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Chromogranin A, B and C: immunohistochemical localization in ovine pituitary and the relationship with hormone-containing cells

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Summary

Chromogranins A, B and C, three distinct groups of proteins found in bovine chromaffin granules, were also found to be present in the pituitary using immunoblotting techniques. Their distribution was therefore studied in the normal ram pituitary using an immunoperoxidase technique applied to semithin serial sections and compared with that of some of the hormones of the anterior pituitary. Chromogranin-immunoreactivity was found in gonadotrophs (all three), thyrotrophs (A with some positive for C) and corticotrophs (a fraction with A and fewer with B and C). The mammotrophs and somatotrophs were negative. Chromogranin C was the only one of the three to be located in the pars nervosa, whilst chromogranin B was rarely found in the pars intermedia. The results suggest that (i) chromogranins A, B and C are not always stored together, (ii) some hormone-containing cells do not contain immunohistologically detectable levels of the chromogranins.

luteinizing hormone; follicle stimulating hormone; thyrotropin stimulating hormone; adrenocorticotrophic hormone; colocalization; sheep

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Introduction

The mechanism by which high concentrations of hormones and neurotransmitters are stored in secretory vesicles is still not entirely known despite being the subject of speculation for many years [1]. The potential role of soluble proteins in this process is being re-assessed as a result of recent reports indicating that acidic proteins formerly thought to be associated with particular tissues are in fact widely distributed throughout the endocrine system. The protein for which this was first established, chromogranin A (CG-A), was isolated from chromaffin granules of the adrenal medulla. It is a glycoprotein rich in acidic amino acids and with an apparent molecular mass of 70–80 kDa [2]. In chromaffin granules CG-A is synthesized as a single proprotein which becomes partly degraded in these organelles by endogenous proteases [3,4]. CG-A was originally thought to be confined to adrenergic tissues; however, a protein present in parathyroid gland (SP-I) was found to be similar, if not identical [5]. Subsequent studies [6–10] established that CG-A had a widespread distribution in endocrine tissues and in brain [9,11,12]. In this latter organ its localization did not exactly reflect that of any known neuropeptide [9].

More recently a second group of acidic proteins was isolated from bovine chromaffin granules and named chromogranin B (CG-B) [13,14]. These proteins are also synthesized as a single proprotein [4], are subsequently significantly sulphated [15], and are finally proteolytically processed by endogenous proteases. Similar or identical proteins are probably found in the chromaffin granules of rat PC12 cells [16,21]. CG-B was recently found in several other endocrine tissues [17,21].

Finally, chromaffin granules contain still another group of acidic proteins which have been named chromogranin C (CG-C) [18,22], TSP 84/86 or secretogranin II [20,21]. The protein was originally found in anterior pituitary [19] where it is present in several of the cell populations of this gland [20]. An antiserum against this protein crossreacts with a protein in adrenal medulla which is different from CG-A and CG-B [22]. Thus, CG-A, CG-B and CG-C are all immunologically distinct [17,18,21,22].

While these studies have established that the three chromogranin proteins are present in several endocrine tissues including the pituitary, there are only limited data available on their co-storage and on their localization with other hormones. In the present study we have used for the first time antisera against all three proteins, as well as antisera against the hormones of the anterior pituitary in an attempt to define their exact localization in this organ. Two specific questions were asked: (i) are the three chromogranins always found together; (ii) which hormones are co-localized with the chromogranins?

Materials and Methods

Preparation of antisera and immunochemical methods

Antisera against CG-A and CG-B were raised as described previously [14,17]. Their specificity and lack of crossreactivity has already been established by two dimensional immunoblots [9,14,17]. CG-C was isolated by two dimensional electrophoresis from

the total sedimentable particles (100 000 × *g*, 30 min) of bovine anterior pituitary. CG-C is one of the major spots with a p*I* of 5.0 and a molecular weight of 86000 (see Fig. 1) [20]. After two-dimensional electrophoresis the Coomassie blue stained spot was excised, frozen in liquid nitrogen and pulverized [see 22]. The powder was extracted three times with 0.1 mM EDTA in 0.2 M NaCl at room temperature for 24 h. After low speed centrifugation at 3700 × *g* for 20 min the supernatant was mixed with two parts of acetone. After 24 h at −18°C this mixture was centrifuged and the pellets were resuspended in 300 μl water. This solution emulsified with Freund's adjuvant was used for i.d. immunization of rabbits. Two dimensional electrophoresis and immunoblots were performed as described previously [14].

Preparation of tissues

Three 2–3-year-old Merino rams were used in this study, all from experimental flocks. The pituitaries were removed no more than 10 min after the death of the animal, cut into 3–5 mm sagittal slices, and immersed in a fixative comprising 0.15% picric acid, 4% paraformaldehyde, 0.05% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 [9,25]. The tissue was fixed for 8 h at room temperature, then immersed in 50% ethanol for 30 min, after which it was washed free of fixative with 0.1 M phosphate buffer, pH 7.3. Blocks of tissue, with no dimension greater than 3 mm, were then cut from the slices and dehydrated through a graded series of ethanol, propylene oxide, and embedded in Durcupan (Fluka, Switzerland). After curing the resin for 48 h at 57°C, serial 1-μm sections were cut on a Huxley LKB microtome. The area of detailed study was initially confined to one particular area to ensure that each of the three animals was treated and studied in a standardized fashion. The area chosen was taken from the dorsum of the gland, in the midline and just caudal to the lower infundibular stem. Sections were cut from other blocks later to confirm and expand the results.

Immunohistochemistry

An indirect antibody avidin-biotin-peroxidase complex method was used [25]. The postembedding immunocytochemical procedure was carried out as described earlier [26]. Primary antisera used, their source and dilution are listed in Table I.

Sections were etched in ethanolic sodium hydroxide for 30 min then washed in three changes of absolute ethanol, two of distilled water, and two of Tris-phosphate-buffered saline (T-PBS). They were then blocked with 20% normal sheep or horse serum for 20 min and incubated with the primary antiserum for 18 h in a humid chamber at 4°C. This was followed by two 10-min washes in T-PBS and one wash in 1% normal sheep/horse serum, diluted in T-PBS. The sections were then incubated with biotinylated sheep anti-rabbit/horse anti-mouse serum (1:200; Vectalabs, CA, U.S.A.) for 45 min, followed by another 45 min in avidin-biotinylated horseradish peroxidase complex (1:200; Vectalabs) after three 10-min washes in T-PBS. After further washing with T-PBS, they were incubated for 5 min in 0.05% 3,3′-diaminobenzidine (DAB; Sigma, U.S.A.) in 50 mM Tris buffer, pH 7.3. Hydrogen peroxide (0.01%) was then added and the reaction stopped after 5 min by washing twice with distilled water. The reaction end-product was intensified by a brief incubation with

TABLE I

Details of primary antisera

Antiserum	Code	Source	Antigen	Dilution	Reference
CG-A	–	rabbit	CG-A	1/300	9
CG-B	–	rabbit	CG-B	1/200	17
CG-C	–	rabbit	CG-C	1/400	22
LH	INN-bLH-1	monoclonal mouse	bLH	1/20	33,34
LH-β	NIAMDD IC-1 AFP54372	rabbit	hLH-β	1/400	–
TSH-β*	NIAMDD IC-1 AFP62423473	rabbit	hTSH-β	1/400	–
ACTH	UCB-Bioproducts (Belgium)i503/002	rabbit	hACTH 1-24	1/1000	–
GH	NIAMDD oGH-2 AFP C0123080	rabbit	NIAMDD oGH 1-3 AFP5285C	1/2000	–
PRL	MacNeilly 2532	rabbit	oPRL	1/2000	–

Each antiserum was first used over a wide range of dilutions. The final dilutions chosen for routine use were those that gave optimal staining of the cells with minimal staining of the background.

* Cross-reactivity of TSH antiserum as determined by RIA:hTSH 4.8%; hFSH intact 0.14%; hLH intact 0.05%. Routinely absorbed for use with 10^{-7} M each of oLHβ and hFSHβ subunits (NIAMDD WRR-2-β and I-2 AFP1194B, respectively) for 12 h at 4°C.

a weak osmium tetroxide solution (0.001%), the slides washed, dehydrated through a graded series of ethanols, xylene, and then mounted in Xam (Gurr, U.K.). Data on staining patterns were obtained using camera lucida drawings of sandwiches of strictly serial sections.

Controls

Method specificity controls included sequential omission of the first or second layer of antiserum, the peroxidase complex, the DAB, or the peroxide. No staining was observed in any such sequence. In most cases preimmune serum of the animal of origin of the primary antibody was applied instead of the immune serum; no staining was observed in this instance either. Antibody specificity was tested by incubation of the antiserum with various antigens as listed in Table II.

Results

Chromogranins in the pituitary

CG-C was found in extracts of sedimentable particles from the ovine anterior pituitary (Fig. 1) and spots attributable to Chromogranins A and B [9,17] were also visible on the same gels (Fig. 1). The antiserum against CG-C also reacted with a smaller component with similar p*I* values, a result typical of the chromogranins and

TABLE II

Results of antisera adsorptions

Antiserum	Adsorbed with						
	chromaffin vesicle lysate	CG-A	LHβ	TSHβ	FSHβ	ACTH 1–24	α-MSH
α CG-A	–		+	+	+	+	+
α CG-B	–		+	+	+	+	+
α CG-C	–		+	+	+	+	+
α LH	+	+	–	+	+	+	+
α LHβ	+	+	–	+	+	+	+
α TSHβ	+	+	±	–	±	+	+
α ACTH	+	+	+	+	+	–	±

+ = no change in staining, – = abolition of staining, ± = regional abolition of staining. See text.
Concentration of all antigens 10^{-7} M. All incubated 12 h at 4°C.
Source of antigens: LH-NIAMDD oLHβWRR-2-β
TSH-NIAMDD hTSHβI-2 AFT3292B } contaminants not known.
FSH-NIAMDD hFSHβI-2 AFP1194B
ACTH 1-24-Synacthen (Ciba-Geigy Australia)
αMSH-αMSH 4057-V (Protein Research Foundation, Japan)

probably due to the action of peptidases in the granules. CG-C is clearly present in greater quantity in the pituitary than either CG-A or CG-B (Fig. 1).

Immunohistochemical localization

Chromogranin immunoreactivity. Antiserum to CG-C stained the pars nervosa with a fine, punctate staining probably of nerve endings (Fig. 2A, b). Antisera to CG-A and CG-C both stained the pars intermedia with the only noticeable difference being the lower intensity of staining for CG-C. The cells were not uniformly stained with either antiserum; they ranged from a faint, diffuse or granular cytoplasmic pattern, to one where the cells were stained intensely, with all cytoplasmic detail obscured (Fig. 2A, a and b). Antiserum to CG-B stained some small, scattered cells weakly (Fig. 2A, c) but these were not common.

Cells in the pars distalis adjacent to remnants of Rathke's pouch stained strongly with all three antisera, CG-B being relatively the strongest. There is a cuboidal epithelium lining these spaces, and the stained cells lay immediately inferior to this, and appeared to be separated from the body of the pars distalis by a septum (Fig. 2B).

There were three main types of cell in the pars distalis stained by the chromogranin antisera: (1) The most common type of cell stained, at least in the area of detailed study, was a discrete, medium-large, ovoid or polygonal cell (Fig. 3). All three antisera stained these cells: antiserum to CG-A stained them variably and, when compared with CG-B and CG-C it was obvious that not all the cells of this type stained for CG-A (Fig. 3a,b). The antiserum to CG-B stained moderately intensely, while

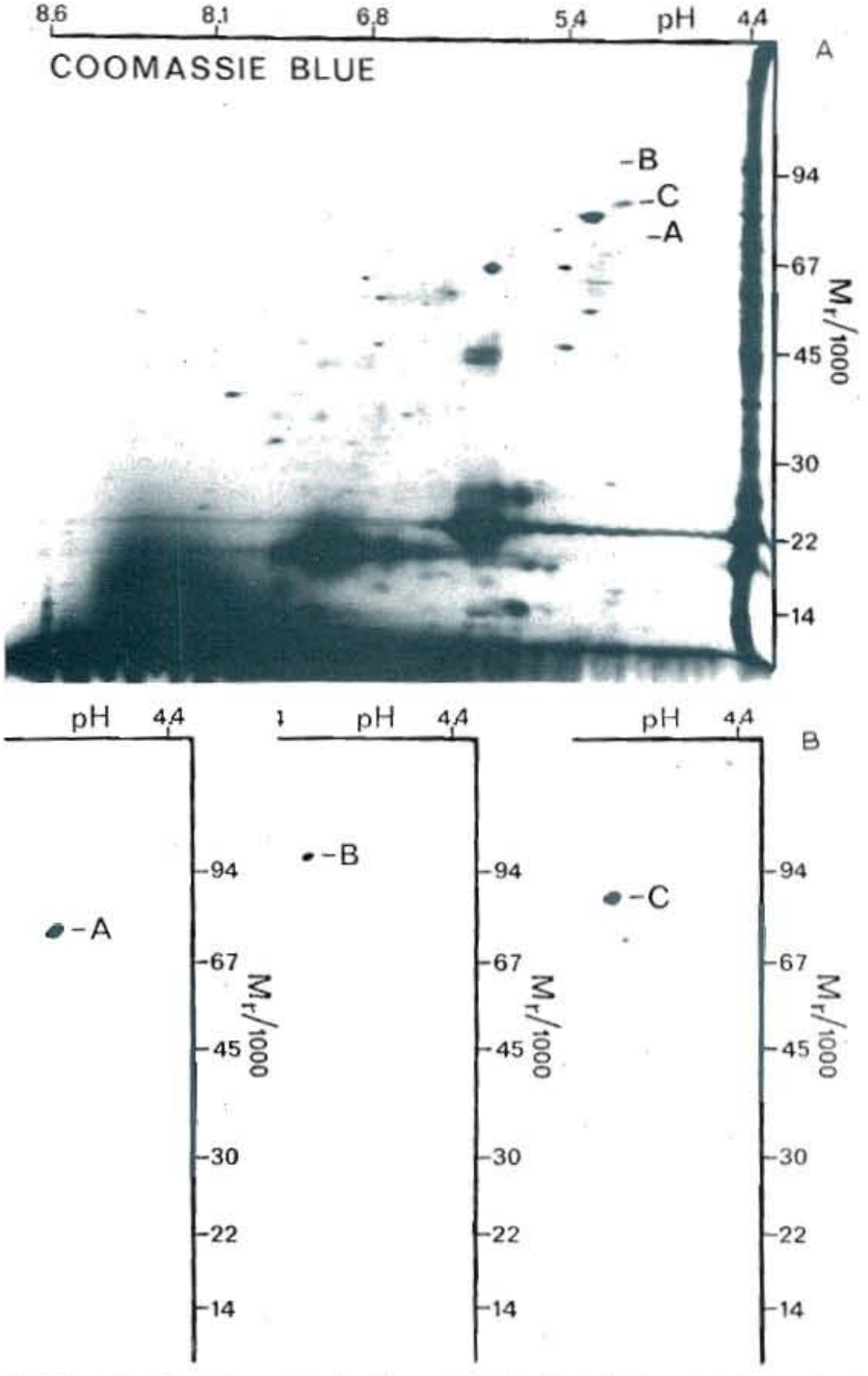

Fig. 1. Two dimensional electrophoresis and immunoblotting of proteins from the ovine anterior pituitary. The sedimentable particles of ovine anterior pituitary were extracted with electrophoresis buffer and directly subjected to two dimensional electrophoresis: (a) Coomassie Blue stain of the proteins. Those reacting with the three antisera (see b) are marked A, B and C. (b) Immunoblot with antisera against bovine chromogranins A, B and C. The faint extra spot shown by the antiserum to chromogranin C is most likely to be a breakdown product of the larger protein. Evidence presented elsewhere has shown that there is no immunological crossreactivity between chromogranins A, B and C [22].

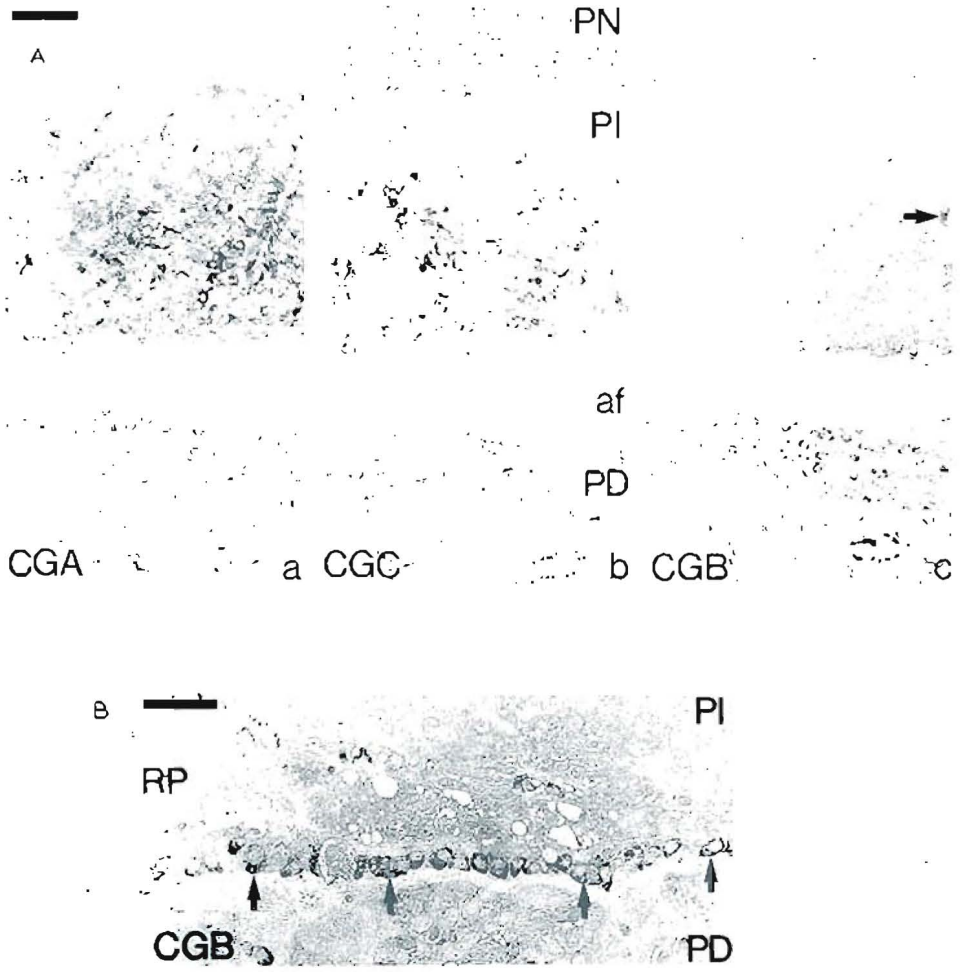

Fig. 2. (A) Consecutive sections through the three lobes of pituitary. Note punctate staining of pars nervosa by CG-C (b), diffuse staining of pars intermedia by CG-A and -C (a,b) and staining by all three of the pars distalis. Arrow (c) indicates cell in pars intermedia stained by CG-B. PN, pars nervosa; PI, pars intermedia; PD, pars distalis; af, artifact produced in tissue processing. Scale: a,b,c same magnification, 50 μm. (B) Arrows indicate column of cells stained by CG-B adjacent to a colloid-filled remnant of Rathke's pouch. Note the epithelium superior, and the connective tissue septum inferior to these cells. RP, Rathke's pouch remnant. Some of these cells also stained with LH antisera. Scale: 20 μm.

the staining for CG-C was very intense. The latter antiserum showed that this particular type of cell occurred in well defined tracts (not shown). (2) The second cell type was found in areas where the previous type was absent. These were small, discrete cells with little cytoplasm. These cells were stained weakly with all three antisera (not shown). (3) The third type was not numerous in the area of detailed study. These were medium-large polymorphic cells which were found in clusters, and which appeared to be randomly scattered throughout this area. They were not stained by the CG-B antiserum. A small number stained faintly for CG-C, but frequently it was

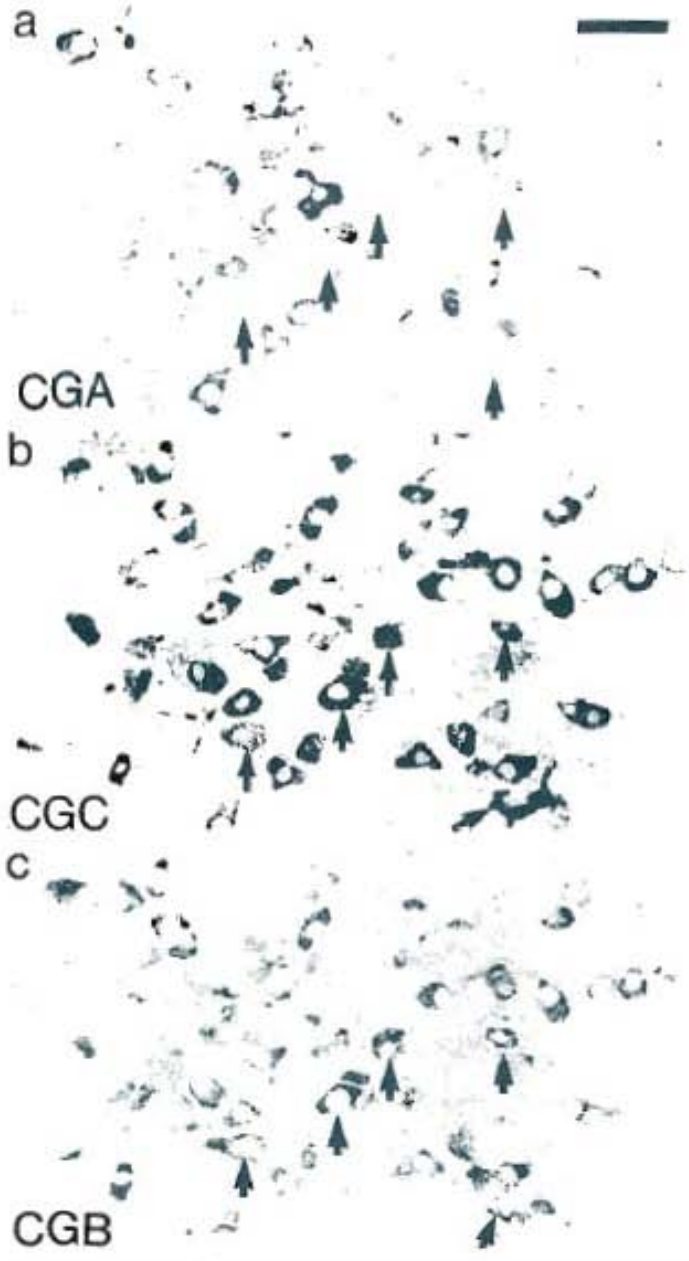

Fig. 3. Consecutive sections of pars distalis stained with the three chromogranin antisera. Arrows indicate cells not stained by CG-A. Note the variability of staining of this antiserum as compared with that of the other two. Scale: a,b,c same magnification, 20 μm.

difficult to discern them from background. More of this type stained for CG-A, but again this was variable (Figs. 4A, 7A and B). These cells were seen in greater numbers in an area rostral to the area of detailed study and stained intensely for CG-A. Here cells with a similar morphology were observed to stain faintly for CG-B (Fig. 8).

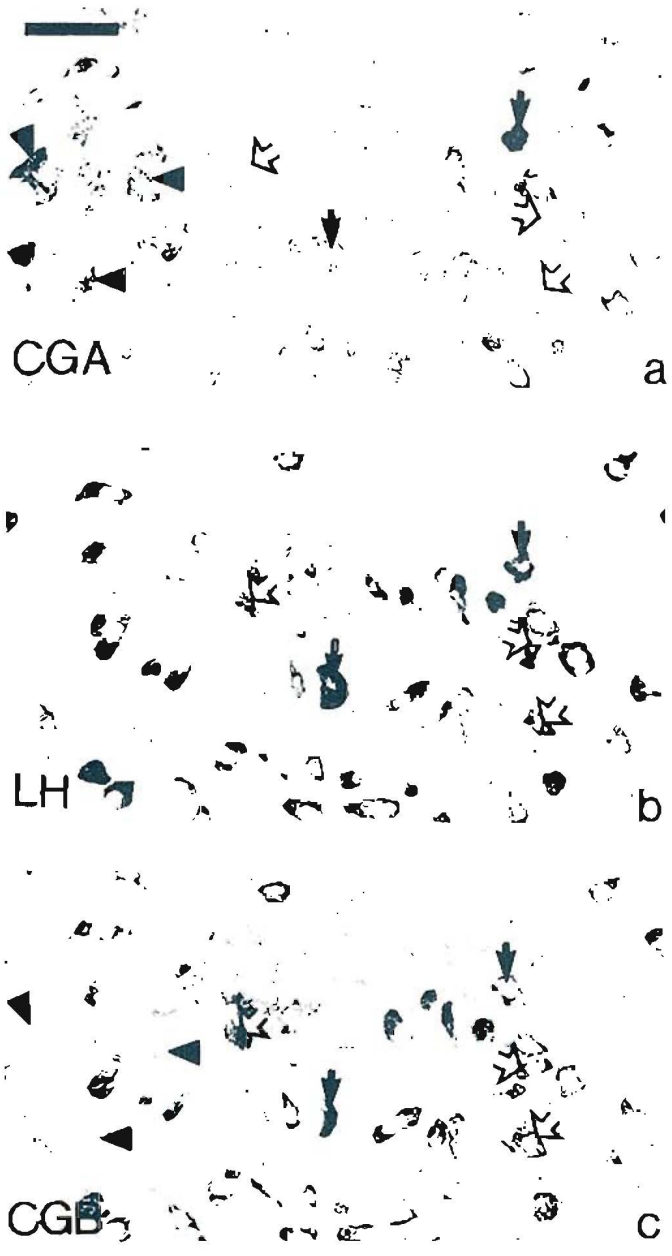

Fig. 4. Series comparing staining of chromogranins and LH. CG-B and LH stain the same cells (b,c). Some of these cells are stained by CG-A (a, solid arrows), but others are not (open arrows). There are in these sections cells stained by CG-A only (a,c; arrowheads); compare Fig. 3. Scale: a,b,c same magnification, 20 μm.

Comparison with pituitary hormone immunoreactivity

(1) Growth hormone, prolactin. The staining obtained with antisera against the hormones did not coincide with that produced with any of the three chromogranins (Fig. 5).

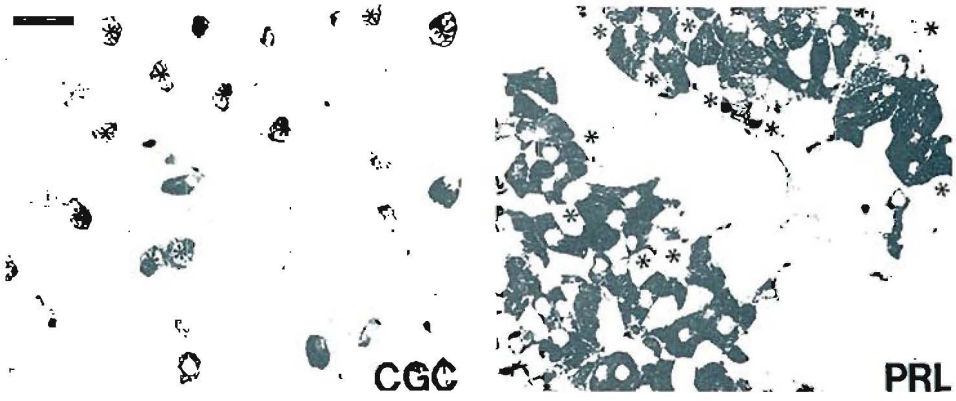

Fig. 5. Serial sections stained with CG-C and prolactin (PRL). Note the lack of coincidence of the two. This was also found with the other two chromogranins (not shown). The cells staining with antisera to CG-C are shown (*). Scale: same magnification, 30 μm.

(2) LH. Two antisera were used against LH; one a polyclonal raised against the human LH beta subunit (see Table I for details); the other a monoclonal raised against bovine LH, whose subsequent characterization indicated that it was directed against a region on the beta subunit [34,35]. The staining pattern obtained with each was identical. The cells stained were the medium-large, ovoid-polygonal type, as described above. Most, if not all, of the cells thus stained by the LH antisera were also stained by the CG-B and CG-C antisera, whilst the agreement with CG-A was more variable (Fig. 4). The antiserum to CG-A was the weakest, however, and this could have led to immunoreactivity below detectable levels.

Some of the cells adjacent to Rathke's pouch remnants were also LH immunoreactive, as were many of the small cell types described above. This latter cell may belong to a different population of gonadotroph, or may simply be the same cell in a different phase.

(3) TSH. There were no TSH-immunoreactive cells in the area of detailed study. Therefore sections were reacted from blocks containing the central mucoid wedge, an area of the rostro-ventral part of the gland. The cells stained here with the TSH antiserum were large and ovoid, and were located in cords arranged about capillaries. Most of these were also CG-A-immunoreactive. A small number reacted weakly for CG-C (Fig. 6), but no coincident staining was observed with CG-B (not shown).

(4) ACTH. The ACTH antiserum was a polyclonal raised against human ACTH 1-24. It stained the pars intermedia intensely; in the area of detailed study of the pars distalis the stained cells were in a few scattered clumps and of a large polymorphic type (Fig. 7A,B). In an area rostral to this, many of these cells were found in large clusters or cords (Fig. 8); thus it appears that they have a patchy regional distribution. When this antiserum was adsorbed with α-MSH, the staining of the pars intermedia was abolished, but little effect was noted in the pars distalis. The intensity of staining of the pars intermedia by the ACTH antiserum (although it may be recognizing α-MSH) was not matched by either CG-A or CG-C. The former stained most cells of this structure, but the other two did not. In the pars distalis, only some of the ACTH-

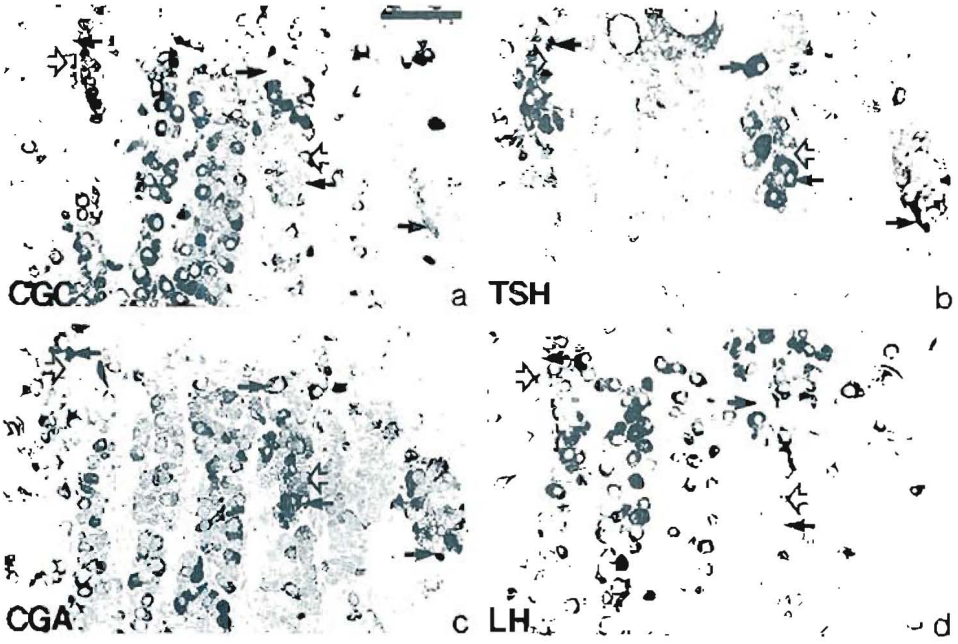

Fig. 6. Series comparing staining of chromogranins, LH and TSH. Solid arows indicate the position of cells stained by CG-A and TSH. Only one of these clusters is stained by CG-C. Open arrows indicate cells not stained by TSH: these are more strongly stained by CG-C and LH. These cells are found within clusters of TSH-stained cells. Note the closer similarity of CG-C and LH, although the sections are too distant to allow direct comparison. Scale: a,b,c,d same magnification, 50 μm.

positive cells were also positive for CG-A; a few isolated cells also stained faintly with CG-C. In the area rostral to this – that is the one with the large populations of ACTH- and CG-A-immunoreactive cells – the two patterns did not overlap. Here it was observed that the ACTH-, GH- and CG-A-positive cells intertwined with each other, with some of the ACTH cells also being positive for CG-B. The staining achieved with CG-C in this area was indistinct, and its interpretation was difficult (Fig. 8).

Discussion

These results indicate that chromogranins A, B and C have a selective distribution in the normal ram pituitary, that they overlap, but that they are not always found in the same cell. The pars nervosa is only immunoreactive for CG-C; CG-B rarely stains the pars intermedia although CG-A and -C are both present; all three are often associated with cells stained by LH antisera; CG-A is often the sole chromogranin in TSH-staining cells, whilst the relationship of the three with ACTH-staining cells varies with a few staining for CG-A and fewer staining for the others.

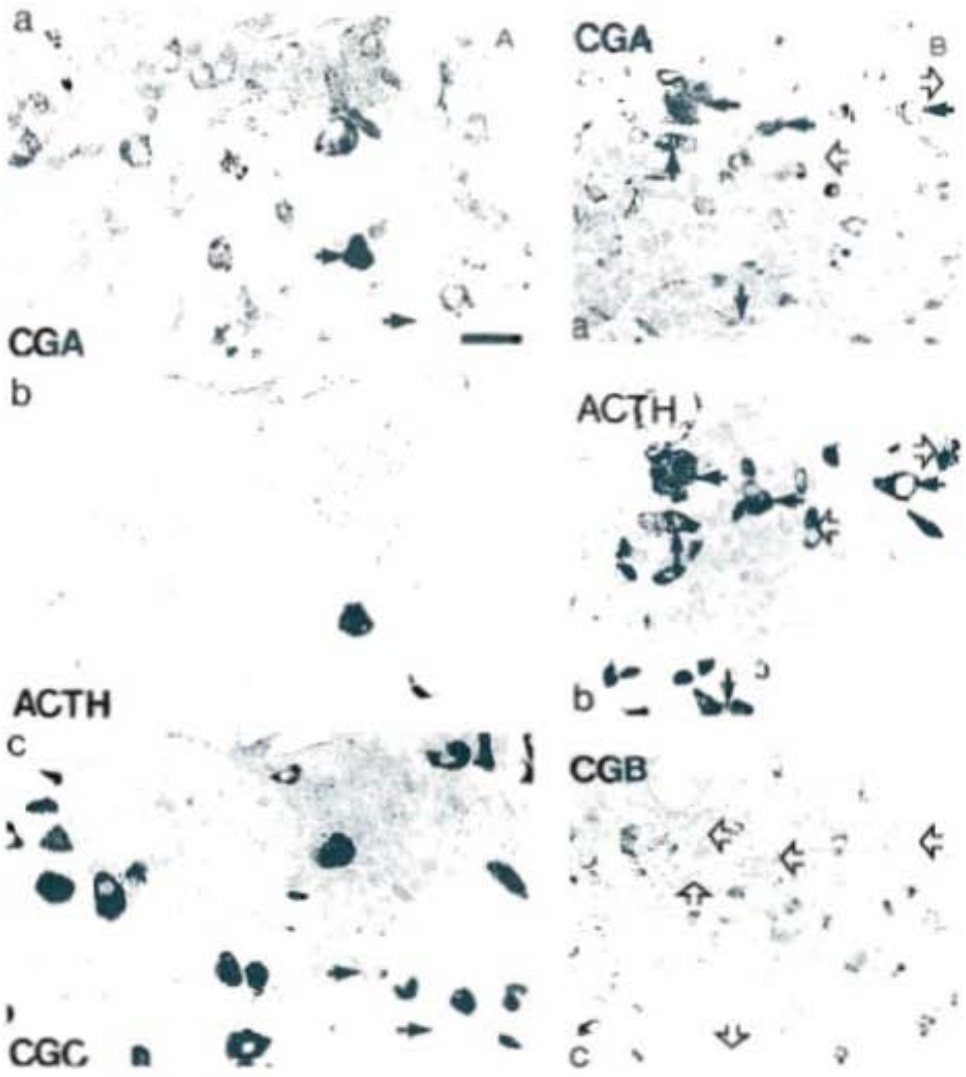

Fig. 7. (A) Serial sections comparing staining of CG-A, -C and ACTH. Arrows indicate cells stained by ACTH (b) which are also stained by CG-A (a).These cells are very weakly stained by CG-C (c). Scale: a,b,c same magnification, 20 μm. (B) Serial sections from similar area of 7A. Working from the ACTH stained section first (b): solid arrows indicate cells stained by CG-A; open indicate those not stained by -A (a). The position of these cells is indicated in the CG-B stained section (c) by open arrows. None of the ACTH cells are stained here. This was also the case for CG-C (not shown). Scale: a,b,c same magnification, 50 μm.

Chromogranin and gonadotrophs

This study does not differentiate between LH and FSH containing cells as we were unable to obtain a satisfactory FSH-beta antiserum. However, the storage of these two hormones in these cells has been the subject of many studies in the past decade, especially since the development of beta chain specific antisera. Nakane in 1970 [27] described in the rat pituitary a population of peripherally placed cells containing

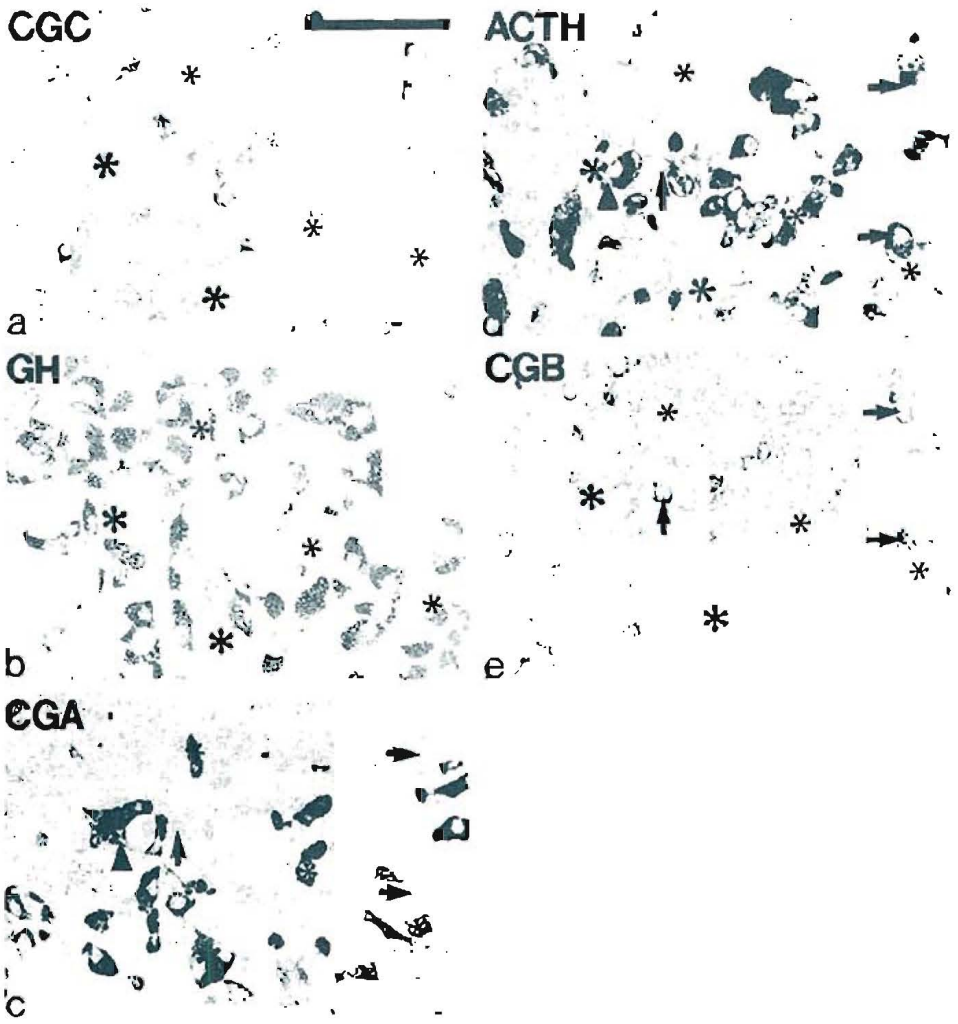

Fig. 8. Series taken from an area rostral to that shown in Fig. 7A, B. Working from the CG-A stained section first (c): note the intensity of staining of these cells; some are asterisked. By using these as markers, it is possible to discern that these cells are intertwined with GH-staining cells (b) and ACTH-staining cells (d). There is one exception to this: an arrowhead indicates the position of a cell stained by ACTH and CG-A. The staining achieved with CG-C (a) and CG-B (e) is indistinct; it is however possible to demonstrate cells stained by both ACTH and CG-B (solid arrows). The cells stained by CG-A are not stained by CG-B, nor are they convincingly by CG-C. Scale: a,b,c,d,e same magnification, 50 μm.

both hormones, with those centrally located only containing one or the other. Moriarty [28] described three populations of putative gonadotroph in the rat; a large ovoid type I cell which contained both, an angular/stellate cell type II in which either or both hormones could be found, and a type III cell in which only FSH could be localized. Herbert [29] demonstrated that the two hormones were colocalized in the same cells in the rhesus monkey pituitary; Pelletier [30] found that 90% of the gonadotrophs contained both hormones in the human, whilst Baker [31] in the mouse

and Dacheux [32] in the pig found both in most of the gonadotrophs. El Etreby and Fath El Bab [33] in the beagle dog could delineate a separate FSH containing population, but found that cells adjacent to the pars intermedia stained for both. Whilst similar work in the sheep is lacking it would appear on the basis of the above results, that most of the LH-positive cells observed in this study would contain FSH as well. Since we have colocalized the three chromogranins with LH in this study, we can conclude that the gonadotrophs contain chromogranins.

The variability of staining obtained with CG-A of the gonadotrophs is noteworthy, especially when compared with the uniformity of staining with CG-B and particularly CG-C. It seems unlikely that this difference is due to different sensitivities of the antisera to CG-A, -B and -C. Although staining for CG-C was generally the strongest, which is consistent with the relatively high amounts of the antigen revealed by electrophoretic analysis, the antiserum to CG-A reacted with some cells more strongly than the other two antisera. Alternatively CG-A may simply be a more labile protein than the other two and the effect of fixation could be variable. It is possible however that the levels of CG-A in these cells may vary, implying a different physiological function for this protein in the face of more constant levels of CG-B and CG-C.

Chromogranin and thyrotrophs

The antiserum used against TSH (an anti-human TSH-beta) exhibited considerable crossreactivity with both LH- and FSH-beta. Thus it was routinely adsorbed with these to enable the delineation of cells, the location and morphology of which satisfied our criteria for their being thyrotrophs. Most of these cells stained for CG-A in agreement with previous results [6,36] but only a few stained for CG-C and none for CG-B. These cells have been reported to stain strongly for CG-C in the rat pituitary [20]. Some chromogranin was thus found in each of the glycoprotein hormone producing cells.

Chromogranin and corticotrophs

Only a fraction of the ACTH-positive cells stained for the chromogranins. In rat and bovine glands CG-A is apparently absent from the corticotrophs [6,8], whereas there were some positive cells in human tumours derived from these cells [36,37]. CG-C has been reported in some rat corticotrophs [20]. This study suggests either a level too low to detect consistently or that only a subpopulation of the cells contains any chromogranin. In fact Moriarty [28] described an angular cell, which was designated as a corticotroph, which also contained both ACTH and FSH. It is not possible without further study to define whether the proportion of ACTH-stained cells which stained for chromogranin were of this type. The fact that both CG-A and CG-C were localized in the pars intermedia, a major site in the sheep for the metabolism of pro-opiomelanocortin [38] suggests that the chromogranins have a link with this hormone. It is noteworthy that the adrenal chromaffin cells, the classical cells of chromogranin production, have also been suggested as having the capacity to synthesise the proopiomelanocortin peptides in the same species [39].

The finding of intensely CG-A-positive cells intertwined with GH- and ACTH-

positive cells is intriguing. Nakane [27] has described juxtaposed ACTH and GH cells in the rat. The CG-A-positive cells were not stained by any of our other hormonal antisera. The question arises as to their role. It could be that they were secretory cells in a resting phase, though why CG-A was not detected in the neighbouring cells, and the ACTH cells in particular, is not known. It may simply be that these cells do not secrete one of the classical hormones of the pars distalis, and in that case could not be classified due to the lack of an appropriate antiserum. It is unlikely that they are in fact nonsecretory cells, supporting cells for example, because of the presence in them of CG-A.

Chromogranins, mammotrophs and somatotrophs

We found no evidence that these cells contain any of the three chromogranins. For CG-A, this is in agreement with work on the rat [6] and human [36] pituitary. For CG-C, our results are similar to those reported in rat [21] although it was originally suggested that all endocrine cells except for somatotrophs were positive [20].

In this study, the first to use antisera against all three chromogranins together, we could not establish that they were always co-stored. Furthermore in the anterior pituitary not all the hormone-containing cells contained detectable amounts of the chromogranins. It seems unlikely that these results were due to different sensitivities of the antisera or to problems introduced by fixation. All antisera gave strong staining of a variety of cells within tissue blocks and sections treated in the same way. Thus, the major differences in immunohistochemical staining are most likely to be due to different levels of the antigens in these cells.

The function or reason for the presence of chromogranins in some endocrine cells is not known. However, the distinct patterns of their localization, such as reported here for all three and in the brain for CG-A, suggests a fairly specific function for the proteins and appropriate experiments need now to be designed to determine their role.

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References

1 Smith, A.D., Storage and secretion of hormones, In Gilliland, I., Francis, J. (Eds.), The Scientific Basis of Medicine Annual Reviews, University of London, Athlone Press, 1972, pp. 74–102.

2 Winkler, H. and Carmichael, S. W., The chromaffin granule. In Poisner, A.M., Trifaro, J. M. (Eds.), The secretory granule. Elsevier/North-Holland, Amsterdam, 1982, pp. 3–79.
3 Kilpatrick, L., Gavine, F., Apps, D., Phillips, J., Biosynthetic relationship between the major matrix proteins of the adrenal chromaffin granules, FEBS Lett., 164 (1983) 383–388.
4 Falkensammer, G., Fischer-Colbrie, R., Richter, K., Winkler, H., Cell-free and cellular synthesis of chromogranin A and B of bovine adrenal medulla, Neuroscience, 14 (1985) 735–746.
5 Cohn, D.V., Zangerle, R., Fischer-Colbrie, R., Chu, L.L.H., Elting, J.J., Hamilton, J.W., Winkler, H., Similarity of secretory protein I from the parathyroid gland to chromogranin A from the adrenal medulla, Proc. Natl. Acad. Sci. USA, 79 (1982) 6056–6059.
6 Cohn, D.V., Elting, J.J., Frick, M., Elde, R., 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 (1984) 1963–1974.
7 O'Connor, D.T., Chromogranin: widespread immunoreactivity in polypeptide producing tissues and in serum, Regul. Peptides, 6 (1983) 263–280.
8 O'Connor, D.T., Burton, D., Deftos, L.J., Chromogranin A: immunohistology reveals its universal occurrence in normal polypeptide hormone producing endocrine glands, Life Sci., 33 (1983) 1657–1663.
9 Somogyi, P., Hodgson, A.J., DePotter, R.W., Fischer-Colbrie, R., Schober, M., Winkler, H., Chubb, I.W., Chromogranin immunoreactivity in the central nervous system. Immunochemical characterization, distribution and relationship to catecholamine and enkephalin pathways, Brain Res. Rev., 8 (1984) 193–230.
10 Wilson, B.S., Lloyd, R.V., Detection of chromogranin in neuroendocrine cells with a monoclonal antibody, Am. J. Pathol., 115 (1984) 458–468.
11 O'Connor, D.T., Frigon, R.P., Chromogranin A, the major catecholamine storage vesicle soluble protein, J. Biol. Chem., 259 (1984) 3237–3247.
12 Nolan, J.A., Trojanowski, J.Q., Hogue-Angeletti, R., Neurons and neuroendocrine cells contain chromogranin: detection of the molecule in normal bovine tissues by immunochemical and immunohistochemical methods, J. Histochem. Cytochem., 33 (1985) 791–798.
13 Winkler, H., Falkensammer, G., Patzak, A., Fischer-Colbrie, R., Schober, M., Weber, A., Life cycle of the catecholaminergic vesicles: from biogenesis to secretion. In Visi, E.S., Magyar, K. (Eds.), Regulation of Transmitter Function. Basic and Clinical Aspects, Akademiai Kiado, Budapest, 1984, pp. 65–73.
14 Fischer-Colbrie, R., Frischenschlager, I., Immunological characterization of secretory proteins of chromaffin granules: chromogranins A, chromogranins B, and enkephalin-containing peptides, J. Neurochem., 44 (1985) 1854–1861.
15 Falkensammer, G., Fischer-Colbrie, R., Winkler, H., Biogenesis of chromaffin granules: incorporation of sulfate into chromogranin B and into a proteoglycan, J. Neurochem., 45 (1985) 1475–1480.
16 Lee, R.W.H., Huttner, W.B., Tyrosine-O-sulfated proteins of PC12 phaeochromocytoma cells and their sulfation by a tyrosylprotein sulfotransferase, J. Biol. Chem., 258 (1983) 11326–11334.
17 Fischer-Colbrie, R., Lassman, H., Hagn, C., Winkler, H., Immunological studies on the distribution of chromogranin A and B in endocrine and nervous tissues, Neuroscience, 16 (1985) 547–556.
18 Winkler, H., Apps, D.K., Fischer-Colbrie, R., The molecular function of adrenal chromaffin granules: established facts and controversial results, Neuroscience, (1985) in press.
19 Rosa, P., Zanini, A., Purification of a sulfated secretory protein from the adenohypophysis. Immunochemical evidence that similar macromolecules are present in other glands, Eur. J. Cell Biol., 31 (1983) 94–98.
20 Rosa, P., Fumagalli, G., Zanini, A., Huttner, W.B., The major tyrosine-sulfated protein of the bovine anterior pituitary is a secretory protein present in gonadotrophs, thyrotrophs, mammotrophs, and corticotrophs, J. Cell Biol., 100 (1985) 928–937.
21 Rosa, P., Hille, A., Lee, R.W.H., Zanini, A., DeCamilli, P., Huttner, W.B., Secretogranins I and II: Two tyrosine-sulfated secretory proteins common to a variety of cells secreting peptides by the common regulated pathway, J. Cell Biol., 101 (1985) 1999–2011.
22 Fischer-Colbrie, R., Hagn, C., Kilpatrick, L., Winkler, H., Chromogranin C: a third component of the acidic proteins in the chromaffin granules, J. Neurochem. (1986) in press.

23 Hager, D.A., Burgess, R.R., Elution of proteins from sodium dodecyl sulfate-polyacrylamide gels. Removal of sodium dodecyl sulfate and renaturation of enzymatic activity: results with Sigma subunit of *Escherichia coli* RNA polymerase, wheatgerm DNA topoisomerase, and other enzymes, Anal. Biochem., 109 (1980) 76–86.
24 Somogyi, P., Takagi, H., A note on the use of picric acid-paraformaldehyde-glutaraldehyde fixative for correlated light and electron microscopic immunocytochemistry, Neuroscience, 7 (1982) 1779–1783.
25 Hsu, S.-M., Raine, L., Fanger, H., Use of avidin-biotin-peroxidase complex[ABC] in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody[PAP] procedures, J. Histochem. Cytochem., 29 (1981) 577–580.
26 Somogyi, P., Hodgson, A.J., Smith, A.D., Nunzi, M.G., Gorio, A., Wu, J.-Y., Different populations of GABA-ergic neurons in the visual cortex and hippocampus of cats contain somatostatin or cholecystokinin-immunoreactive material, J. Neurosci., 4 (1984) 2590–2603.
27 Nakane, P., Classifications of anterior pituitary cell types with immunoenzyme histochemistry, J. Histochem. Cytochem., 18 (1970) 9–20.
28 Moriarty, G.C., Immunocytochemistry of the pituitary glycoprotein hormones, J. Histochem. Cytochem., 24 (1976) 846–863.
29 Herbert, D.C., Immunocytochemical evidence that luteinizing hormone[LH] and follicle stimulating hormone[FSH] are present in the same cell type in the rhesus monkey pituitary gland, Endocrinology, 98 (1976) 1554–1557.
30 Pelletier, G., Leclerc, R., Labrie, F., Identification of gonadotrophic cells in the human pituitary by immunoperoxidase technique, Mol. Cell. Endocrinol., 6 (1976) 123–128.
31 Baker, B.L., Gross, D.S., Cytology and distribution of secretory cell types in the mouse hypophysis as demonstrated with immunocytochemistry, Am. J. Anat., 153 (1978) 193–216.
32 Dacheux, F., Ultrastructural localization of gonadotrophic hormones in the porcine pituitary using the immunoperoxidase technique, Cell Tissue Res., 191 (1978) 219–232.
33 El Etreby, M.F., Fath El Bab, M.R., Localization of gonadotrophic hormones in the dog pituitary gland. A study using immunoenzyme histochemistry and chemical staining, Cell Tissue Res., 183 (1977) 167–175.
34 Kofler, R., Kalchschmid, E., Berger, P., Wick, G., Production and characterization of monoclonal antibodies against bovine luteinizing hormone, Immunobiology, 160 (1981) 196–207.
35 Kofler, R., Immunofluorescence localization of glycoprotein hormones in the rat anterior pituitary gland using monoclonal antibodies and polyclonal antisera, J. Histochem. Cytochem., 30 (1982) 645–649.
36 DeStephano, D.B., Lloyd, R.V., Pike, A.M., Wilson, B.S., Pituitary adenomas. An immunohistochemical study of hormone production and chromogranin localisation, Am. J. Pathol., 116 (1984) 464–472.
37 Lloyd, R.V., Wilson, B.S., Kovacs, K., Ryan, N., Immunohistochemical localisation of chromogranin in human hypophyses and pituitary adenomas, Arch. Pathol. Lab. Med., 109 (1985) 515–517.
38 Jackson, S., Hope, J., Estivariz, F., Lowry, P.J., Nature and control of peptide release from the pars intermedia. In Evered, D., Lawrensen, G., (Eds.), Peptides of the pars intermedia, Ciba Symposium 81, Pitman Medical Press, Bath, 1981, pp. 141–153.
39 Evans, C.J., Erdelyi, E., Weber, E., Barchas, J.D., Identification of proopiomelanocortin-derived peptides in the human adrenal medulla, Science, 221 (1983) 957–960.