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REVIEW ARTICLE

Biochemistry of the chromogranin A protein family

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INTRODUCTION

The secretory granules of adrenal medullary chromaffin cells synthesize, store and then liberate catecholamines by exocytosis after cell stimulation with acetylcholine, itself liberated from the splanchnic nerve (for review, see Viveros, 1975).

Apart from adrenaline and noradrenaline, chromaffin granules contain a great number of molecules, essentially proteins, which are co-secreted with the catecholamines. The soluble acidic proteins of chromaffin granules have been collectively called chromogranins (Blaschko *et al.*, 1967). Chromogranin A (CGA) is the major protein, representing 40 % of the soluble protein of bovine adrenal medullary chromaffin granules (Winkler, 1976). Since it was first characterized about 20 years ago (Helle, 1966), CGA has been much studied. Its physicochemical properties have been analysed in detail. However, it was only in 1986 that two independent groups obtained for the first time the complete primary sequence of CGA of the bovine chromaffin granule (Benedum *et al.*, 1986; Iacangelo *et al.*, 1986). The recent discovery of a negative feedback control of insulin (Tatemoto *et al.*, 1987) and catecholamine (Simon *et al.*, 1988) release by peptide derivatives of CGA and the presence of an anti-CGA immunoreactivity in numerous endocrine and nervous tissues, support the concept that this protein might have a major function in the maintenance of physiological equilibria of organisms. This article summarizes the work of 20 years of research on CGA of bovine chromaffin cells and those of other species. Recent data showing that CGA is not restricted to chromaffin cells but is also present in numerous other endocrine and neuroendocrine cells will be analysed and discussed. Finally, the value of CGA to pathology in the diagnosis of certain types of neuroendocrine tumours will be mentioned.

CHROMOGRANIN A OF BOVINE ADRENAL MEDULLARY CHROMAFFIN CELLS

Structural properties

CGA of bovine chromaffin granules is a protein of molecular mass between 70 and 75 kDa, as shown by analytical ultracentrifugation (Smith & Winkler, 1967) or by SDS gel electrophoresis (Winkler, 1976). Determination of the primary sequence of CGA by Benedum *et al.* (1986) and Iacangelo *et al.* (1986) however permits the attribution of a real molecular mass of 48 kDa (Fig. 1). Taking into account post-translational modifications, this value corresponds to that (53 kDa) obtained by Kirshner (1974) using the equilibrium sedimentation method in the presence of guanidine hydrochloride. Thus, the molecular mass of CGA calculated from its primary sequence gives a value considerably less than that determined by polyacrylamide-gel electrophoresis in the presence of SDS. Translation of CGA mRNA *in vitro* gives a protein which migrates, under denaturing conditions, as a component of 75 kDa. CGA is thus a protein with a real molecular mass close to 50 kDa but which migrates in SDS gel electrophoresis with an apparent molecular mass of 75 kDa. This difference between apparent and real molecular mass of CGA might result from the weak binding of SDS to the protein, thus considerably reducing its migration rate.

Benedum *et al.* (1986) and Iacangelo *et al.* (1986) succeeded, thanks to molecular cloning techniques, in obtaining a cDNA clone to CGA. The cDNA clone hybridizes to a mRNA of 2100 bases. The total length of the mRNA, without taking into account its poly(A) tail, is 1881 bases according to Benedum *et al.* (1986) or 1929 bases according to Iacangelo *et al.* (1986). The open reading frame codes for a protein of 449 amino acids (Fig. 1). The first 18 amino acid residues constitute the signal sequence responsible for the targeting and the passage of the nascent polypeptide to and through the membrane of rough endoplasmic reticulum. After cleavage of the signal sequence, the CGA polypeptide chain comprises 431 amino acids, corresponding to an unmodified protein of 48 kDa.

The amino acid composition of the protein coded by the CGA cDNA clone is in agreement with that deduced from the cDNA nucleotide sequence. Its amino acid composition is unusual in that it has a high content (24.1 %) of acidic amino acids. These values are in perfect agreement with data on amino acid composition previously reported (for references, see Winkler, 1976). In addition, analysis of the CGA sequence does not support the data of Settleman *et al.* (1985*b*) on amino acid sequence homologies within the CGA molecule. While the glutamic acid residues are present all along the CGA sequence, they are preferentially grouped in several regions of the molecule. The high density of negative charges confers an acidic isoelectric pH of 4.5–5.0 on CGA. CGA is, on the other hand, a protein rich in proline (about 10 %) and cysteine is particularly rare: only three cysteine residues, localized in the first 60 amino acids, are present (Fig. 1).

In 1967, Smith & Winkler noted that the molecular mass of CGA estimated by gel filtration appeared much greater than that obtained by analytical ultracentrifugation. Under their conditions, the elution volume of the protein corresponded to a protein of 500 kDa. This

Abbreviations used: CGA, chromogranin A; CGAF, chromogranin A protein family; AChE, acetylcholinesterase.

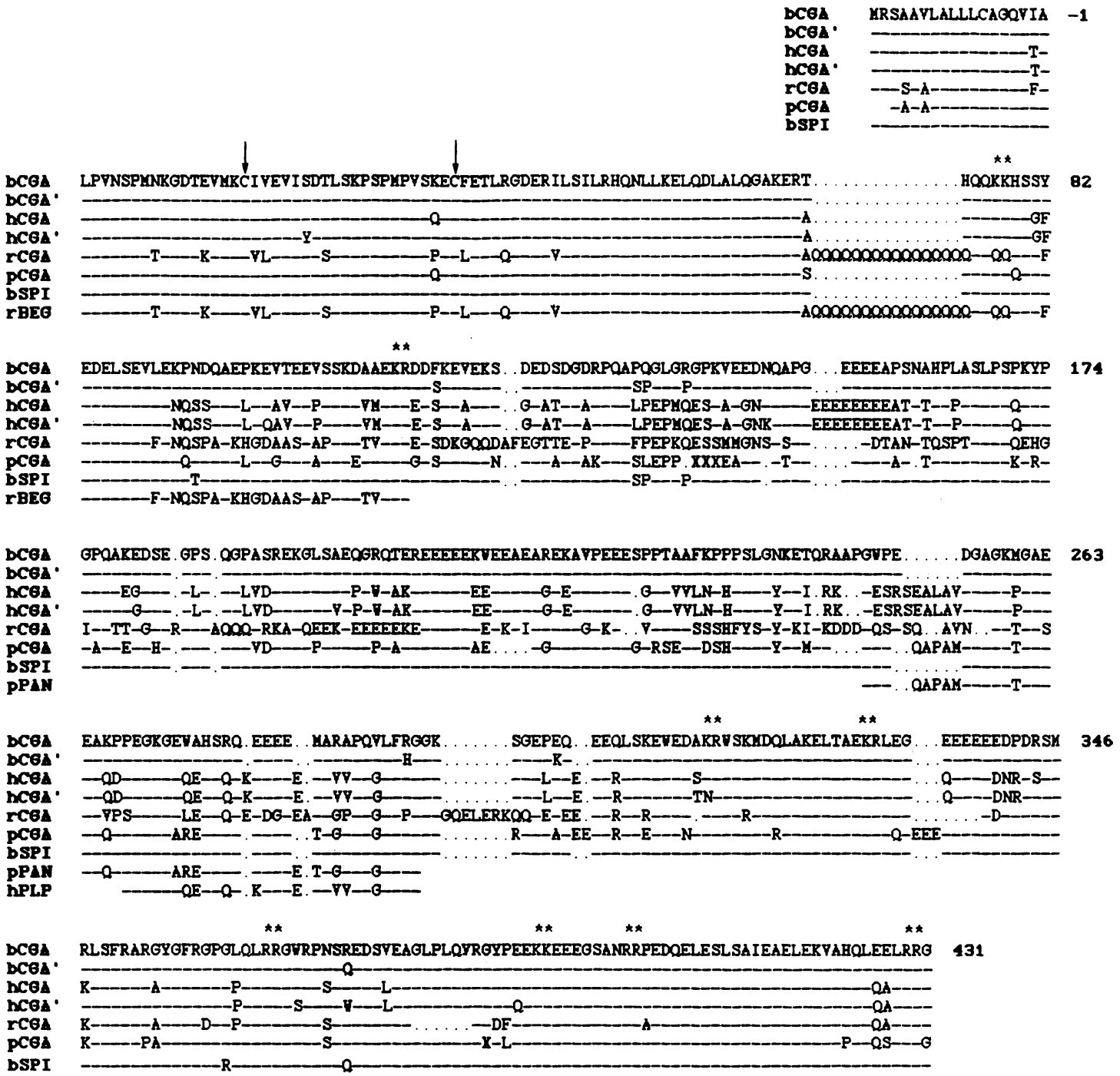

Fig. 1. Comparison of amino acid sequences of bovine, human, rat and porcine chromogranin A, bovine secretory protein I, porcine pancreastatin and human pancreastatin-like peptide

bCGA and bCGA′ are the bovine chromogranin A sequences as reported by Iacangelo *et al.* (1986) and by Benedum *et al.* (1986) respectively; hCGA and hCGA′ are the human chromogranin A sequences as reported by Konecki *et al.* (1987) and by Helman *et al.* (1988) respectively; rCGA, rat chromogranin A sequence (Iacangelo *et al.*, 1988*a*); pCGA, porcine chromogranin A sequence (Iacangelo *et al.*, 1988*b*); bSPI, bovine secretory protein I sequence (Ahn *et al.*, 1987); rBEG, rat *β*-granin sequence (Hutton *et al.*, 1988); pPAN, porcine pancreastatin sequence (Tatemoto *et al.*, 1987); hPLP, human pancreastatin-like peptide sequence (Sekiya *et al.*, 1988). Numbers on bCGA sequence indicate the number of amino acids; peptide signal sequences are shown separately at the top right of the Figure. All amino acids in bCGA sequence (used as reference) are given using the single letter code. Amino acids in other sequences which are identical to bCGA sequence are marked with dashes. Residues which are not identical are denoted with their respective letter. X in the sequence indicates some ambiguity. Dots have been introduced to obtain the best alignment. Asterisks on the bCGA sequence reveal potential dibasic cleavage sites. The two cysteine residues in the protein sequence are indicated by arrows, while a third cysteine residue (not labelled) is found in the peptide signal sequence.

unusual behaviour of CGA on gel filtration chromatography has recently been confirmed (Fischer-Colbrie & Frischenschlager, 1985; Fischer-Colbrie & Schober, 1987; Simon *et al.*, 1988); the protein is consistently eluted in fractions containing proteins of molecular masses several times higher (300–400 kDa) than that determined using other techniques. In fact, these results are explained by the special tertiary structure of CGA approaching that of a 'random coil polypeptide', an hypothesis that studies *in vitro* and *in vivo* by nuclear magnetic resonance have confirmed (Daniels *et al.*, 1978).

The relatively large spatial volume occupied by the molecule may be attributed to electrostatic repulsion of the numerous negative charges. The predicted secondary structure of CGA, based on the primary sequence, supports this model (Grimes *et al.*, 1987). It is possible that the molecule has a few α-helix-forming regions; however, the groups of amino acid residues that might form an α-helix structure are made up of many residues with the same charge (mostly glutamic acid) which would oppose the formation of a stable α-helix. In addition, the β-sheet percentage content in CGA molecule is very low. Analysis of the primary structure of CGA in terms of secondary and tertiary structures shows that this protein is not globular but possesses a disorganized structure termed 'random coil'.

CGA is a glycoprotein containing 5.4% carbohydrate (Kiang *et al.*, 1982; Apps *et al.*, 1985). Methylation and specific sugar degradation have shown the protein to be associated through *O*-glycosidic linkages with tri- and tetra-saccharides constituted of galactose, *N*-acetylgalactosamine and sialic acids. CGA does not seem to be *N*-glycosylated since tunicamycin, an *N*-glycosylation inhibitor, has no effect on sugar chain synthesis (Rosa *et al.*, 1985). CGA does not bind to concanavalin A nor other mannose- or galactose-specific lectins. However, treatment of CGA with neuraminidase enables the protein to bind galactose-specific but not mannose-specific lectins. Galactose is situated in the penultimate position in CGA sugar chains. As shown by the primary structure of CGA, potential *O*-glycosidic (serine and threonine residues) binding sites are present all along the molecule. It is thus impossible to predict which are *O*-glycosylated. On the other hand, studying the primary sequence reveals no possibility of *N*-glycosylation (Kornfeld & Kornfeld, 1985). These data are in agreement with biochemical analyses (Kiang *et al.*, 1982).

Settleman *et al.* (1985*b*) have shown that CGA is a phosphoprotein. Measurement of phosphorylated amino acids gives a ratio of five phosphorylated serine residues per molecule of CGA. No phosphothreonine or phosphotyrosine residues were detected. Immunological analysis of dephosphorylated CGA show that antisera recognize both dephosphorylated and native CGA forms equally well. Rosa *et al.* (1985) have studied CGA phosphorylation in isolated chromaffin cells and found incorporation essentially on serine residues with a weak incorporation on threonine residues. Tyrosine residues are not labelled. Cote *et al.* (1986) have shown that [^{32}P]phosphate incorporation into CGA is increased when chromaffin cells are stimulated by secretagogues. However, the significance of this result is still obscure.

The incorporation of [^{35}S]sulphate into CGA is relatively weak (Falkensammer *et al.*, 1985*a*), an observation confirmed by Rosa *et al.* (1985) who, in addition, showed that sulphate is bound to *O*-glycans and not to tyrosine residues as is frequently the case. Secreted proteins bear sulphated tyrosine residues when these are preceded mostly by acidic amino acids (Lee & Huttner, 1985). None of the four tyrosine residues of CGA are preceded by such amino acids.

The chromogranin A stored in the secretory granules of chromaffin cells maintained in primary culture, and subsequently secreted, is a carboxymethylated protein (Nguyen *et al.*, 1987). Since carboxymethylase is localized exclusively in the cytosol of chromaffin cells (Diliberto *et al.*, 1976), CGA methylation is a co-translational phenomenon. Bovine erythrocyte carboxymethylase incorporates 660 pmol of methyl groups per mg of CGA in 10 min. The stoichiometry of CGA methylation *in vitro* is 0.26 mol of methyl groups per mol of protein (Veeraragavan *et al.*, 1988). The biological significance of CGA methylation is at the moment unknown.

Binding capacity

From experiments with specific dyes for calcium-binding proteins and labelling with ^{45}Ca, CGA appears to be a 'calci-protein' (Reiffen & Gratzl, 1986*a*,*b*). CGA binds intragranular calcium with a weak affinity but with a high capacity (Reiffen & Gratzl, 1986*b*). The affinity constant (K_d) is 54 μM and the calcium-binding capacity is about 18 mol of calcium per mol of CGA using its real molecular mass. CGA–calcium binding is influenced by Mg^{2+}, pH and ionic strength (Gratzl, 1987). Analysis of the CGA primary sequence shows that this contains regions specific to calcium-binding proteins (Iacangelo *et al.*, 1986).

Electrostatic interactions between soluble acidic proteins and ATP have been observed by nuclear magnetic resonance in chromaffin granules (Daniels *et al.*, 1978; Sharp & Sen, 1979). Similar interactions exist between CGA and catecholamines (Uvnas & Aborg, 1977, 1980). However, the latter results should be treated with caution, and further data with purified CGA are necessary to confirm these conclusions.

Helle postulated that CGA is the major protein of the chromaffin granule membrane (Helle & Serck-Hanssen, 1969; Helle, 1971). In contrast, Winkler *et al.* (1970) found very little in their preparations of membrane granules. However, more recently Settleman *et al.* (1985*a*) have again raised the question of intragranular CGA localization by stating that CGA is not only a soluble protein but is also a constitutive protein of the granule membrane. Nevertheless, two observations tend to contradict their results: (1) CGA is found exclusively in the aqueous phase when membranes are treated with Triton-X114 while intrinsic membrane proteins such as dopamine β-hydroxylase or cytochrome b_{561} remain in the organic phase (Pryde & Phillips, 1986); (2) ultrastructural immunocytochemistry demonstrates that only the soluble component of chromaffin granules reacts with anti-CGA serum (Aunis *et al.*, 1980; see also Ehrhart *et al.*, 1986). It appears that the extraction and washing procedures employed by Settleman *et al.* (1985*a*) were inadequate to remove soluble CGA adsorbed to membranes during subcellular fractionation and granule lysis.

Apart from the signal sequence, CGA is an extremely hydrophilic protein. This hydrophilic nature enables the protein to remain soluble after boiling (Rosa *et al.*, 1985). Hydrophobicity tests have shown that CGA lacks significant hydrophobic regions.

Biosynthesis

To study CGA biosynthesis *in vitro*, mRNAs were isolated from bovine adrenal medulla and translated in a cell-free wheat germ system (Kilpatrick *et al.*, 1983; Falkensammer *et al.*, 1985*b*) or in rabbit reticulocyte lysates (Falkensammer *et al.*, 1985*b*). In both systems, two components are immunoprecipitated by anti-CGA sera. These two polypeptides of apparent molecular masses 73 kDa and 75 kDa have slightly different isoelectric pH values from endogenous CGA. In the presence of microsomes, these two precursors are converted to a protein of 72 kDa by removal of two signal sequences each with a different length. The relation between the two CGA precursor polypeptides is not yet established. The suggestion that these two precursors result from the initiation of the translation at different AUG codons of a single mRNA or two CGA mRNAs has not been confirmed by data on the translation *in vitro* of the mRNA of 2100 bases (Benedum *et al.*, 1986; Iacangelo *et al.*, 1986). Degradation of mRNAs or their products during translation *in vitro* may be responsible for the presence of the two precursors. However the results of Kilpatrick *et al.* (1983) and Falkensammer *et al.* (1985*b*) do not confirm those of Serck-Hanssen & O'Connor (1984). In fact, these authors consider that a large number of CGA precursors are produced *in vitro*. It would appear that the detection of numerous precursors results from employing a non-specific anti-CGA serum which immunoprecipitates other non-related proteins as well as CGA. Falkensammer *et al.* (1985*b*) have described CGA synthesis in freshly isolated chromaffin cells. CGA thus obtained is a single protein of molecular mass 72 kDa and isoelectric pH 5.2. During prolonged incubation, this labelled protein becomes larger and more acidic (molecular mass 75 kDa; isolectric pH 5.0). These changes probably result from post-translational modifications.

Eiden *et al.* (1987*a*) developed a radioimmunoassay to quantify CGA levels in chromaffin cells in primary culture. Nicotine stimulation of chromaffin cells provokes the liberation of catecholamines and CGA into the external medium. When CGA levels are measured in cells and in the culture medium after nicotine treatment for 72 h, it is noted that CGA release is not compensated by an increase of CGA biosynthesis; the result is a clear-cut reduction of intracellular CGA. CGA has also been measured in cells treated with forskolin for 72 h. Forskolin, which produces a rise in intracellular cyclic AMP, provokes CGA secretion that is not accompanied by a compensatory increase in intracellular levels of CGA. These results seem to indicate that for CGA in cultured chromaffin cells, no coupling exists between stimulus, secretion and synthesis. However, the biosynthetic regulation of CGA in chromaffin cells in primary culture has been recently examined (Simon *et al.*, 1989) by measuring CGA synthesis after radioactive amino acid incorporation and subsequent immunoprecipitation. It appears that in fact the rate of CGA synthesis is directly related to the secretory activity of the cells. Cellular stimulation by secretagogues activates CGA synthesis. This activation is calcium-dependent and it is known that the calcium is derived from the external medium (Artalejo *et al.*, 1987). It may be concluded that the decrease of intracellular CGA level due to secretion is compensated by an increased synthesis in order, so it seems, to maintain constant intracellular CGA levels. Such conclusions disagree with those of Eiden and co-workers, but, since CGA is easily degraded in the extracellular medium (Simon *et al.*, 1988), the technique employed by Eiden and co-workers may in fact underestimate the amount of released CGA.

While forskolin does not alter the synthetic rate of CGA (Eiden *et al.*, 1987*a*; Simon *et al.*, 1989), 12-*O*-tetradecanoylphorbol 13-acetate (TPA), a protein kinase C activator, increases it significantly (Simon *et al.*, 1989). The increase in CGA synthesis induced by phorbol ester probably results from protein kinase C activation. This conclusion is supported by the observation that sphingosine, a protein kinase C inhibitor, prevents the increase in CGA synthesis provoked by nicotine stimulation of cells.

Thus, the synthesis of CGA of bovine chromaffin granules appears to be regulated, at least in part, by protein kinase C, though the intracellular message has not yet been identified. Since CGA mRNA levels remain constant in primary chromaffin cell cultures, even after cell stimulation (Waschek *et al.*, 1987), protein kinase C might regulate CGA synthesis in normal cells at a translational step.

Evidence for and characterization of a family of chromogranin A

Anti-CGA sera, obtained by injecting animals with native CGA purified from secretory granules, react not only with this molecular form but also with other proteins and polypeptides which are also present in secretory granules (Fig. 2). This observation was described for the first time by Hortnagl *et al.* in 1974.

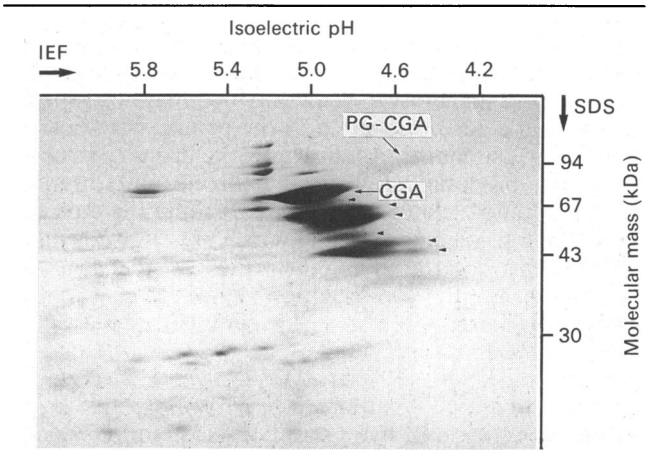

Fig. 2. Two-dimensional gel electrophoretogram of soluble proteins of bovine chromaffin granule lysate

IEF, isoelectrofocusing in the first dimension; SDS, sodium dodecyl sulphate slab gel electrophoresis of focused proteins on 8–15% polyacrylamide gel in the second dimension. pH of isoelectric focusing gel (abscissa) and position of protein marker (ordinate) are indicated; 1 mg of protein from chromaffin granule matrix was deposited on the IEF gel. Slab gel was stained with Coomassie Brilliant Blue to visualize proteins. Chromogranin A (CGA) and proteoglycan–chromogranin A (PG-CGA) are indicated. Arrowheads indicate endogenous CGA breakdown products immunoreactive with an antiserum directed against native 74 kDa CGA molecule (Ehrhart *et al.*, 1986; Grube *et al.*, 1986; Eiden *et al.*, 1987*a*; Simon *et al.*, 1988).

A diffuse component with a heterogenous pI (4.0–5.0) and high apparent molecular mass (85–100 kDa) is specifically recognized by anti-CGA antibodies (Kilpatrick *et al.*, 1983; Rosa *et al.*, 1985; J.-P. Simon & D. Aunis, unpublished work: Fig. 2). This component is actually a proteoglycan (proteoglycan-CGA; for proposed nomenclature see Eiden *et al.*, 1987*b*), the nature of which has been elucidated by radioactive labelling and selective digestion with chondroitinase ABC. The glycan chain consists of chondroitin 4,6-sulphate and dermatan sulphate which represent respectively about 25 and 50% of the total carbohydrates (Kiang *et al.*, 1982). Little is known about the core protein of this proteoglycan. The amino acid composition is similar to that of CGA (Kiang *et al.*, 1982) though the peptide maps are quite different (Banerjee & Margolis, 1982) suggesting that the proteoglycan protein core is structurally distinct from CGA in spite of certain similarities of the two molecules. The function of the proteoglycan–CGA is at present unknown, although an intragranular role as a condensing factor might be suggested.

Anti-CGA sera recognize proteins of molecular mass lower than that of CGA. The various antisera recognize between five and nine molecular species of molecular species of molecular masses respectively between 43 and 74 kDa (Simon *et al.*, 1988) and between 17.4 and 75 kDa (Kilpatrick *et al.*, 1983; Fischer-Colbrie & Frischenschlager, 1985). This diversity of results can be explained by the variability of epitopes recognized by each antiserum. All proteins recognized by anti-CGA antibodies are characterized by very similar isoelectric points; however, CGA-generated peptides with molecular mass lower than 17.4 kDa do not cross-react with anti-CGA antibodies due to the loss of epitopes. In 1976, Winkler suggested that polypeptides recognized specifically by anti-CGA antibodies are degradation products of CGA, which are present *in vivo* in chromaffin granules. Results of studies on CGA biosynthesis *in vitro* have refuted the suggestion that these species derive from the same gene family. CGA represents about 53% of total CGA immunoreactive protein of the chromaffin granule (Hagn *et al.*, 1986*b*; Simon *et al.*, 1988). When the anti-CGA immunoreactivity of various fractions obtained by centrifugation of a crude granule preparation on a 1.0–2.2 M continuous sucrose gradient (J. P. Simon & D. Aunis, unpublished work) is tested, a homogeneous distribution of CGA and its degradation products is found in all fractions. This result appears to indicate that CGA is co-stored with its degradation products and that immunoreactive species are present in the same ratios in both immature and mature granules. The CGA molecules would thus appear to be cleaved early *in vivo*. The same ratio is, in addition, found for the extracellular fraction after cell stimulation (J. P. Simon & D. Aunis, unpublished work). Studies of cellular CGA synthesis appear, however, to indicate that the moelcule is not processed proteolytically immediately after its synthesis. In fact, after a 2 h chase period, degradation products resulting from newly synthesized CGA cannot be detected in granules (Falkensammer *et al.*, 1985*b*). Most recent data appear to show that CGA is still undegraded 18–20 h after its biosynthesis (Phillips, 1987; Simon *et al.*, 1989). Apparently endogenous CGA degradation is a slow process. CGA appears to be continually degraded into polypeptides of lower molecular mass until it is secreted, although the mechanisms which control CGA degradation are not known at the present time.

Examination of the sequence of CGA reveals the presence of eight pairs of basic amino acids, six of which are found in the *C*-terminal region of the molecule (Benedum *et al.*, 1986; Iacangelo *et al.*, 1986). Each pair of basic amino acids represents a potential cleavage site for trypsin-like endoproteases (Fig. 1). The endogenous CGA degradation products might arise from cleavage of the molecule at such sites. Given the random coil conformation of the protein and its degradation products, it is difficult to compare the sizes of endogenous immunoreactive proteins and polypeptides with those predicted by the sequence of CGA. Nevertheless, a relatively close correlation exists between native CGA and theoretically possible polypeptides and those found *in vivo*, on the basis that CGA is cleaved sequentially at dibasic amino acid sites starting from the *C*-terminal end (Fischer-Colbrie *et al.*, 1987).

The production of antibodies directed against synthetic CGA peptides should afford unambiguous conclusions on whether dibasic amino acid pairs are really the cleavage sites *in vivo*. Other types of cleavage sites cannot be excluded, in particular those close to serine residues. In fact, Wohlfarter *et al.* (1988) have shown with sera directed against synthetic peptides present in the bovine CGA sequence that intragranular degradation of native CGA into sub-species could start at cleavage sites at either the *N*-terminal or *C*-terminal ends.

It is reasonable to suppose that native CGA is degraded in the chromaffin granule; this degradation should be due to specific proteases present in the chromaffin granule matrix. However, very little is known at the moment on the nature of these proteases. The presence of several enzymes with peptidase activity has been demonstrated in chromaffin granules. These enzymes are involved in the process of transforming enkephalin precursors (proenkephalins) into enkephalins. These are essentially endopeptidases, including the serine proteases (Linberg *et al.*, 1984), with trypsin-like activity (Evangelista *et al.*, 1982) and exopeptidases with carboxypeptidase activity (Hook *et al.*, 1982; Fricker & Snyder, 1982). The involvement of such intragranular enzymes in CGA degradation and the formation of the CGA family are not yet established.

An enzyme likely to cleave CGA has been recently identified: it is acetylcholinesterase (AChE; EC 3.1.1.7). Its activity is detectable in the matrix and the membrane of chromaffin granules (Somogyi *et al.*, 1975; Gratzl *et al.*, 1981; Burgun *et al.*, 1986). Recent data have shown that, apart from its esterase activity, AChE has the capacity to hydrolyse peptide bonds (Chubb *et al.*, 1980, 1983; Chubb & Millar, 1984; Ismael *et al.*, 1986). Small *et al.* (1986) demonstrated that AChE from electric eel can cleave CGA into two polypeptides of molecular masses identical to those of the endogenous breakdown products of CGA. These authors showed, in addition, that trypsin acts in the same way, suggesting that AChE could have a peptidase activity similar to that of trypsin. The sensitivity of AChE and trypsin to certain peptidase inhibitors differ greatly. However the presence of AChE activity in chromaffin granules is contested by certain authors (for discussion, see Winkler *et al.*, 1986); the characterization of granular AChE should be further studied to validate the hypothesis concerning its role in proteolytic degradation of CGA. Settleman *et al.* (1985*b*) have reported preliminary data indicating that CGA is

degraded in chromaffin granules by a calcium-dependent mechanism, but unfortunately this result has not been confirmed. Three hypotheses may be advanced on the timing of the appearance of CGA breakdown products: (1) CGA is degraded immediately after its synthesis by proteases not necessarily present in chromaffin granule; (2) CGA is degraded in immature granules by intragranular proteolytic enzymes, the activity of which might depend on intra- or extragranular factors yet to be characterized; (3) CGA is continually degraded from the time of its synthesis to its liberation into the extracellular medium by a slow process. Only detailed studies in which the appearance of CGA breakdown products are analysed in conjunction with the biogenesis of the chromaffin granule will confirm one or other of the hypotheses.

Functions of chromogranin A

The soluble compartment of each mature secretory granule contains more than a dozen different molecular species (Winkler & Westhead, 1980) including CGA (5000 molecules per granule), nucleotide triphosphates (930000 molecules of ATP) and catecholamines (3000000 molecules). In spite of hyperosmotic concentrations of hydrophilic molecules, the chromaffin granule is a system in osmotic equilibrium with its surrounding cytoplasm (Blaschko *et al.*, 1956).

What is the mechanism by which this iso-osmolarity is maintained? Helle *et al.* (1985) postulated that the CGA family contributes to the regulation of osmotic pressure in chromaffin granules by interacting with other intragranular constituents. The experiments reported by these authors were performed by directly measuring the osmotic pressure exerted by the soluble phase of the granule. Since no study has been performed with purified CGA or the CGA family, it would be premature to suggest that CGA and its degradation products control the osmotic equilibrium between the granule and the cytosol. Nevertheless, even if CGA has no specific role in binding catecholamines, it might have a function in stabilizing granule contents, as suggested by Sharp & Sen (1979).

CGA is able to bind calcium (Reiffen & Gratzl, 1986*a*,*b*). This affinity for calcium might be partly responsible for the low amounts of free calcium in the chromaffin granule (4 mM), even though the total intragranular calcium concentration is 40 mM. Chromaffin granules could play an important role in decreasing cytosolic calcium (Kostron *et al.*, 1977). Stimulation of bovine chromaffin cells with secretagogues provokes a massive entry of extracellular calcium into the cell interior (Kao & Schneider, 1986; Artalejo *et al.*, 1987). After stimulation, the intracellular free calcium concentration very quickly returns to basal levels and chromaffin granules may be in part responsible for the rapid sequestration of cytosolic calcium. Intragranular binding of calcium might be assured by CGA.

Recently, data have been reported on a role of CGA as a prohormone. It appears that peptides resulting from the breakdown of native CGA are able to inhibit the secretion of chromaffin cells in primary culture (Simon *et al.*, 1988). CGA and its degradatin products were purified from the soluble fraction of chromaffin granules. This CGA family (CGAF) was found to inhibit nicotine-induced secretion, but only if cells had been preincubated with CGAF for about 60 min. The fact that preincubation is a prerequisite for the inhibitory capacity of the CGAF and the observation that the ratio between CGA and its immunoreactive degradation products is modified during this preincubation step suggest that the CGAF-derived peptides rather than the CGAF are involved in inhibiting secretion. This has been unambiguously demonstrated with peptides obtained by trypsinolysis of CGAF, since such resultant peptides inhibit secretion without the necessity for the preincubation step. At the present time, neither the nature nor the metabolism of this or these peptides, nor the nature of the enzymes responsible for the formation of these peptides are known. The active peptide or peptides may be formed by CGA cleavage at dibasic sites but this awaits proof. Thus CGA of the chromaffin granule would be a prohormone, a precursor of one or several peptides yet to be characterized which would play a regulatory role in catecholamine secretion, and thus in perturbed homeostasis in stress situations.

CHROMOGRANIN A OF THE ADRENAL MEDULLA OF OTHER SPECIES

Antibodies against CGA of bovine adrenal medullary chromaffin granules can be used to demonstrate the strict localization of CGA in the granules of bovine chromaffin cells (Fig. 3). These antibodies have also been shown to detect CGA of chromaffin granules of other species; CGA appears to be a molecule the structure of which has been highly conserved during evolution, a fact confirmed by studies of its sequence.

Chromogranin A of human chromaffin cells

CGA of human adrenal medullary chromaffin granules has been characterized by immunoblots (Lloyd & Wilson, 1983; Hagn *et al.*, 1986*a*; Schober *et al.*, 1987). It migrates as a doublet of apparent molecular masses 76 and 78 kDa, and a component with a slightly lower molecular mass also reacts with anti-CGA sera. It appears that CGA of human chromaffin granules is not degraded to the same extent as that of bovine adrenal chromaffin granules. Partial (Kruggel *et al.*, 1985) and total (Konecki *et al.*, 1987) primary sequences of CGA from human pheochromocytomas (a tumour of the adrenal medulla) are identical. Human CGA is a protein of 439 amino acids (Fig. 1) with a real molecular mass of 49 kDa. The mRNA codes for a molecule of 457 amino acids, the first 18 amino acids constituting the signal sequence. Comparison of its sequence with that of bovine chromaffin cell CGA reveals that the *N*-terminal domains are highly conserved between the two species; the eight dibasic amino acid pairs, potential cleavage sites of the protein, are also present. 83% homology exists between the mRNA nucleotide sequences coding for human and bovine CGA. The middle portion of the human protein shows remarkable sequence variation (36%) with regard to the bovine protein. These results have been confirmed by Helman *et al.* (1988) (see Fig. 1). The nucleotide sequence of human cDNA as reported by Helman *et al.* (1988) is 86% identical with that of bovine CGA cDNA. The sequence determined by Konecki *et al.* (1987) differs by 2% from that reported by Helman *et al.* (1988).

Human pheochromocytomas contain two mRNAs that hybridize with CGA cDNA probes. The major mRNA (2100 bases) is identical with that of bovine chromaffin cells. The second mRNA detected by the cDNA probe comprises 1600 bases; its role is unknown.

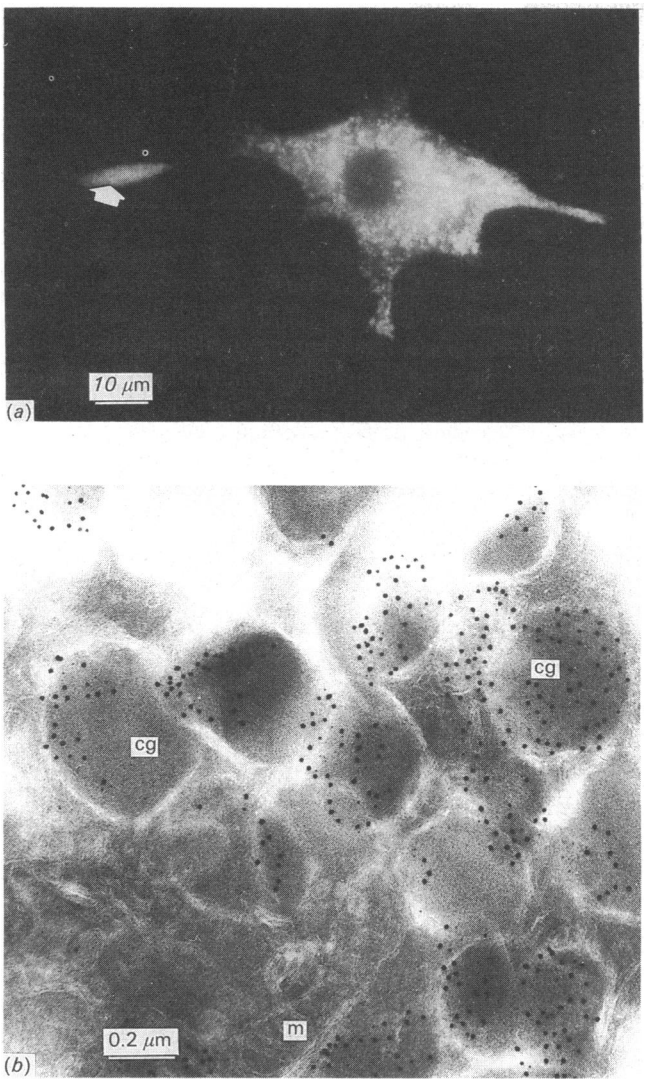

Fig. 3. Immunocytochemical localization of chromogranin A in cultured bovine chromaffin cells

(*a*) Immunochemical staining by specific anti-CGA antibody of bovine adrenal chromaffin cells maintained in culture. Anti-CGA antibody was revealed with fluorescein-coupled sheep anti-rabbit immunoglobulins. Note the granular pattern of staining within the cytoplasm and the intense fluorescence in extension tip (arrow). (*b*) Ultrathin frozen sections from isolated cultured chromaffin cells were labelled with anti-CGA serum and 12-nm gold-conjugated protein A (see Patzak *et al.*, 1987). Chromogranin A was found in electron-dense granules (cg) but not in mitochondria (m).

Recently, the human CGA gene has been localized on chromosome 14 (Murray *et al.*, 1987). The CGA gene is probably a single copy and not a member of a dispersed multigene family. These results have been partly confirmed by the studies of Konecki *et al.* (1987) who showed that a single CGA gene with intron sequences exists in the haploid genome. It is of interest to note that the CGA gene does not seem to be co-localized with the genes of the hormones associated with CGA in secretory granules (Murray *et al.*, 1987).

Chromogranin A of rat chromaffin cells

The CGA of rat chromaffin cells is not the major protein of secretory granules. The protein has a higher apparent molecular mass (80 kDa) than that of the bovine chromaffin cell CGA (Hutton *et al.*, 1985; Rosa *et al.*, 1985). Fischer-Colbrie & Schober (1987) have shown that CGA isolated from rat pheochromocytomas has a molecular mass of 86 kDa.

Iacangelo *et al.* (1988*a*) established the primary structure of rat pre-CGA from the nucleotide sequence of an adrenal medullary cDNA clone (Fig. 1). This cDNA encodes for a protein of 466 amino acids (including the hydrophobic signal sequence of 18 amino acids which is 83% homologous with the corresponding bovine signal peptide). The actual molecular mass of the protein, such as can be deduced from its cDNA, is 50.2 kDa. Comparison of the primary sequences of rat and bovine CGA reveals similar features. Pairs of dibasic amino acids are present in both proteins, seven of the ten pairs having identical positions. The *C*- and *N*-terminal ends are highly conserved in the two molecules. Rat CGA possesses two potential *N*-linked glycosylation sites.

CGA levels in rat adrenal medullas have been determined *in vivo* after nervous stimulation (Sietzen *et al.*, 1987). When rats are treated with insulin or reserpine, CGA levels remain slightly below control levels. After hypophysectomy, CGA is reduced to low levels, and this is not compensated by new synthesis. This observation appears to be confirmed by measurements of CGA mRNA levels. Splanchnic nerve activation by insulin treatment *in vivo* has no effect on CGA mRNA levels, but in contrast, a significant reduction is observed after hypophysectomy. These data would tend to suggest that glycocorticoids regulate the expression of CGA in rat chromaffin cells at a pre-translational step (Fischer-Colbrie *et al.*, 1988; Iacangelo *et al.*, 1988*a*).

Chromogranin A in chromaffin cells of other vertebrates

Rieker *et al.* (1988) showed that chromaffin cells or tissues from sheep, pig, horse, guinea-pig, chicken, mouse and from frog contain one or several proteins recognized by antibodies against bovine CGA. However, variations in the immunostaining patterns are observed in all species investigated. Analysis by immunoblots reveals a strongly immunoreactive band of 75 kDa (sometimes a doublet) corresponding to native CGA. In contrast, the profile and staining intensity of endogenous degradation products significantly vary from one species to another. A band that could correspond to proteoglycan-CGA is detected in some but not all species. The process of CGA degradation seems to exist in most species studied and its significance is probably the same as that proposed for bovine CGA.

CHROMOGRANIN A IN OTHER TISSUES

Recent studies have shown that the secretory vesicles of a large variety of cells, namely endocrine and nerve cells, contain proteins similar to CGA. The protein has been detected in these tissues (Tables 1 and 2) by immunohistochemistry, using polyclonal or monoclonal antibodies in general produced against CGA from bovine adrenal medullary chromaffin granules. The use of CGA cDNA probes has also enabled the cells producing the protein to be identified. Many contradictory results have

Table 1. Chromogranin A immunoreactivity in normal and tumoral endocrine tissues

+, positive; −, negative.

Normal endocrine tissues		Endocrine tumours	
Adrenal medulla	+	Pheochromocytoma	+
Parathyroid gland		Parathyroid adenoma	+
Chief cells			
Human	+		
Bovine	+		
Thyroid gland		Medullary carcinoma of thyroid	+
Thyroid C cells			
Human	+		
Bovine	+,−		
Rat	+,−		
Thyroid follicular cells			
Human	+		
Endocrine pancreas		Insulinoma	+
Glucagon cells			
Human	+		
Bovine	+		
Cat	+		
Rat	+		
Insulin cells			
Human	+,−		
Bovine	+		
Cat	+		
Rat	−		
Somatostatin cells			
Human	−		
Bovine	+		
Cat	+		
Rat	−		
Pancreatic polypeptide cells			
Human	+,−		
Bovine	+,−		
Cat	−		
Rat	−		
Pituitary			
Anterior lobe			
Gonadotroph cells			
Human	+		
Bovine	+		
Ovine	+		
Rat	+		
Thyrotroph cells			
Human	+		
Bovine	+		
Ovine	+		
Rat	+		
Somatotroph cells			
Human	−		
Bovine	+		
Ovine	+,−		
Rat	−		
Corticotroph cells			
Human	−		
Bovine	−		
Ovine	+,−		
Rat	−		
Mammotroph cells			
Human	−		
Bovine	−		
Ovine	−		
Rat	−		
Intermediate lobe			
Human	−		
Bovine	+		
Ovine	+		
Rat	−		
Posterior lobe			
Human	−		
Bovine	−		
Ovine	−		
Rat	−		
Gut		Intestine carcinoid	+
Enterochromaffin cells			
Human	+		
Bovine	+		
Gastric EC-like cells			
Human	+		
Bovine	+		
Pyloric gastrin G			
Human	+		
Bovine	+		
Glicentin/PPY L cells			
Bovine	−		
Pyloric D cells			
Bovine	−		

Table 2. Chromogranin A immunoreactivity in nervous tissues, immune system, lung and Merkel cells

+, positive; −, negative.

Nervous tissues		Other tissues and cells	
Brain		Immune system	
Telecephalon		Spleen	
Cerebral cortex		Bovine	+,−
Bovine	+	Rat	+
Sheep	+	Thymus	
Hippocampus		Bovine	+
Bovine	+	Rat	+
Sheep	+	Lymph node	
Rat	+	Rat	+
Basal ganglia		Merkel cell	
Bovine	+	Porcine	+
Sheep	+	Cat	+
Claustrum		Mouse	−
Bovine	−	Guinea pig	−
Sheep	−	Lung	
Septum		Human	+
Sheep	+	Monkey	+
Amygdala		Porcine	+
Sheep	+	Rat	−
Diencephalon		Rabbit	−
Thalamus			
Sheep	+		
Hypothalamus			
Sheep	+,−		
Mesencephalon			
Sheep	+		
Pons and dorsal tegmentum			
Locus coeruleus			
Sheep	+		
Cerebellum			
Sheep	+		
Bovine	+		
Sympathetic ganglion cells			
Bovine	+		
Rat	+		
Splenic nerve			
Bovine	+		

been published however on the presence of CGA in other tissues and cells. Three major methodological problems should be considered: (1) tissue fixation appears to be critical for the conservation of epitopes recognized by anti-CGA antibodies; (2) the anti-CGA specificity of the immune serum employed is of utmost importance; (3) when the molecular mass of an antigen recognized by anti-CGA is estimated, special precautions should be taken to avoid proteolytic degradation, since CGA is particularly sensitive to proteolysis due to its random coil structure.

Chromogranin A of endocrine tissues

Parathyroid gland. A protein called SP-I (secretory protein-I), which is found in large amounts in secretory granules of the parathyroid gland, is recognized by antibodies directed against CGA: in parallel, anti SP-I antibodies react with CGA (Cohn *et al.*, 1982). The presence of anti-CGA immunoreactivity in parathyroid granules has subsequently been confirmed by other groups (Nolan *et al.*, 1985; Lassmann *et al.*, 1986; Hearn, 1987). Purified SP-I from bovine parathyroids consists of at least two glycoproteins of nearly similar size (72 and 70 kDa) The difference in size might be accounted for by their carbohydrate composition. SP-I contains about 30% acidic amino acid residues and has an isoelectric pH of 4.5. Comparison of the peptides produced from bovine SP-I and CGA by chemical and enzymic hydrolysis confirms the homology of these two proteins (Hamilton *et al.*, 1986).

Ahn *et al.* (1987), who have established the primary structure of bovine SP-I (Fig. 1), suggested that the same gene might code for both SP-I and CGA, since a mRNA of 2100 bases is detected by hybridization with a CGA cDNA probe in both parathyroid tissue and the adrenal medulla. It seems clear now that nucleotide sequences of SP-I cDNA and CGA cDNA are nearly identical.

Very recently, it has been shown that the protein SP-I is a competitive inhibitor of an enzyme called IRCM-serine protease 1, isolated from porcine pituitary anterior and intermediate lobes. This enzyme can cleave peptidic molecules (e.g. hormones) at their pairs of basic amino acids (Seidah *et al.*, 1987). These authors suggest that the protein SP-I might play an important role as a regulator of post-translational processing of prohormones.

Thyroid gland. CGA has been detected in C cells of both the thyroid gland and in tumours derived from them (O'Connor *et al.*, 1983*a*; Cohn *et al.*, 1984; Rindi *et al.*, 1986; O'Connor & Deftos, 1987; Hearn, 1987; Schmid *et al.*, 1987), although other studies have not confirmed these observations (Fischer-Colbrie *et al.*, 1985; Lassmann *et al.*, 1986). CGA appears to be localized in regions containing calcitonin-positive cells. CGA purified from C cells of human tumours has a molecular mass of 78 kDa. Deftos *et al.* (1986) isolated from human thyroid carcinomas a mRNA of about 2300 bases which codes for a 72 kDa protein immunoreactive with anti-CGA antibodies.

Endocrine pancreas. Anti-CGA immunoreactivity has been demonstrated in endocrine pancreas by numerous authors (O'Connor *et al.*, 1983*a*; Cohn *et al.*, 1984; Wilson & Lloyd, 1984; Varndell *et al.*, 1985). However, contradictory results have been published on the types of endocrine cells containing CGA. The data of Ehrhart *et al.* (1986) considerably clarify the previous results. These authors analysed in detail the cellular and subcellular distribution of CGA in bovine pancreatic islets of Langerhans. Anti-CGA immunoreactivity was detected in cells producing insulin, glucagon and somatostatin. Ultrastructural studies using an immunogold technique show that CGA-like immunoreactivity was confined exclusively to hormone-containing secretory granules. However, while the hormones are principally localized in the central part of vesicles, CGA is found essentially at the periphery (Ehrhart *et al.*, 1986). This localization, which is peculiar to CGA, has also been observed in human pancreatic islets (Hearn, 1987), but its significance is not clear. Studies on the localization of CGA in the endocrine pancreas of a dozen mammalian species demonstrate that, apart from a few exceptions, all endocrine pancreatic cells of these mammals are immunoreactive (Grube *et al.*, 1986). The A cells are the most immunoreactive; nevertheless, each species has its own pattern of CGA-immunoreactive cell types.

Analysis of the CGA of bovine endocrine pancreas by electrophoresis shows that the major immunoreactive band migrates identically to bovine adrenal medullary CGA (Yoshie *et al.*, 1987). Immunoblotting also reveals the presence of an additional species migrating more slowly than the 75 kDa CGA (proteoglycan–CGA). Besides this, an immunoreactive species with a lower molecular mass than CGA is observed, which is probably a degradation product. These data are in good agreement with results obtained on human pancreas (Hagn *et al.*, 1986*a*), but disagree with those of Hutton *et al.* (1985, 1987*a*,*b*) on rat pancreas. These authors isolated from both normal rat pancreas and insulinomas a 20 kDa protein (called β-granin) which is strongly recognized by antibodies against bovine chromaffin granule CGA and which is co-localized with insulin in rat pancreatic B cells. The sequence of β-granin has been determined (Fig. 1) and it was found to be identical to the *N*-terminal sequence of rat adrenal medulla CGA (Iacangelo *et al.*, 1988*a*; Hutton *et al.*, 1988). It is probable that β-granin is a proteolytic fragment of CGA solely expressed in rat endocrine pancreas. 'Pulse-chase' experiments in rat insulinomas show that β-granin derives from a precursor which has been found to have a molecular mass (80–100 kDa), peptide map and immunoreactivity identical to those of the native CGA in rat adrenal medulla. This precursor appears to be rapidly converted soon after its passage through Golgi apparatus into a stable 20 kDa protein after initial cleavage by a calcium-dependent endoprotease at one of its pairs of basic amino acids. The precursor is not a stable constituent of rat endocrine pancreatic cells; rather β-granin, which is more stable, seems to be the major component of secretory granules of rat insulinomas (Hutton *et al.*, 1987*a*,*b*). The cDNA corresponding to the mRNA coding for the precursor form of rat insulinoma β-granin has a sequence identical to that of rat adrenal medulla CGA (Hutton *et al.*, 1988).

Recently, Tatemoto *et al.* (1987) purified a peptide of 49 amino acids from porcine endocrine pancreas, called pancreastatin. The primary structure of porcine CGA, deduced from the sequence of an adrenal medulla CGA cDNA clone (Fig. 1) shows that CGA is the potential precursor of porcine pancreastatin (Iacangelo *et al.*, 1988*b*). The CGAs of rat (Hutton *et al.*, 1988; Iacangelo *et al.*, 1988*a*), ox (Eiden, 1987) and man (Konecki *et al.*, 1987; Helman *et al.*, 1988) contain a sequence which according to species, is 59–71 % identical with that of porcine pancreastatin. The observed differences are due to species variations, thus strongly suggesting that pancreastatin could be derived from CGA itself rather than a similar protein.

The primary structure of a peptide found in certain human endocrine pancreatic tumours (pancreatic glucagonoma) has been determined after its identification by a specific antibody raised against porcine pancreastatin. The sequence of this human 28 amino-acid pancreastatin-like peptide (Fig. 1) is 75 % homologous with that of porcine pancreastatin (Sekiya *et al.*, 1988).

Pancreastatin inhibits the rapid phase of insulin release when the rat pancreas is stimulated with glucose (Tatemoto *et al.*, 1987; Efendic *et al.*, 1987); it also seems capable of inhibiting acid secretion from rat gastric parietal cells (Delvalle *et al.*, 1987). This result supports the idea that CGA might be the precursor of a group of peptides regulating the activity of certain endocrine cells (see also Simon *et al.*, 1988).

Anterior and intermediate lobes of the pituitary gland. CGA is present only in the anterior and intermediate lobes of the pituitary (O'Connor *et al.*, 1983*b*; Somogyi *et al.*, 1984; Lassmann *et al.*, 1986). A detailed study of the anterior lobe of several species (ox, sheep, rat, human) reveals the presence of a CGA-like protein in gonadotropin- and thyrotropin-producing cells. Somatotropin-secreting cells are weakly stained in the ox and sheep, but not at all in man and rat (O'Connor *et al.*, 1983*b*; Cohn *et al.*, 1984; Lloyd *et al.*, 1985; Rundle *et al.*, 1986). The pituitary protein recognized by antiserum against chromaffin cell CGA has a molecular mass and an isoelectric pH identical to those of the adrenal medulla CGAs of the species concerned (Somogyi *et al.*, 1984; Rosa *et al.*, 1985; Hagn *et al.*, 1986*a*), although O'Connor (1983) and Nolan *et al.* (1985) reported different data. The nucleotide sequences of the CGA cDNAs from the adrenal medulla and pituitary are quasi-identical and analysis of genomic DNA suggests that pituitary and adrenal CGA are products of the same gene (Ahn *et al.*, 1987).

Endocrine cells of the digestive tube. A large number of endocrine cells of the gastrointestinal tract immunoreact with anti-CGA antibodies (Varndell *et al.*, 1985; Facer

et al., 1985; Rindi *et al.*, 1986; Grube *et al.*, 1987; Hearn, 1987; Qian *et al.*, 1988). The enterochromaffin cells of the small intestine are very intensely immunostained (man, ox). In the same species, cells of the digestive epithelium which secrete entero-glucagon and gastrin also react with anti-CGA sera. In all cases, immunoreactivity is restricted to the secretory vesicles.

Other non-nervous tissues. Anti-CGA immunoreactivity has also been detected in rat and bovine spleen, thymus (lymphocytes are not stained) and lymph nodes (Nolan *et al.*, 1985; Hogue Angeletti & Hickey, 1985). The presence of CGA in lymph organs thus demonstrates the relationship between the immune and endocrine systems. The presence of CGA in cat and porcine Merkel cells, granular cells of the epithelium the function of which is not yet known, has been reported by Gauweiler *et al.* (1988). The immunoreactivity of these cells depends on the developmental stage of the animal. Lauweryns *et al.* (1987) demonstrated immunoreactivity towards anti-CGA in monkey lung and also in the lung of human foetus. Weiler *et al.* (1987) found that the antigen responsible for the immunostaining from human bronchial carcinoids is a protein of molecular mass identical to that of CGA from chromaffin cells. Immunoblots revealed, in addition, large amounts of a proteoglyan–CGA. More recently, Murray *et al.* (1988) described the co-regulation of secretion and cytoplasmic CGA mRNA in a cell line derived from a human lung cancer. In this cell line, treatment of cells with phorbol 12-myristate 13-acetate results in a significant dose-dependent increase of CGA secretion and of CGA-specific cytoplasmic mRNA level.

Reiker *et al.* (1988) characterized a protein of about 74 kDa which is present in an organ of the carp, the so-called 'head kidney', and in two endocrine glands of the lobster situated close together near the eye socket. This protein is recognized by antibodies against bovine chromaffin granule CGA. The demonstration of anti-CGA immunoreactivity in the ultimobranchial glands and in some other endocrine glands of the rainbow trout and coho salmon (Deftos *et al.*, 1987) suggests a wide distribution of CGA very early in evolution.

Chromogranin A in nervous cells and tissues

CGA is present in the central and peripheral nervous system (Somogyi *et al.*, 1984). Anti-CGA immunoreactivity was detected throughout the brain (cerebral cortex, amygdala, cerebellum, locus coeruleus, hippocampus, basal ganglion, spinal cord). In spite of this wide distribution, the brain contains about 1000-fold less CGA than the adrenal medulla, the tissue in which CGA is most abundant (O'Connor & Frigon, 1984). In the brain, CGA is found in noradrenergic neurons, in neurons containing enkephalins, dynorphin and neo-endorphin (Somogyi *et al.*, 1984) and in cholinergic neurons (Somogyi *et al.*, 1984; Volknandt *et al.*, 1987). However, these neurons are not systematically CGA-positive. O'Connor & Frigon (1984) showed that bovine brain CGA is most prominent in neuronal cytosol rather than in synaptosomal fractions which are rich in neurotransmitter storage vesicles. The 74 kDa CGA detected in mammalian brain extracts might be cleaved into non-immunoreactive peptides in nerve terminals. CGA mRNA is detectable in bovine striatum, and in the rat central nervous system the mRNAs have been detected in the cerebral cortex and in the hippocampus (Iacangelo *et al.*, 1988*a*).

In 1969, Banks *et al.* noticed an antigen which was recognized by anti-CGA immune serum in noradrenergic vesicles of bovine splenic nerve. The CGA-like protein of bovine splenic nerve has recently been characterized (Neuman *et al.*, 1984; Fischer-Colbrie *et al.*, 1985; Hagn *et al.*, 1986*b*): it has a molecular mass of 75 kDa and an isoelectric pH of 5.0.

CHROMOGRANIN A OF INVERTEBRATES

The ciliated protozoan *Paramecium tetraurelia* produces secretory granules (trichocysts) which liberate acidic proteins. Antibodies against bovine chromaffin granule CGA and those produced against chemically synthesized CGA-related peptides recognize certain proteins of trichocysts. Conversely, antibodies directed against trichocyst proteins cross-react with bovine chromaffin granule CGA. The anti-CGA antibodies recognize trichocyst proteins of molecular masses between 15 and 25 kDa. Larger proteins of 53 and 49 kDa which are also recognized in crude extracts of *Paramecium tetraurelia* could correspond to unprocessed precursors of the 15–25 kDa proteins found in mature trichocysts (Peterson *et al.*, 1988). Other data confirm the relationship between certain proteins of *Paramecium tetraurelia* and mammalian CGA: *Paramecium* mRNAs hybridize with bovine CGA cDNA clone (Peterson *et al.*, 1988).

CHROMOGRANIN A AND NEUROENDOCRINE TUMOURS

Tumours of neuroendocrine tissues may be detected by measurement of plasma CGA levels. In general, the CGA concentration is relatively stable in the plasma of normal individuals but increases dramatically in patients with certain tumours of endocrine origin. The rise in plasma CGA levels reflects the increased secretion rate of peptides or hormones by (neuro)endocrine tumour tissues. The demonstration of a rise in plasma CGA levels in patients with certain endocrine tumours could be of great clinical value, particularly for the early diagnosis of these tumours (O'Connor & Bernstein, 1984; O'Connor & Deftos, 1986, 1987) but such an approach should be developed more systematically.

CONCLUSIONS

Twenty years ago, when chromogranin A was described for the first time, it was thought that this protein was limited to chromaffin granules of the adrenal medulla. Since this time, it has become clear that it is widely distributed in mammalian endocrine and neuroendocrine tissues. It is also found in certain tissues of the lower vertebrates, such as shellfish, demonstrating its early appearance during evolution; it seems, in addition, to be highly conserved.

This protein is localized in specialized organelles, secretory vesicles, together with neurotransmitters or hormone characteristic of each endocrine cell type. Each type of endocrine or neuroendocrine cell has its own system of proteolytic enzymes, capable of processing chromogranin A into specific peptides; these peptides, in certain cases, may modulate hormone secretion from the cell type in which they are produced.

Future avenues of research should verify this hypothesis and emphasize the potential prohormone role of chromogranin A in secretory regulation mechanisms in endocrine and neuroendocrine cells.

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REFERENCES

Ahn, T. G., Cohn, D. V., Gorr, S. U., Ornstein, D. L., Kashdan, M. A. & Levine, M. A. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 5043–5047

Apps, D. K., Phillips, J. H. & Purves, F. C. (1985) Neuroscience **16**, 477–485

Artalejo, C. R., Garcia, A. G. & Aunis, D. (1987) J. Biol. Chem **262**, 915–926

Aunis, D., Hesketh, J. E. & Devilliers, G. (1980) J. Neurocytol. **9**, 255–274

Banerjee, S. & Margolis, R. U. (1982) J. Neurochem. **39**, 1700–1703

Banks, P., Helle, K. B. & Mayor, D. (1969) Mol. Pharmacol. **5**, 210–212

Benedum, U. M., Baeuerle, P. A., Konecki, D. S., Frank, R., Powell, J., Mallet, J. & Huttner, W. B. (1986) EMBO J. **5**, 1495–1502

Blaschko, H., Born, G. V. R., D'Ioro, A. & Eade, N. R. (1956) J. Physiol. (London) **183**, 548–557

Blaschko, H., Comline, R. S., Schneider, F. H., Silver, M. & Smith, A. D. (1967) Nature (London) **215**, 58–59

Burgun, C., Martinez de Munoz, D. & Aunis, D. (1986) Biochim. Biophys. Acta **839**, 219–227

Chubb, I. W., Hodgson, A. J. & White, G. H. (1980) Neuroscience **5**, 2065–2072

Chubb, I. W., Ranieri, E., White, G. H. & Hodgson, A. J. (1983) Neuroscience **10**, 1369–1377

Chubb, I. W. & Millar, T. J. (1984) Clin. Exp. Hypertension **6**, 79–90

Cohn, D. V., Zangerle, R., Fischer-Colbrie, R., Chu, L. L. H., Elting, J. J., Hamilton, J. W. & Winkler, H. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 6056–6059

Cohn, D. V., Elting, J. J., Frick, M. & Elder, R. (1984) Endocrinology (Baltimore) **114**, 1963–1974

Cote, A., Doucet, J. P. & Trifaro, J. M. (1986) Neuroscience **19**, 629–645

Daniels, A. J., Williams, R. J. P. & Wright, P. E. (1978) Neuroscience **3**, 573–585

Deftos, L. J., Murray, S. S., Burton, D. W., Parmer, R. J. & O'Connor, D. T. (1986) Biochem. Biophys. Res. Commun. **137**, 418–423

Deftos, L. S., Bjornsson, B.Th., Burton, D. W., O'Connor, D. T. & Copp, D. H. (1987) Life Sci. **40**, 2133–2136

Delvalle, J., Debas, H., Tatemoto, K. & Yamada, T. (1987) Gastroenterology **92**, 1368–1374

Diliberto, E. D., Viveros, O. H. & Axelrod, J. (1976) Proc. Natl. Acad. Sci. U.S.A. **73**, 4050–4054

Efendic, S., Tatemoto, K., Mutt, V., Quan, C., Chang, D. & Ostenson, C. G. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 7257–7260

Ehrhart, M., Grube, D., Bader, M. F., Aunis, D. & Gratzl, M. (1986) J. Histochem. Cytochem. **34**, 1673–1682

Eiden, L. E. (1987) Nature (London) **325**, 301

Eiden, L. E., Iacangelo, A., Hsu, C. M., Hotchkiss, A., Bader, M. F. & Aunis, D. (1987*a*) J. Neurochem. **49**, 65–74

Eiden, L. E., Huttner, W. B., Mallet, J., O'Connor, D. T., Winkler, H. & Zanini, A. (1987*b*) Neuroscience **21**, 1019–1021

Evangelista, R., Ray, P. & Lewis, R. V. (1982) Biochem. Biophys. Res. Commun. **106**, 895–902

Facer, P., Bishop, A. E., Lloys, R. V., Wilson, B. S., Henessy, R. J. & Polak, J. M. (1985) Gastroenterology **89**, 1366–1373

Falkensammer, G., Fischer-Colbrie, R. & Winkler, H. (1985*a*) J. Neurochem. **45**, 1475–1480

Falkensammer, G., Fischer-Colbrie, R., Richter, K. & Winkler, H. (1985*b*) Neuroscience **14**, 735–746

Fischer-Colbrie, R. & Frischenschlager, I. (1985) J. Neurochem. **44**, 1854–1861

Fischer-Colbrie, R. & Schober, M. (1987) J. Neurochem. **48**, 262–270

Fischer-Colbrie, R., Lassmann, H., Hagn, C. & Winkler, H. (1985) Neuroscience **16**, 547–555

Fischer-Colbrie, R., Hagn, C. & Schober, M. (1987) Ann. N.Y. Acad. Sci. **493**, 121–134

Fischer-Colbrie, R., Iacangelo, A. & Eiden, L. E. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 3240–3244

Fricker, L. D. & Snyder, S. H. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 3886–3890

Gauweiler, B., Weihe, E., Hartschuh, W. & Yanaihara, N. (1988) Neurosci. Lett. **89**, 121–126

Gratzl, M. (1987) in Stimulus–Secretion Coupling in Chromaffin Cells (Rosenheck, K. & Lelkes, P. I., eds.), vol. 1, pp. 111–123, CRC Press, Boca Raton, FL

Gratzl, M., Krieger-Bauer, H. & Ekerdt, R. (1981) Biochim. Biophys. Acta **649**, 355–366

Grimes, M. I., Iacangelo, A., Eiden, L. E., Godfrey, B. & Herbert, E. (1987) Ann. N.Y. Acad. Sci. **493**, 351–378

Grube, D., Aunis, D., Bader, M. F., Cetin, Y., Jorns, A. & Yoshie, S. (1987) Histochemistry **85**, 442–452

Hagn, C., Schmid, K. W., Fischer-Colbrie, R. & Winkler, H. (1986*a*) Lab. Invest. **55**, 405–411

Hagn, C., Klein, R. L., Fischer-Colbrie, R., Douglas, B. H. & Winkler, H. (1986*b*) Neurosci. Lett. **67**, 295–300

Hamilton, J. W., Chu, L. L. H., Rouse, J. B., Reddig, K. & MacGregor, R. R. (1986) Arch. Biochem. Biophys. **244**, 16–26

Hearn, S. A. (1987) J. Histochem. Cytochem. **35**, 795–801

Helle, K. B. (1966) Mol. Pharmacol. **2**, 298–310

Helle, K. B. (1971) Biochim. Biophys. Acta **245**, 80–93

Helle, K. B. & Serck-Hanssen, G. (1969) Pharmacol. Res. Commun. **1**, 25–29

Helle, K. B., Reed, R. K., Pihl, K. E. & Serck-Hanssen, G. (1985) Acta Physiol. Scand. **123**, 21–33

Helman, L. J., Ahn, T. G., Levine, M. A., Allison, A., Cohen, P. S., Cooper, M. J., Cohn, D. V. & Israel, M. A. (1988) J. Biol. Chem. **263**, 11559–11563

Hogue Angeletti, R. & Hickey, W. F. (1985) Science **230**, 89–90

Hook, V. Y. H., Eiden, L. E. & Brownstein, M. J. (1982) Nature (London) **295**, 341–342

Hortnagl, H., Lochs, H. & Winkler, H. (1974) J. Neurochem. **22**, 197–199

Hutton, J. C., Hansen, F. & Peshavaria, M. (1985) FEBS Lett. **188**, 336–340

Hutton, J. C., Davidson, H. W., Grimaldi, K. A. & Peshavaria, M. (1987*a*) Biochem. J. **244**, 449–456

Hutton, J. C., Davidson, H. W. & Peshavaria, M. (1987*b*) Biochem. J. **244**, 457–464

Hutton, J. C., Nielsen, E. & Kastern, W. (1988) FEBS Lett. **236**, 269–274

Iacangelo, A., Affolter, H. U., Eiden, L. E., Herbert, E. & Grimes, M. (1986) Nature (London) **323**, 82–86

Iacangelo, A., Okayama, H. & Eiden, L. E. (1988*a*) FEBS Lett. **227**, 115–121

Iacangelo, A., Fischer-Colbrie, R., Koller, K. J., Brownstein, M. J. & Eiden, L. E. (1988*b*) Endocrinology (Baltimore) **122**, 2339–2341

Ismael, Z., Millar, T. J., Small, D. H. & Chubb, I. W. (1986) Brain Res. **376**, 230–238
Kao, L. S. & Schneider, A. S. (1986) J. Biol. Chem. **261**, 4881–4888
Kiang, W. L., Krusius, T., Finne, J., Margolis, R. U. & Margolis, R. K. (1982) J. Biol. Chem. **257**, 1651–1659
Kilpatrick, L., Gavine, F., Apps, D. & Phillips, J. H. (1983) FEBS Lett. **164**, 383–388
Kirshner, N. (1974) Adv. Cytopharmacol. **2**, 265–272
Konecki, D. S., Benedum, U. M., Gerdes, H. H. & Huttner, W. B. (1987) J. Biol. Chem. **262**, 17026–17030
Kornfeld, R. & Kornfeld, S. (1985) Annu. Rev. Biochem. **54**, 631–664
Kostron, H., Winkler, H., Geissler, D. & Konig, P. (1977) J. Neurochem. **28**, 487–493
Kruggel, W., O'Connor, D. T. & Lewis, R. V. (1985) Biochem. Biophys. Res. Commun. **127**, 380–383
Lassmann, H., Hagn, C., Fischer-Colbrie, R. & Winkler, H. (1986) Histochem. J. **18**, 380–386
Lauweryns, J. M., Van Ranst, L., Lloyd, R. V. & O'Connor, D. T. (1987) J. Histochem. Cytochem. **35**, 113–118
Linberg, I., Yang, H. Y. T. & Costa, E. (1984) J. Neurochem. **42**, 1411–1419
Lee, R. W. H. & Huttner, W. B. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 6143–6147
Lloyd, R. V. & Wilson, B. S. (1983) Science **222**, 628–630
Lloyd, R. V., Wilson, B., Kovacs, K. & Ryan, N. (1985) Arch. Pathol. Lab. Med. **109**, 515–517
Murray, S. S., Deaven, L. L., Burton, D. W., O'Connor, D. T., Mellon, P. L. & Deftos, L. J. (1987) Biochem. Biophys. Res. Commun. **142**, 141–146
Murray, S. S., Burton, D. W. & Deftos, L. J. (1988) Endocrinology (Baltimore) **122**, 495–499
Neuman, B., Wiedermann, C. J., Fischer-Colbrie, R., Schober, M., Sperk, G. & Winkler, H. (1984) Neuroscience **13**, 921–931
Nguyen, M. H., Harbour, D. & Gagnon, C. (1987) J. Neurochem. **49**, 38–44
Nolan, J. A., Trojanowski, J. Q. & Hogue Angeletti, R. (1985) J. Histochem. Cytochem. **33**, 791–798
O'Connor, D. T. (1983) Regul. Peptides **6**, 263–280
O'Connor, D. T. & Bernstein, K. N. (1984) N. Engl. J. Med. **311**, 764–770
O'Connor, D. T. & Deftos, L. J. (1986) N. Engl. J. Med. **314**, 1145–1151
O'Connor, D. T. & Deftos, L. J. (1987) Ann. N.Y. Acad. Sci. **493**, 379–386
O'Connor, D. T. & Frigon, R. P. (1984) J. Biol. Chem. **259**, 3237–3247
O'Connor, D. T., Burton, D. & Deftos, L. J. (1983*a*) J. Clin. Endocrin. Metab. **57**, 1084–1086
O'Connor, D. T., Burton, D. & Deftos, L. J. (1983*b*) Life Sci. **33**, 1657–1663
Patzak, A., Aunis, D. & Langley, O. K. (1987) Exp. Cell Res. **171**, 346–356
Peterson, J. B., Nelson, D. L., Ling, E. & Hogue Angeletti, R. (1987) J. Biol. Chem. **262**, 17264–17267
Phillips, J. H. (1987) In Stimulus–Secretion Coupling in Chromaffin Cells (Rosenheck, K. & Lelkes, P. I., eds.) vol. 1, pp. 31–54, CRC Press, Boca Raton, FL
Pryde, J. G. & Phillips, J. H. (1986) Biochem. J. **233**, 525–533
Qian, J., Hickey, W. F. & Hogue Angeletti, R. (1988) J. Neuroimmunol. **17**, 159–165
Reiffen, F. U. & Gratzl, M. (1986*a*) FEBS Lett **195**, 327–330
Reiffen, F. U. & Gratzl, M. (1986*b*) Biochemistry **25**, 4402–4406
Rieker, S., Fischer-Colbrie, R., Eiden, L. E. & Winkler, H. (1988) J. Neurochem. **50**, 1066–1073
Rindi, G., Buffa, R., Sessa, F., Tortora, O. & Solcia, E. (1986) Histochemistry **85**, 19–28
Rosa, P., Hille, A., Lee, R. W. H., Zanini, A., De Camilli, P. & Huttner, N. B. (1985) J. Cell. Biol. **101**, 1999–2011
Rundle, S., Somogyi, P., Fischer-Colbrie, R., Hagn, C., Winkler, H. & Chubb, I. W. (1986) Regul. Peptides **16**, 217–233
Schmid, K. W., Fischer-Colbrie, R., Hagn, C., Jasani, B., Williams, E. D. & Winkler, H. (1987) Am. J. Surg. Pathol. **11**, 551–556
Schober, M., Fischer-Colbrie, R., Schmid, K. W., Bussolati, G., O'Connor, D. T. & Winkler, H. (1987) Lab. Invest. **57**, 385–391
Seidah, N. G., Hendy, G. N., Hamelin, J., Paquin, J., Lazure, C., Metters, K. M., Rossier, J. & Chretien, M. (1987) FEBS Lett. **211**, 144–150
Sekiya, K., Ghatei, M. A., Minamino, N., Bretherton-Watt, D., Matsuo, H. & Bloom, S. R. (1988) FEBS Lett. **228**, 153–156
Serck-Hanssen, G. & O'Connor, D. T. (1984) J. Biol. Chem. **259**, 11597–11600
Settleman, J., Nolan, J. & Hogue Angeletti, R. (1985*a*) J. Biol. Chem. **260**, 1641–1644
Settleman, J., Fonseca, R., Nolan, J. & Hogue Angeletti, R. (1985*b*) J. Biol. Chem. **260**, 1645–1651
Sharp, R. R. & Sen, R. (1979) Biochim. Biophys. Acta **538**, 155–163
Sietzen, M., Schober, M., Fischer-Colbrie, R., Scherman, D., Sperk, G. & Winkler, H. (1987) Neuroscience **22**, 131–139
Simon, J. P., Bader, M. F. & Aunis, D. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 1712–1716
Simon, J. P., Bader, M. F. & Aunis, D. (1989) Biochem. J. **260**, 915–922
Small, D. H., Ismael, Z. & Chubb, I. W. (1986) Neuroscience **19**, 289–295
Smith, A. D. & Winkler, H. (1967) Biochem. J. **103**, 483–492
Somogyi, P., Chubb, I. W. & Smith, A. D. (1975) Proc. R. Soc. London B **191**, 271–283
Somogyi, P., Hodgson, A. J., De Potter, R. W., Fischer-Colbrie, R., Schober, M., Winkler, H. & Chubb, I. W. (1984) Brain Res. Rev. **8**, 193–230
Tatemoto, K., Efendic, S., Mutt, V., Makk, G., Feistner, G. & Barchas, J. D. (1987) Nature (London) **324**, 476–478
Uvnas, B. & Aborg, C. H. (1977) Acta Physiol. Scand. **99**, 476–483
Uvnas, B. & Aborg, C. H. (1980) Acta Physiol. Scand. **109**, 345–354
Varndell, J. M., Lloyd, R. V., Wilson, B. S. & Polak, J. M. (1985) Histochem. J. **17**, 981–992
Veeraragavan, K., Coulombe, R. & Gagnon, C. (1988) Biochem. Biophys. Res. Commun. **152**, 732–738
Viveros, O. H. (1975) Handb. Physiol. **6**, 389–426
Volknandt, W., Schober, M., Fischer-Colbrie, R., Zimmermann, H. & Winkler, H. (1987) Neurosci. Lett. **81**, 241–244
Waschek, J. A., Pruss, R. M., Siegel, R. E., Eiden, L. E., Bader, M. F. & Aunis, D. (1987) Ann. N.Y. Acad. Sci. **493**, 308–323
Weiler, R., Feichtinger, H., Schmid, K. W., Fischer-Colbrie, R., Grimelius, L., Cedermark, B., Papotti, M., Bussolati, G. & Winkler, H. (1987) Virchows Arch A **412**, 103–109
Wilson, B. S. & Lloyd, R. V. (1984) Am. J. Pathol. **115**, 458–468
Winkler, H. (1976) Neuroscience **1**, 65–80
Winkler, H. & Westhead, E. (1980) Neuroscience **5**, 1803–1823
Winkler, H., Hortnagl, H. & Smith, A. D. (1970) Biochem. J. **118**, 303–310
Winkler, H., Apps, D. K. & Fischer-Colbrie, R. (1986) Neuroscience **18**, 261–290
Wohlfarter, T., Fischer-Colbrie, R., Hogue Angeletti, R., Eiden, L. E. & Winkler, H. (1988) FEBS Lett. **231**, 67–70
Yoshie, S., Hagn, C., Ehrhart, M., Fischer-Colbrie, R., Grube, D., Winkler, H. & Gratzl, M. (1987) Histochemistry **87**, 99–106