regulating cell growth and development? *Trends biochem. Sci.* **15,** 213–216.
313. Small D. H., Ismael Z. and Chubb I. W. (1986) Acetylcholinesterase hydrolyses chromogranin A to yield low molecular weight peptides. *Neuroscience* **19,** 289–295.
314. Smeekens S. P., Avruch A. S., LaMendola J. and Chan S. J. (1991) Identification of a cDNA encoding a second putative prohormone convertase related to PC2 in AtT20 cells and islets of Langerhans. *Proc. natn. Acad. Sci. U.S.A.* **88,** 340–344.
315. Smith A. D. (1968) Biochemistry of adrenal chromaffin granules. In *The Interaction of Drugs and Subcellular Components on Animal Cells* (ed. Campbell P. N.), pp. 239–292. Churchill, London.
316. Smith A. D., de Potter W. P., Moerman E. J. and de Schaepdryver A. F. (1970) Release of dopamine β-hydroxylase and chromogranin A upon stimulation of the splenic nerve. *Tiss. Cell* **2,** 547–568.
317. Smith A. D. and Winkler H. (1967) Purification and properties of an acidic protein from chromaffin granules of bovine adrenal medulla. *Biochem. J.* **103,** 483–492.
318. Smith W. J. and Kirshner N. (1967) A specific soluble protein from the catecholamine storage vesicles of bovine adrenal medulla. Purification and chemical characterization. *Molec. Pharmac.* **3,** 52–62.
319. Somogyi P., Hodgson A. J., De Potter R. W., Fischer-Colbrie R., Schober M., Winkler H. and Chubb I. W. (1984) Chromogranin immunoreactivity in the central nervous system. Immunochemical characterization, distribution and relationship to catecholamine and enkephalin pathways. *Brain Res. Rev.* **8,** 193–230.
319a. Song J., Jin L., Chandler W. F., England B. G., Smart J. B., Landefeld T. D. and Lloyd R. V. (1990) Gonadotropin-releasing hormone regulates gonadotropin β-subunit and chromogranin-B messenger ribonucleic acids in cultured chromogranin-A-positive pituitary adenomas. *J. clin. Endocr. Metab.* **71,** 622–630.
320. Steiner H. J., Schmid K. W., Fischer-Colbrie R., Sperk G. and Winkler H. (1989) Co-localization of chromogranin A and B, secretogranin II and neuropeptide Y in chromaffin granules of rat adrenal medulla studied by electron microscopic immunocytochemistry. *Histochemistry* **91,** 473–477.
321. Steiner H. J., Weiler R., Ludescher C., Schmid K. W. and Winkler H. (1990) Chromogranins A and B are co-localized with atrial natriuretic peptides in secretory granules of rat heart. *J. Histochem. Cytochem.* **38,** 845–850.
322. Strieder N., Ziegler E., Winkler H. and Smith A. D. (1968) Some properties of soluble proteins from chromaffin granules of different species. *Biochem. Pharmac.* **17,** 1553–1556.
323. Takatsuki K., Schneider A. B., Shin K. Y. and Sherwood L. M. (1981) Extraction, purification, and partial characterization of bovine parathyroid secretory protein. *J. biol. Chem.* **256,** 2342–2345.
324. Takayama H. and Sitkovsky M. V. (1987) Antigen receptor-regulated exocytosis in cytotoxic T lymphocytes. *J. exp. Med.* **166,** 725–743.
325. Takiyyuddin M. A., Baron A. D., Cervenka J. H., Barbosa J. A., Neumann H. P. H., Parmer R. J., Sullivan P. A. and O'Connor D. T. (1991) Suppression of chromogranin-A release from neuroendocrine sources in man: pharmacological studies. *J. clin. Endocr. Metab.* **72,** 616–622.
326. Takiyyuddin M. A., Cervenka J. H., Sullivan P. A., Pandian M. R., Parmer R. J., Barbosa J. A. and O'Connor D. T. (1990) Is physiologic sympathoadrenal catecholamine release exocytotic in humans? *Circulation* **81,** 185–195.
327. Takiyyuddin M. A., Cervenka J. H., Pandian M. R., Stuenkel C. A., Neumann H. P. H. and O'Connor D. T. (1990) Neuroendocrine sources of chromogranin A in normal man: clues from selective stimulation of endocrine glands. *J. clin. Endocr. Metab.* **71,** 360–369.
328. Tamamura H., Ohta M., Yoshizawa K., Ono Y., Funakoshi A., Miyasaka K., Tateishi K., Jimi A., Yajima H., Fujii N. and Funakoshi S. (1990) Isolation and characterization of a tumor-derived human protein related to chromogranin A and its *in vitro* conversion to human pancreastatin-48. *Eur. J. Biochem.* **191,** 33–39.
329. Tateishi K., Funakoshi A., Wakasugi H., Iguchi H., Shinozaki H., Abe M., Funakoshi S., Tamamura H., Yajima H. and Matsuoka Y. (1989) Plasma pancreastatin-like immunoreactivity in various diseases. *J. clin. Endocr. Metab.* **69,** 1305–1308.
330. Tateishi K., Miyasaka K., Shinozaki H., Funakoshi S., Matsuoka Y. and Funakoshi A. (1990) Pancreastatin-like immunoreactivity in urine. *J. clin. Endocr. Metab.* **71,** 842–845.
331. Tatemoto K., Efendic S., Mutt V., Makk G., Feistner G. J. and Barchas J. D. (1986) Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. *Nature* **324,** 476–478.

332. Thomas L., Leduc R., Thorne B. A., Smeekens S. P., Steiner D. F. and Thomas G. (1991) Kex2-like endoproteases PC2 and PC3 accurately cleave a model prohormone in mammalian cells: evidence for a common core of neuroendocrine processing enzymes. *Proc. natn. Acad. Sci. U.S.A.* **88,** 5297–5301.
333. Tisserand-Jochem E. M., Lopez E., Milet C., Vidal B., Magnac C., Eyquem A. and Cohn D. V. (1987) Co-localization and secretion of parathyrin of stannius corpuscles (immunoreactive parathyroid hormone) and of secretory glycoproteins including SP-I in the European eel (*Anguilla angilla* L.). *Bone Min.* **2,** 163–169.
334. Tooze J., Tooze S. A. and Fuller S. D. (1987) Sorting of progeny coronavirus from condensed secretory proteins at the exit from the trans-Golgi network of AtT20 cells. *J. Cell Biol.* **105,** 1215–1226.
335. Turkewitz A. P., Madeddu L. and Kelly R. B. (1991) Maturation of dense core granules in wild type and mutant *Tetrahymena thermophila*. *Eur. molec. Biol. Org. J.* **10,** 1979–1987.
336. Udenfriend S., Cooper J. R., Clark C. T. and Baer J. E. (1953) Rate of turnover of epinephrine in the adrenal medulla. *Science* **117,** 663–665.
337. Udenfriend S. and Kilpatrick D. L. (1983) Biochemistry of the enkephalins and enkephalin-containing peptides. *Archs Biochem. Biophys.* **221,** 309–323.
338. Usellini L., Finzi G., Riva C., Capella C., Mochizuki T., Yanaihara C., Yanaihara N. and Solcia E. (1990) Ultrastructural identification of human secretin cells by the immunogold technique. Their costorage of chromogranin A and serotonin. *Histochemistry* **94,** 113–120.
339. Varndell I. M., Lloyd R. V., Wilson B. S. and Polak J. M. (1985) Ultrastructural localization of chromogranin: a potential marker for the electron microscopical recognition of endocrine cell secretory granules. *Histochem. J.* **17,** 981–992.
339a. Videen J. S., Mezger M. S., Chang Y.-M. and O'Connor D. T. (1992) Calcium and catecholamine interaction with adrenal chromogranins. *J. biol. Chem.* **267,** 3066–3073.
340. Vittoria A., La Mura E., Cocca T. and Cecio A. (1990) Serotonin-, somatostatin- and chromogranin A-containing cells of the urethro-prostatic complex in the sheep. An immunocytochemical and immunofluorescent study. *J. Anat.* **171,** 169–178.
341. Vittoria A., Paino G., La Mura E., Budetta G. and Cecio A. (1989) Chromogranin- and somatostatin-containing neuroendocrine cells in the porcine uterus. An immunocytochemical study. *Anat. Histol. Embryol.* **18,** 136–142.
342. Volknandt W., Schober M., Fischer-Colbrie R., Zimmermann H. and Winkler H. (1987) Cholinergic nerve terminals in the rat diaphragm are chromogranin A immunoreactive. *Neurosci. Lett.* **81,** 241–244.
343. Wand G. S., Takiyyuddin M., O'Connor D. T. and Levine M. A. (1991) A proposed role for chromogranin A as a glucocorticoid-responsive autocrine inhibitor of pro-opiomelanocortin secretion. *Endocrinology* **128,** 1345–1351.
344. Watanabe T., Uchiyama Y. and Grube D. (1991) Topology of chromogranin A and secretogranin II in the rat anterior pituitary: potential marker proteins for distinct secretory pathways in gonadotrophs. *Histochemistry* **96,** 285–293.
345. Watkinson A., Jonsson A. C., Davison M., Young J., Lee C. M., Moore S. and Dockray G. J. (1991) Heterogeneity of chromogranin A-derived peptides in bovine gut, pancreas and adrenal medulla. *Biochem. J.* **276,** 471–479.
346. Watkinson A., O'Sullivan A. J., Burgoyne R. D. and Dockray G. J. (1990) Differential accumulation of catecholamines, proenkephalin A-derived and chromogranin A-derived peptides in the medium after chronic nicotine stimulation of cultured bovine adrenal chromaffin cells. *Peptides* **11,** 435–441.
346a. Watkinson A. and Robinson I. (1992) Reserpine-induced processing of chromogranin A in cultured bovine adrenal chromaffin cells. *J. Neurochem.* **58,** 877–883.
347. Weiler R., Cidon S., Gershon M. D., Tamir H., Hogue-Angeletti R. and Winkler H. (1989) Adrenal chromaffin granules and secretory granules from thyroid parafollicular cells have several common antigens. *Fedn. Eur. biochem. Socs Lett.* **257,** 457–459.
348. Weiler R., Fischer-Colbrie R., Schmid K. W., Feichtinger H., Bussolatti G., Grimelius L., Krisch K., Kerl H., O'Connor D. T. and Winkler H. (1988) Immunological studies on the occurrence and properties of chromogranin A and B and secretogranin II in endocrine tumors. *Am. J. Surg. Path.* **12,** 877–884.
349. Weiler R., Lassmann H., Fischer P., Jellinger K. and Winkler H. (1990) A high ratio of chromogranin A to synaptin/synaptophysin is a common feature of brains in Alzheimer and Pick disease. *Fedn. Eur. biochem. Socs Lett.* **263,** 337–339.
350. Weiler R., Marksteiner J., Bellmann R., Wohlfarter T., Schober M., Fischer-Colbrie R., Sperk G. and Winkler H. (1990) Chromogranins in rat brain: characterization, topographical distribution and regulation of synthesis. *Brain Res.* **532,** 87–94.
351. Weiler R., Meyerson G., Fischer-Colbrie R., Laslop A., Påhlman S., Floor E. and Winkler H. (1990) Divergent changes of chromogranin A/secretogranin II levels in differentiating human neuroblastoma cells. *Fedn. Eur. biochem. Socs Lett.* **265,** 27–29.
352. Weiler R., Steiner H.-J., Fischer-Colbrie R., Schmid K. W. and Winkler H. (1991) Undegraded chromogranin A is present in serum and enters the endocytotic lysosomal pathway in kidney. *Histochemistry* **96,** 395–399.
353. Westermann R., Luetcke A. and Unsicker K. (1991) The neurotrophic activity of chromogranin A. *Biol. Chem.* Hoppe-Seyler **372,** 889.
354. Westermann R., Stögbauer F., Unsicker K. and Lietzke R. (1988) Calcium-dependence of chromogranin A-catecholamine interaction. *Fedn. Eur. biochem. Socs Lett.* **239,** 203–206.
355. Wiedenmann B. and Huttner W. B. (1989) Synaptophysin and chromogranins/secretogranins—widespread constituents of distinct types of neuroendocrine vesicles and new tools in tumor diagnosis. *Virch. Arch. B. Cell Path.* **58,** 95–121.
356. Wiedenmann B., Waldherr R., Buhr H., Hille A., Rosa P. and Huttner W. B. (1988) Identification of gastroenteropancreatic neuroendocrine cells in normal and neoplastic human tissues with antibodies against synaptophysin, chromogranin A, secretogranin I (chromogranin B), and secretogranin II. *Gastroenterology* **95,** 1364–1374.
357. Williams K. R., Hemmings H. C., LoPresti M. B., Konigsberg W. H. and Greengard P. (1986) DARPP-32, a dopamine- and cyclic AMP-regulated neuronal phosphoprotein. *J. biol. Chem.* **261,** 1890–1903.
358. Wilson B. S. and Lloyd R. V. (1984) Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. *Am. J. Path.* **115,** 458–468.
359. Wilson B. S., Phan S. H. and Lloyd R. V. (1986) Chromogranin from normal human adrenal glands: purification by monoclonal antibody affinity chromatography and partial N-terminal amino acid sequence. *Regul. Pept.* **13,** 207–223.

360. Wilson S. P., Corcoran J. J. and Kirshner N. (1991) Comparative incorporation of proenkephalin-derived peptides, chromogranin A, and dopamine β-hydroxylase into chromaffin vesicles. *J. Neurochem.* **57,** 870–875.
361. Winkler H. (1977) The biogenesis of chromaffin granules. *Neuroscience* **2,** 657–683.
362. Winkler H. (1976) The composition of adrenal chromaffin granules: an assessment of controversial results. *Neuroscience* **1,** 65–80.
363. Winkler H. (1988) Occurrence and mechanism of exocytosis in adrenal medulla and sympathetic nerve. In *Handbook of Experimental Pharmacology* 90/I (eds Trendelenburg U. and Weiner N.), pp. 43–118. Springer, New York.
364. Winkler H., Apps D. K. and Fischer-Colbrie R. (1986) The molecular function of adrenal chromaffin granules: established facts and unresolved topics. *Neuroscience* **18,** 261–290.
365. Winkler H. and Carmichael S. W. (1982) The chromaffin granule. In *The Secretory Granule* (eds Poisner A. M. and Trifaró J. M.), pp. 3–79, Elsevier.
366. Winkler H., Falkensammer G., Patzak A., Fischer-Colbrie R., Schober M. and Weber A. (1984) Life cycle of the catecholaminergic vesicles: from biogenesis to secretion. In *Regulation of Transmitter Function: Basic and Clinical Aspects* (eds Vizi E. S. and Magyar K.), pp. 65–73. Akadémiai Kiadó, Budapest.
367. Winkler H., Hörtnagl H., Schöpf A. J., Hörtnagl H. and Zur Nedden G. (1971) Bovine adrenal medulla: synthesis and secretion of radioactively labelled catecholamines and chromogranins. *Naunyn-Schmiedeberg's Arch. Pharmac.* **271,** 193–203.
368. Winkler H., Hörtnagl H., Hörtnagl H. and Smith A. D. (1970) Membranes of the adrenal medulla. Behaviour of insoluble proteins of chromaffin granules on gel electrophoresis. *Biochem. J.* **118,** 303–310.
369. Winkler H., Schöpf A. J., Hörtnagl H. and Hörtnagl H. (1972) Bovine adrenal medulla: subcellular distribution of newly synthesized catecholamines, nucleotides and chromogranins. *Naunyn-Schmiedeberg's Arch. Pharmac.* **273,** 43–61.
370. Winkler H., Schneider F. H., Ruefener C., Nakane P. K. and Hörtnagl H. (1974) Membranes of the adrenal medulla: their role in exocytosis. In *Advances in Cytopharmacology* (eds Ceccarelli B., Clementi F. and Meldolesi J.), pp. 127–139. Raven Press, New York.
371. Winkler H. and Westhead E. (1980) The molecular organization of adrenal chromaffin granules. *Neuroscience* **5,** 1803–1823.
372. Winkler H., Ziegler E. and Strieder N. (1966) Studies on the proteins from chromaffin granules of ox, horse and pig. *Nature* **211,** 982–983.
373. Wohlfarter T., Fischer-Colbrie R., Hogue-Angeletti R., Eiden L. E. and Winkler H. (1988) Processing of chromogranin A within chromaffin granules starts at C- and N-terminal cleavage sites. *Fedn. Eur. biochem. Socs Lett.* **231,** 67–70.
374. Wu H. J., Rozansky D. J., Parmer R. J., Gill B. M. and O'Connor D. T. (1991) Structure and function of the chromogranin A gene. Clues to evolution and tissue-specific expression. *J. biol. Chem.* **266,** 13,130–13,134.
375. Yoo S. H. and Albanesi J. P. (1990) Ca^{2+}-induced conformational change and aggregation of chromogranin A. *J. biol. Chem.* **265,** 14,414–14,421.
376. Yoo S. H., Albanesi J. P. and Jameson D. M. (1990) Fluorescence studies of nucleotide interactions with bovine adrenal chromogranin A. *Biochim. biophys. Acta* **1040,** 66–70.
377. Yoo S. H. and Albanesi J. P. (1991) High capacity, low affinity Ca^{2+} binding of chromogranin A. Relationship between the pH-induced conformational change and Ca^{2+} binding property. *J. biol. Chem.* **266,** 7740–7745.
378. Yoshie S., Hagn C., Ehrhart M., Fischer-Colbrie R., Grube D., Winkler H. and Gratzl M. (1987) Immunological characterization of chromogranins A and B and secretogranin II in the bovine pancreatic islet. *Histochemistry* **87,** 99–106.
379. Ziegler M. G., Kennedy B., Morrissey E. and O'Connor D. T. (1990) Norepinephrine clearance, chromogranin A and dopamine β hydroxylase in renal failure. *Kidney Int.* **37,** 1357–1362.

(*Accepted* 16 *March* 1992)