Histochemistry (1987) 87:99–106

Histochemistry

Immunological characterization of chromogranins A and B and secretogranin II in the bovine pancreatic islet

S. Yoshie[1], C. Hagn[2], M. Ehrhart[3], R. Fischer-Colbrie[2], D. Grube[1], H. Winkler[2], and M. Gratzl[3]*

[1] Abteilung für Allgemeine Anatomie, Medizinische Hochschule Hannover, D-3000 Hannover, Federal Republic of Germany
[2] Pharmakologisches Insitut, Universität Innsbruck, A-6020 Innsbruck, Austria
[3] Abteilung Anatomie und Zellbiologie, Universität Ulm, Postfach 4066, D-7900 Ulm, Federal Republic of Germany

Accepted February 28, 1987

Summary. Antisera against chromogranin A and B and secretogranin II were used for analysing the bovine pancreas by immunoblotting and immunohistochemistry. All three antigens were found in extracts of fetal pancreas by one dimensional immunoblotting. A comparison with the soluble proteins of chromaffin granules revealed that in adrenal medulla and in pancreas antigens which migrated identically in electrophoresis were present. In immunohistochemistry, chromogranin A was found in all pancreatic endocrine cell types with the exception of most pancreatic polypeptide-(PP-) producing cells. For chromogranin B, only a faint immunostaining was obtained. For secretorgranin II, A- and B-cells were faintly positive, whereas the majority of PP-cells exhibited a strong immunostaining for this antigen. These results establish that chromogranins A and B and secretogranin II are present in the endocrine pancreas, but that they exhibit a distinct cellular localization.

Introduction

The acidic soluble proteins of bovine chromaffin granules have been collectively named chromogranins (Blaschko et al. 1967). Their main component, chromogranin A, has been isolated and characterized (Helle 1966; Smith and Winkler 1967; Smith and Kirschner 1967) and finally the primary amino acid sequence has been established (Benedum et al. 1986; Iancangelo et al. 1986). Chromogranin A is synthesized as a proprotein which within chromaffin granules is processed by endogenous proteases (Kilpatrick et al. 1983; Falkensammer et al. 1985a). Thus, mature chromaffin granules contain a family of immunologically related chromogranin A proteins (see Winkler et al. 1986). In addition to these chromogranins A, chromaffin granules contain at least two other groups of acidic proteins (see Winkler et al. 1986). According to a recent nomenclature proposal (Eiden et al. 1987), we are using the terms chormogranins B and secretorgranins II for these two protein families (proproteins plus endogenous breakdown products).

Chromogranin A and B and secretogranin II are widely distributed in endocrine tissues (Cohn et al. 1982; O'Connor 1983; Wilson and Lloyd 1984; Fischer-Colbrie et al. 1985; Rindi et al. 1986; Lassmann et al. 1986; Rosa et al. 1985) and all three antigens have been demonstrated immunohistochemically in the endocrine pancreas of several species (O'Connor et al. 1983; Lloyd et al. 1984; Fischer-Colbrie et al. 1985; Lassmann et al. 1986; Hagn et al. 1986; Rindi et al. 1986).

Recently, the cellular distribution of chromogranin A has been studied in detail for the bovine endocrine pancreas (Ehrhart et al. 1986) and for the endocrine pancreas of nine other mammalian species (Grube et al. 1986). In bovine pancreatic islets insulin-, glucagon-, and somatostatin-producing cells exhibited chromogranin A-like immunoreactivities. They were exclusively confined to the secretory vesicles. Most of the pancreatic polypeptide-(PP-) producing cells, however, were unreactive towards chromogranin A antiserum.

As yet it is unclear, whether the pancreatic chromogranin A has the same molecular form as chromogranin A isolated from the adrenal medulla. Proteins with a molecular size of about 20 kD reacting with chromogranin A antibodies have been observed in secretory vesicles isolated from rat insulinomas and in extracts of whole bovine pancreas (Nolan et al. 1985; Hutton et al. 1985). On the other hand, immunoblot studies with extracts of human endocrine pancreas indicated that the molecular size (75000) of the pancreatic chromogranin A was identical to that of the adrenal antigen (Hagn et al. 1986).

In the present paper we analyze the molecular properties of chormogranin A, chormogranin B and secretogranin II of the bovine pancreatic islet by immunochemical techniques and determine their cellular distribution by immunohistochemistry.

Materials and methods

Immunoblotting procedures

Fresh pancreatic tissue from fetal calves (body weight about 6 kg) was obtained at the local slaughterhouse in Ulm. It was kept in an icecold medium (20 m*M* Mops, 0.5 m*M* EGTA, 340 m*M* sucrose, 1 m*M* PMSF, pH 7.3) and transported to the laboratory. The tissue was chopped into small pieces using sissors. Homogenization of the fetal tissue (10 g tissue/30 ml medium) was performed in a loose-fitting Teflon-to-glass homogenizer by 5 downward strokes. The homogenate was centrifuged at $2500 \times g_{av}$ for 5 min. The supernatant was centrifuged for 1 h at $100000 \times g_{av}$. The pellet was resuspended in water (tenfold volume), boiled for 5 min and spun down for 10 min at $2500 \times g_{av}$. This procedure increases the yield of heat stable chromogranins/secretogranins (Rosa et al. 1985) and prevents proteolytic artifacts. The lyophilized supernatant was used for the electrophoresis. Analogous results were obtained when frozen pancreatic tissue was first freeze-dried, sliced with a razor into small pieces and homogenized with water followed by boling for 5 min. Soluble proteins of chromaffin granules

* To whom offprint requests should be sent

Table 1. Source and working dilutions of the antisera used for immunohistochemistry

Antiserum	*Source*	*Working dilution*	*Ref.*
Bovine chromogranin A	R. Fischer-Colbrie, Innsbruck	1:1000–1:4000	1
Bovine chromogranin B	R. Fischer-Colbrie, Innsbruck	1:1000	1
Bovine secretogranin II	R. Fischer-Colbrie, Innsbruck	1:1000–1:2000	2
Insulin A564	Dakopatts, Hamburg	1:1000–1:2000	–
Insulin LAA	Novo, Denmark	1:1000–1:4000	3
Glucagon A565	Dakopatts, Hamburg	1:2000–1:4000	4
Somatostatin	H. Etzrodt, Ulm	1:4000–1:8000	3, 4
Bovine pancreatic polypeptide (bPP)	R.E. Chance, Indianapolis	1:4000–1:8000	3, 4

References: 1 = Fischer-Colbrie and Frischenschalger 1985; 2 = Fischer-Colbrie et al. 1986; 3 = Grube et al. 1983; 4 = Grube et al. 1986

were isolated as already described (Fischer-Colbrie et al. 1985). Immunoreplicas were obtained by the method of Burnette (1981) as described previously (Fischer-Colbrie and Frischenschlager 1985). Instead of 5% bovine serum albumin 2% lipid free instant milk was used as a blocking reagent. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (Laemmli 1970) with an acrylamide gradient ranging from 10 to 17% and two-dimensional electrophoresis (according to O'Farrel 1975) were performed as already described in detail (Fischer-Colbrie et al. 1984).

Immunohistochemistry

Tissue preparation. Small tissue specimens of the bovine pancreas were quenched in melting Freon 22 precooled with liquid nitrogen, freeze-dried, and fixed by vapor-phase *p*-formaldehyde. Subsequently, the specimens were immersed in propylene oxide and embedded in epoxy resin (araldite). Specimens from the bovine adrenal medulla, which served as reference organ, were treated in the same way. Serial semithin sections were cut at 0.5 μm and mounted on microscopic slides by heat (90° C, 30 min). The resin was removed from the sections by Na-methoxide (Mayor et al. 1961).

Antisera. The antisera against chromogranin A and B, and secretogranin II were raised in the Department of Pharmacology (Innsbruck). The antisera against the established pancreatic hormones (insulin, glucagon, somatostatin, pancreatic polypeptide = PP) were either purchased commercially or kindly provided by other groups. All of these antisera (except the insulin antiserum A564) have been characterized during previous immunohistochemical studies. All antisera are listed in Table 1. The insulin antiserum A564 – as compared to the insulin antiserum LAA (Novo, Denmark) – showed immunostaining of pancreatic insulin (B-) cells exclusively.

Immunohistochemical protocol

Throughout all investigations the peroxidase-anti-peroxidase (PAP) technique of Sternberger (1979) was used. This technique has been modified and standardized for the application on semithin sections (Grube 1980). Serial semithin sections were sequentially immunostained for the chromogranins/secretogranin II or for the established pancreatic hormones. The working dilutions of the antisera (see Table 1) were determined by running ascending dilutions of the antisera from 1:1000 to 1:16000.

For immunohistochemistry of the chromogranins the buffer used as diluent and as rinsing solution turned out to be of crucial importance. Thus, the use of phosphate-buffered saline (PBS) produced immunostaining of low intensities in both the adrenal medulla and the pancreas. Therefore, 50 mM *Tris*-HCl (pH 7.6) was used as diluent for the chromogranin antisera and as rinsing solution.

Specifity controls

All controls to prevent non-specific immunostaining caused by the method or by antibody non-specifities were performed as recommended in the literature (Sternberger 1979; Grube 1980). The controls confirmed the specificities of all immunohistochemical findings (see also Grube et al. 1986).

Results

Immunoblotting

These studies were performed with boiled extracts (see method section) of fetal bovine pancreas. Figure 1 presents the results. With an antiserum against chromogranin A a strong reaction was obtained. In electrophoresis the major immunoreactive band behaved identically to chromogranin A of adrenal origin. In the pancreatic extracts a band migrating slower than chromogranin A also stained significantly. Such staining could also be seen with chromaffin granules when higher protein amounts were applied (results not shown). It has already been established that this band corresponds to a special form of chromogranin A, containing highly sulfated proteoglycan chains (Falkensammer et al. 1985b; Rosa et al. 1985). Figure 1 demonstrates that the pancreas contains a relatively high concentration of this special form of chromogranin A. In addition, both chromaffin granules and pancreas contain several smaller immunoreactive bands which at least for chromaffin granules have been shown to represent breakdown products formed by endogenous proteolytic processing of chromogranin A (see Winkler et al. 1986).

Chromogranin B and secretogranin II were also detected in immunoblots (Fig. 1). However the reaction was significantly less intense and therefore more protein had to be applied and longer exposure times had to be chosen. For both antigens the pancreas contained immunoreactive bands which correspond to the largest component present in chromaffin granules. For secretogranin II, one strongly staining band of M_r 31000 was present, which had no counterpart in chromaffin granules. In two dimensional immunoblots (Fig. 2) this immunoreactive band had a pI similar to that of the larger component, which is typical for breakdown products formed by endogenous proteases (see Winkler et al. 1986). The position (pI and M_r) of the larger component (SG II in Fig. 2) is identical to that of the adrenal antigen (compare Fischer-Colbrie et al. 1986).

Immunohistochemistry

Adrenal medulla. In the bovine adrenal medulla the antiserum against chromogranin A revealed an intense immunostaining of endocrine cells. All of these cells were also heavily immunoreactive for chromogranin B (Fig. 3a, b). For

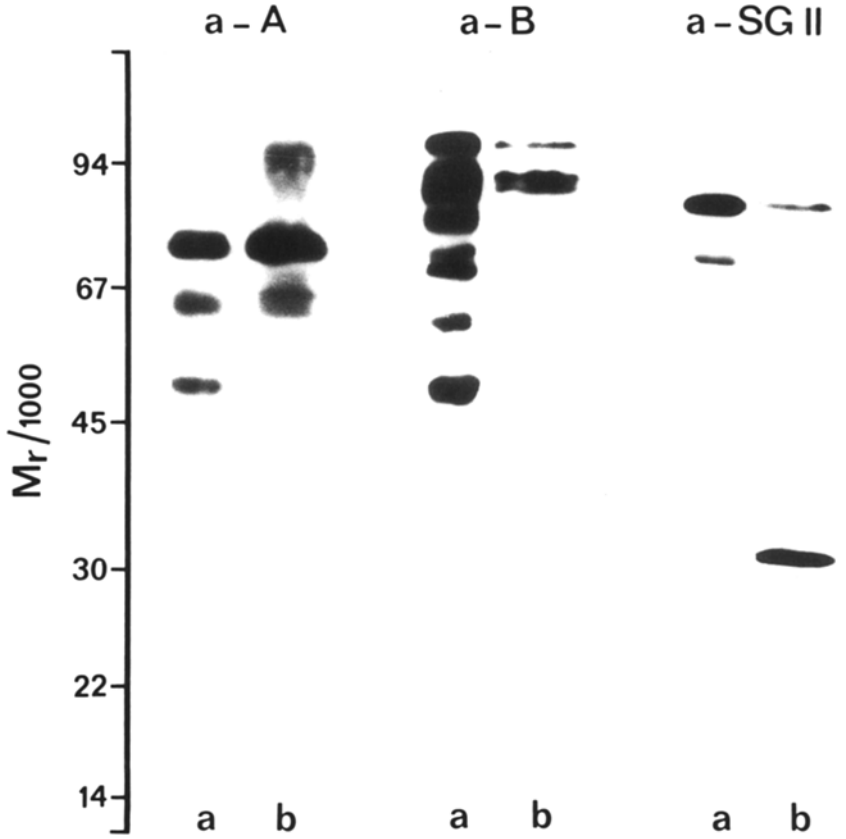


Fig. 1. Immunoblotting of soluble proteins of bovine chromaffin granules and of extracts of bovine fetal pancreas. The samples were subjected to one dimensional sodium dodecyl sulphate gel electrophoresis (acrylamide gradient from 10 to 17%) followed by immunoblotting. Antisera used were: Anti-chromogranin A (*a−A*), anti-chromogranin B (*a−B*), anti-secretogranin II (*a-SG II*). For each antiserum soluble proteins of chromaffin granules (**a**) and boiled extracts (218 µg protein for *a−A* and 715 µg for *a−B* and *a−SG II*) of fetal pancreas (**b**) were compared. In chromaffin granules the smaller immunoreactive proteins are due to breakdown products produced by the endogenous proteases of chromaffin granules (see Winkler et al. 1986)

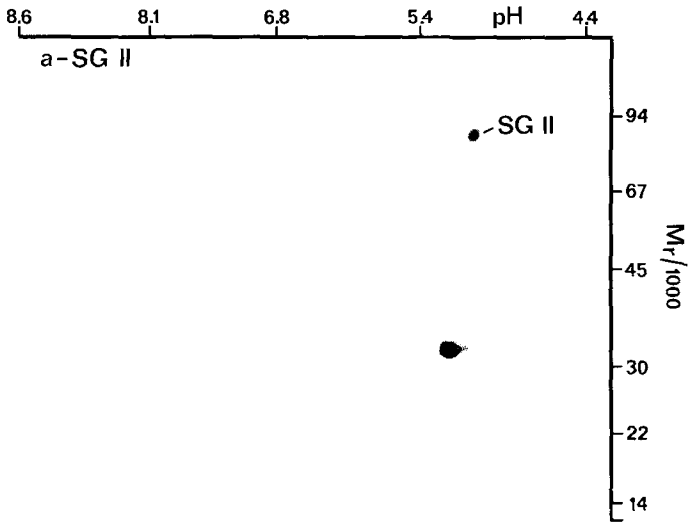


Fig. 2. Immunoblotting of a boiled extract of bovine fetal pancreas. The sample was subjected to two dimensional electrophoresis followed by immunoblotting. An antiserum against secretogranin II (*a-SG II*) was used

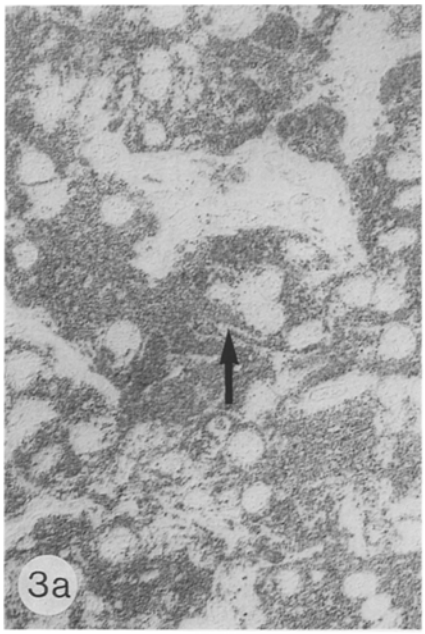
secretogranin II most of the cells exhibited only a weak staining, however, only in a minority of cells a strong immunoreactivity was observed. The latter cells were also immunoreactive for chromogranin A and chromogranin B (Fig. 3).

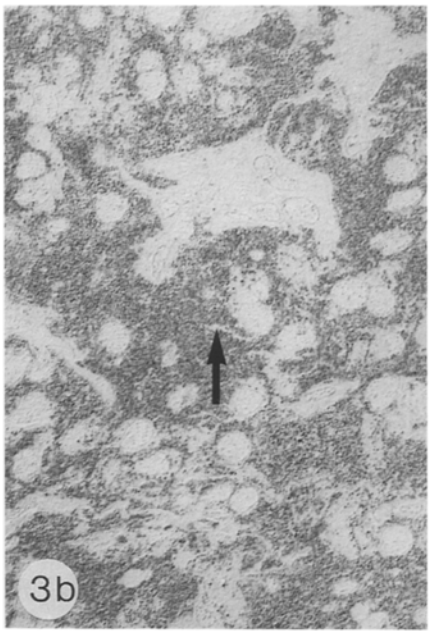
Endocrine pancreas. Confirming previous studies on this subject, the antiserum against chromogranin A immunostained all endocrine cell types of the pancreas except the PP-cells which only sporadically showed chromogranin A-like immunoreactivities (see Ehrhart et al. 1986). For chromogranin B, only very weak and diffuse staining of the islet cells was observed which did not allow to define an exact cellular localization.

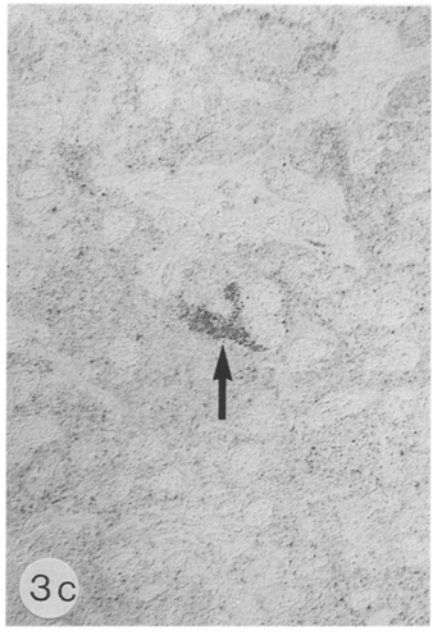
Concerning immunoreactivities for secretogranin II, immunostaining of the various endocrine cell types and the densities of staining were largely dependent on the dilution of the antiserum. When diluted 1:1000 (under these conditions a strong immunostaining was observed in the adrenal medulla), all insulin (B-)cells were only faintly positive for secretogranin II (Fig. 4). At higher dilutions of the secretogranin II antiserum (1:2000), B-cells were very weakly immunoreactive or immunostaining was totally absent (Fig. 6).

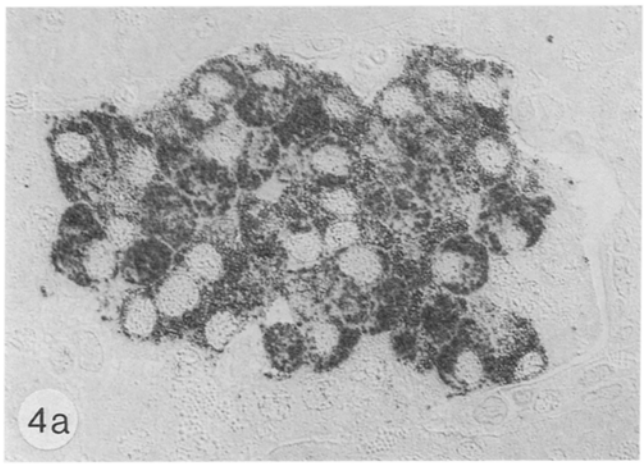
In addition to faintly secretogranin II-immunoreactive B-cells, other endocrine cells showed a dense immunostaining for secretogranin II. These cells turned out to be PP-cells when adjacent semithin sections were immunostained for pancreatic polypeptide (Figs. 5 and 7). In PP-cells, the densities of immunostaining were less dependent on the concentration of the antiserum. Hence, these cells – like adrenal medullary cells – showed secretogranin II-like immunoreactivities even when the antiserum used was diluted up to 1:4000. A certain proportion of secretogranin II-immunoreactive PP-cells exhibited very weak immunoreactivities for chromogranin A (Fig. 5), others were completely negative for chromogranin A (not shown). However, we also observed PP-cells which exhibited dense immunostainings for both chromogranin A and secretogranin II (Fig. 6). Finally, a minority of PP-cells were completely negative for chromogranin A and secretogranin II (see Table 2).

The glucagon (A-) cells were comparable to the B-cells.

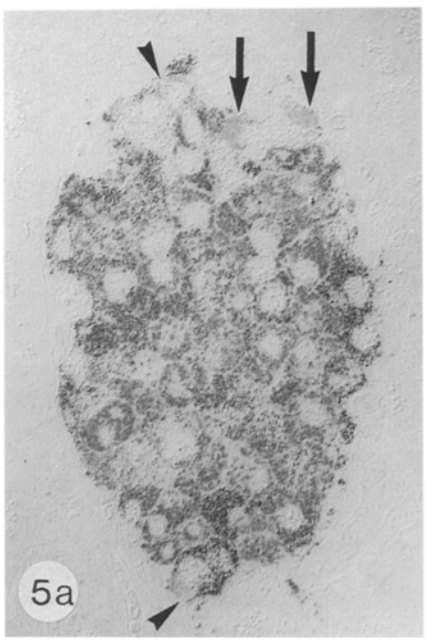

3a

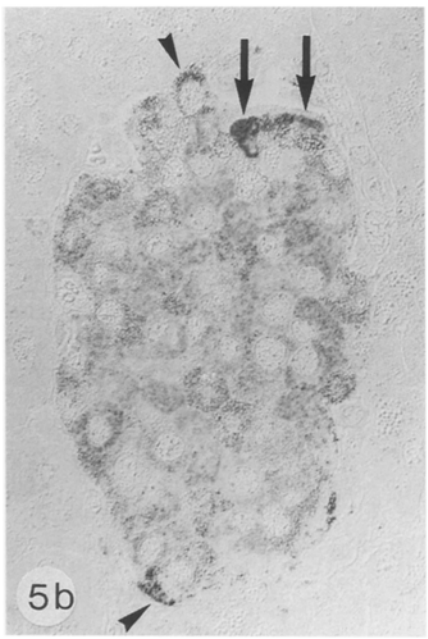

3b

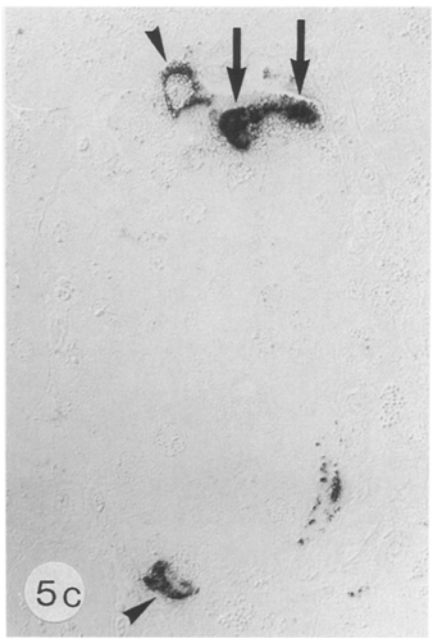

3c


4a


4b


5a


5b


5c

Both showed a moderate or faint immunostaining for secretogranin II only at higher concentrations of the secretogranin II antiserum (Fig. 7). Somatostatin (D-) cells, which show a moderate or faint immunostaining for chromogranin A (see also Ehrhart et al. 1986), were unreactive towards the secretogranin II antiserum (Fig. 8, Table 2).

Discussion

Boiled extracts of fetal bovine pancreas were used for immunoblotting. Boiling enriches the heat-stable chromogranins/secretogranins (Rosa et al. 1985) and in addition is efficient in preventing proteolytic degradation. Fetal pancreas was used since before birth the endocrine tissue represents a relatively high proportion of this organ. With such pancreatic extracts characteristic bands were obtained with antisera against chromogranin A and B and secretogranin II. A comparison with the proteins of chromaffin granules revealed that for each adrenal large molecular weight form a corresponding band exists in the pancreatic extract. This indicates that adrenal medullary and pancreatic endocrine cells synthesize chromogranin/secretogranin molecules of identical sizes. For chromogranin A this confirms results obtained with the human pancreas (Hagn et al. 1986), but disagrees with studies on rat insulinoma granules where the major immunoreactive band was found to be much smaller than the adrenal chromogranin A (Hutton et al. 1985). This may be due to a species difference, but may also be caused by proteolysis during preparation of the sample which is a serious problem in such studies.

Concerning the additional immunoreactive bands differences have been observed between adrenal medulla and the pancreas. In the latter organ a relatively large amount of a higher molecular weight form of chromogranin A is present probably representing a proteoglycan (Rosa et al. 1985; Falkensammer et al. 1985). In addition, the endocrine pancreas contained a significant amount of a smaller component of secretogranin II which was apparently absent from adrenal medulla. This is in accordance with previous observations (Fischer-Colbrie et al. 1985) that the proteolytic processing of the chromogranins varies considerably amongst endocrine organs.

The immunohistochemical findings established that specific staining was confined to the endocrine pancreas. In addition, our studies revealed that the various types of the chromogranins/secretogranin can be localized in different endocrine cell types: in agreement with previous results (Ehrhart et al. 1986; Grube et al. 1986) chromogranin A is mainly contained in A- and B-cells, to a lesser extent in D-cells, and only in a minority of PP-cells. On the other hand, secretogranin II occurs mainly in PP-cells and to a lesser extent in A- and B-cells, but not in D-cells. For chromogranin B the immunohistochemical staining was too weak to allow an exact cellular localization.

How can these immunohistochemical data be correlated with the immunoblotting results? As shown by the latter method chromogranin A was apparently present in the highest concentration. In agreement, a strong immunohistochemical staining was found in a majority of cells. Secretogranin II gave a much weaker reaction in immunoblotting. However as shown by immunohistochemistry it was concentrated in a small subpopulation of cells (PP-cells) which were strongly stained. Chromogranin B gave the weakest reaction in immunoblotting. Apparently within the islets this antigen is not concentrated in any of the cell populations, thus the small amounts present could not be visualized in a reliable way by immunohistochemistry.

Our studies demonstrate that the PP-cells, with respect to the distribution of these antigens are a heterogenous cell population. At least four different types of PP-cells could be discerned following this parameter: *1.* PP-cells with a strong immunostaining for both, chromogranin A and secretogranin II; *2.* PP-cells with a strong immunostaining for secretogranin II and a faint immunostaining for chromogranin A; *3.* PP-cells with a strong immunostaining for secretogranin II and no immunostaining for chromogranin A; *4.* PP-cells containing no immunoreactivities for chromogranin A and secretogranin II. At present, no information is available about the reasons for the heterogeneity of pancreatic PP-cells. Future investigations will show whether the heterogeneity of PP-cells depends on the different types of islets described in the bovine pancreas (see El-Nady et al. 1982; Bonner-Weir and Like 1980).

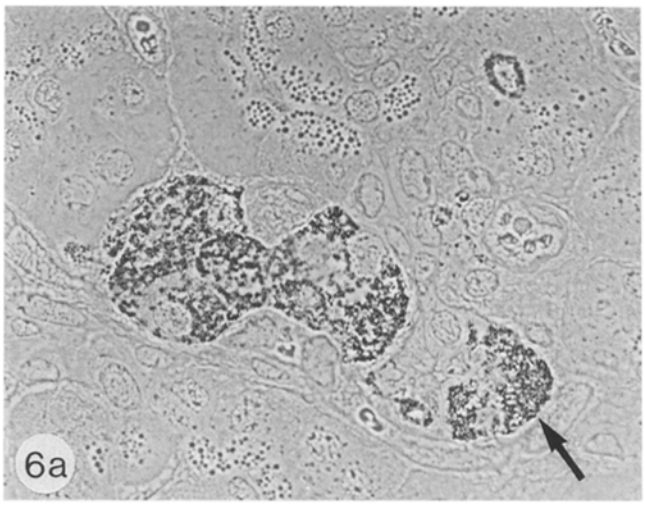
Insulin and glucagon cells – as compared to PP-cells – exhibit a less dense immunostaining for secretogranin II, which require in addition relatively high concentrations of the secretogranin II antiserum. These weak immunoreactivities may be due to the low amounts of secretogranin II in insulin and glucagon cells, or due to the inaccessibility of the epitopes of secretogranin II in the secretory vesicles of B- and A-cells. The hormones themselves could prevent interaction of the antibody with secretogranin II since the immunoreactivities for chromogranins in pancreatic endocrine cells often are inversely related to the densities of the corresponding peptide immunoreactivities (Grube et al. 1986).

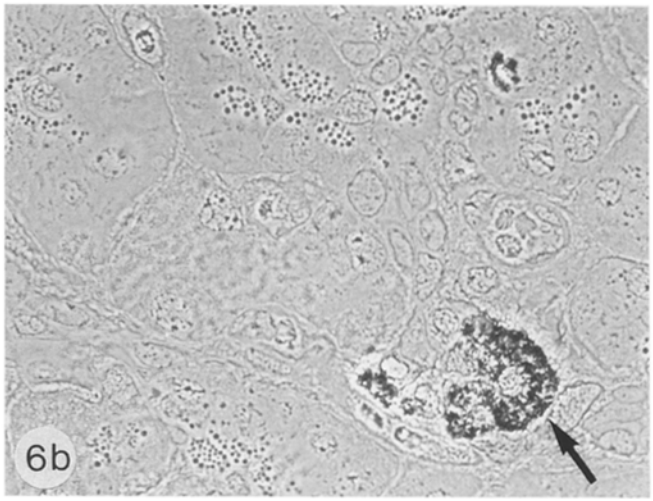
The fact that basically all pancreatic endocrine cell types contain chromogranins and secretogranin II should be seen

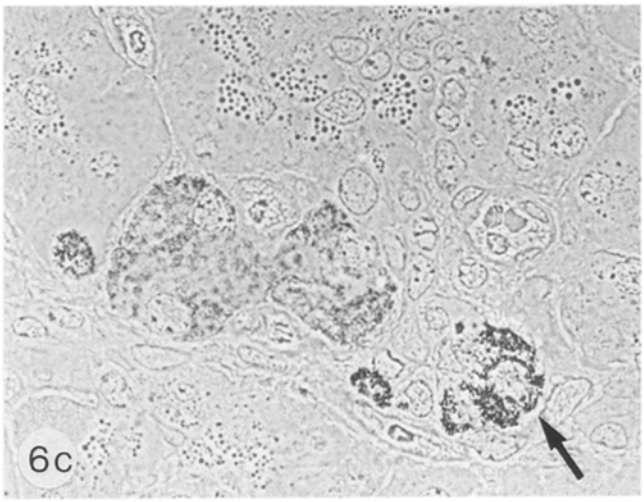
Fig. 3a–c. Chromogranin-like immunoreactivities in the bovine adrenal medulla. Three serial semithin sections were immunostained for chromogranin A (**a**), chromogrnain B (**b**), and secretogranin II (**c**). Adrenal medullary cells are strongly immunoreactive for chromogranin A and B, but most of them are only weakly stained for secretogranin II. *Arrows* in **a, b, c** indicate a cell which shows strong immunoreactivities for all three proteins. Interference phase contrast. × 540

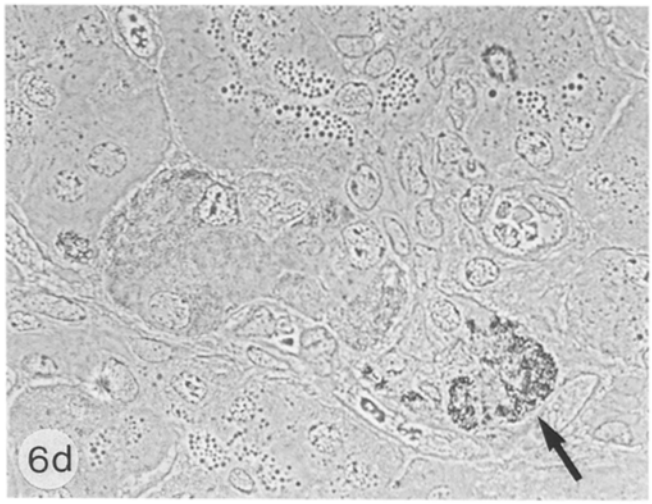
Fig. 4a, b. Immunoreactivities of insulin (B-) cells in the bovine endocrine pancreas for secretogranin II. Two serial semithin sections through an islet of Langerhans were immunostained for insulin (**a**) and secretogranin II (**b**). All B-cells show secretogranin II-immunoreactivities of low or moderate intensity. Interference phase contrast. × 650

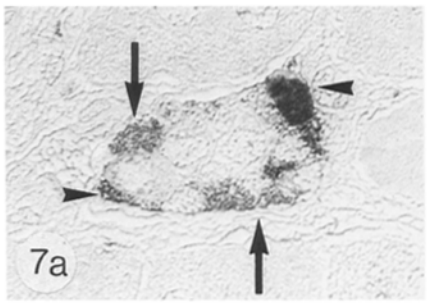
Fig. 5a–c. Immunoreactivities of bovine pancreatic endocrine cells for chromogranin A and secretogranin II. Three serial semithin sections were immunostained for chromogranin A (**a**), secretogranin II (**b**), and pancreatic polypeptide (=PP; **c**). The majority of the islet cells are strongly immunoreactive for chromogranin A and are less densely immunostained for secretogranin II. Endocrine cells showing a dense immunostaining for secretogranin II were identified as PP-cells on an adjacent section; they were only faintly immunoreactive for chormogranin A (*arrows* or *arrow-heads* in **a, b,** and **c**). Interference phase contrast. × 540

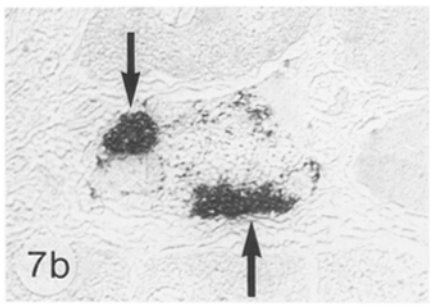

6a

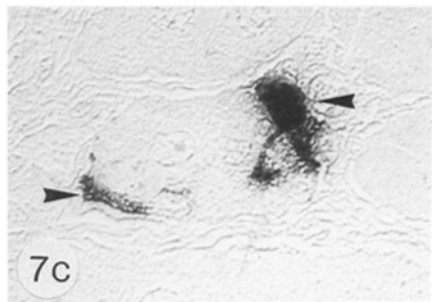

6b

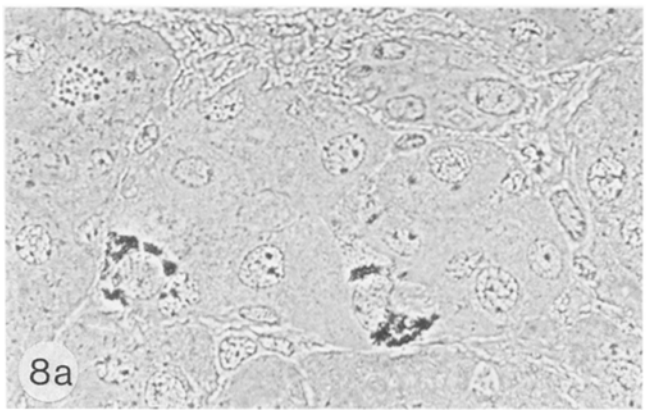

6c


6d


7a


7b


7c


8a

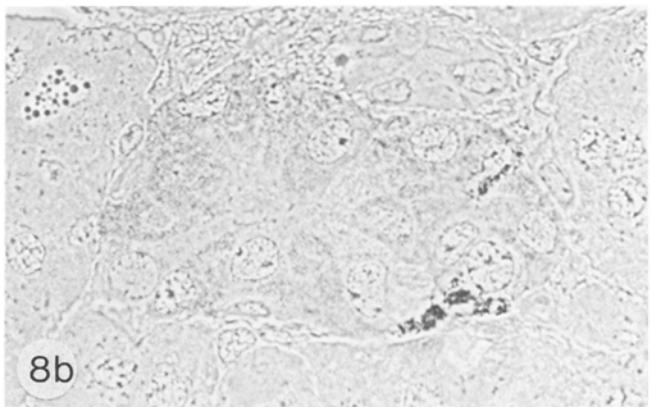

8b

Table 2. Immunoreactivities of the bovine pancreatic endocrine cell types towards chromogranin A and secretogranin II-antisera

Antiserum	Endocrine cell type			
	A	B	D	PP
Chromogranin A	+++	+++	++	++/+/0
Secretogranin II	+	+	0	+++/++/+/0

Explanation of symbols: A glucagon cells; B insulin cells; D somatostatin cells; PP pancreatic polypeptide cells; +++ dense immunostaining; ++ moderate immunostaining; + weak immunostaining; 0 unreactive

in the light of the possible intracellular function of these proteins. Within the chromaffin granules chromogranins may contribute to the osmotic pressure regulation (Helle et al. 1985). Apparently chromogranin A is also involved in the binding of Ca^{2+} within the chromaffin vesicles (Reiffen and Gratzl 1986a, b). Within the secretion granules of pancreatic endocrine cells chromogranin immunoreactivities are mainly localized in the periphery of the granules whereas their center (core) is heavily immunoreactive for the pancreatic hormones (Varndell et al. 1985; Ehrhart et al. 1986; Grube 1986). Interestingly, the intragranular localizations of the chromogranins and of Ca^{2+} (see Ravazzola et al. 1976; Lenzen and Klöppel 1984) are the same. This suggests that also in pancreatic endocrine cells the chromogranins are involved in the binding of Ca^{2+}.

In addition recent studies indicate that the newly discovered putative hormone pancreastatin (Tatemoto et al. 1986) exhibits a striking structural similarity to chromogranin A (Eiden 1987; Huttner and Benedum 1987). Pancreastatin inhibits the first rapid phase of glucose-stimulated insulin secretion (Tatemoto et al. 1986). Thus chromogranin A, secreted by pancreatic islet cells as a precursor of pancreastatin, may have an important function in the regulation of insulin secretion.

Acknowledgements. This work was supported by Deutsche Forschungsgemeinschaft (Gr 681) and by Forschungsschwerpunkt No. 24 of the State of Baden-Württemberg.

For expert technical assistance we are indebted to Mrs. D. von Mayersbach, Mrs. M. Meyer, Mrs. H. Peesel, and Mr. S. Gudat.

We are grateful to the personnel of the Ulm slaughterhouse for their help in obtaining pancreatic tissue. We thank Mrs. M. Henning for her helpful advice on how to word this manuscript and Mrs. B. Mader for typing it.

References

Benedum UM, Baeuerle PA, Konecki DS, Frank R, Powell J, Mallet J, Huttner WB (1986) The primary structure of bovine chromogranin A: A representative of a class of acidic secretory proteins common to a variety of peptidergic cells. EMBO J 5:1495–1502

Blaschko H, Comline RS, Schneider FH, Silver M, Smith AD (1967) Secretion of a chromaffin granule protein, chromogranin, from the adrenal gland after splanchnic stimulation. Nature 215:58–59

Bonner-Weir S, Like AA (1980) A dual population of islets of Langerhans in bovine pancreas. Cell Tissue Res 206:157–170

Burnette WN (1981) "Western Blotting": Electrophoretic transfer of proteins from sodium dodecylsulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal Biochem 112:195–203

Cohn DV, Zangerle R, Fischer-Colbrie R, Chu LLH, Elting JJ, Hamilton JW, Winkler H (1982) Similarity of secretory protein I from parathyroid gland to chromogranin A from adrenal medulla. Proc Natl Acad Sci USA 79:6056–6059

Ehrhart M, Grube D, Bader MF, Aunis D, Gratzl M (1986) Chromogranin A in the pancreatic islet: Cellular and subcellular distribution. J Histochem Cytochem 34:1673–1682

Eiden LE (1987) Is chromogranin a prohormone? Nature 325:301

Eiden LE, Huttner WB, Mallet J, O'Connor DT, Winkler H, Zanini A (1987) A nomenclature proposal for the chromogranin/secretogranin proteins. Neuroscience, in press

El-Nady E, Grube D, Maier V, Castillo O, Hafiez AA, Pfeiffer EF (1982) Two populations of islets in the calf pancreas. Diabetologia 23:165

Falkensammer G, Fischer-Colbrie R, Winkler H (1985a) Biogenesis of chromaffin granules: Incorporation of sulfate into chromogranin B and into a proteoglycan. J Neurochem 45:1475–1480

Fig. 6a–d. Immunoreactivities of bovine pancreatic B- and PP-cells for chromogranin A and secretogranin II. Four serial semithin sections were immunostained for chromogranin A (**a**), pancreatic polypeptide (**b**), and secretogranin II (antiserum dilution 1:1000=**c**; antiserum dilution 1:2000=**d**). All PP-cells (*arrows*) shown in this series exhibit strong immunoreactivities for chromogranin A and secretogranin II. The remaining cells (B-cells, as identified on an adjacent section immunostained for insulin; not shown) show a dense immunostaining for chromogranin A, and a faint immunostaining for secretogranin II. In contrast to PP-cells, B-cells are immunoreactive for secretogranin II only at higher concentrations of the secretogranin II-antiserum. Phase contrast. ×810

Fig. 7a–c. Immunoreactivities of bovine pancreatic glucagon (A−) cells for secretogranin II. Three serial semithin sections were immunostained for secretogranin II (**a**), glucagon (**b**) and pancreatic polypeptide (**c**). Out of the endocrine cells immunoreactive for secretogranin II the less densely immunostained cells were identified as A-cells (*arrows* in **a** and **b**). The endocrine cells showing a strong immunostaining for secretogranin II are PP-cells (*arrow-heads* in **a** and **c**). Interference phase contrast. ×940

Fig. 8a, b. Immunoreactivities of bovine pancreatic somatostatin (D-) cells for secretogranin II. Two serial semithin sections were immunostained for somatostatin (**a**) and secretogranin II (**b**) Both D-cells are non-immunoreactive for secretogranin II. The secretogranin II-immunoreactive endocrine cell (**b**) was identified as a PP-cell on an adjacent section immunostained for pancreatic polypeptide (not shown). Phase contrast. ×940

Falkensammer G, Fischer-Colbrie R, Richter K, Winkler H (1985b) Cell-free and cellular synthesis of chromogranin A and B of bovine adrenal medulla. Neuroscience 14:735–746

Fischer-Colbrie R, Frischenschlager I (1985) Immunological characterization of secretory proteins of chromaffin granules: Chromogranins A, chromogranins B, and enkephalin-containing peptides. J Neurochem 44:1854–1861

Fischer-Colbrie R, Zangerle R, Frischenschlager I, Weber A, Winkler H (1984) Isolation and immunological characterization of a glycoprotein from adrenal chromaffin granules. J Neurochem 42:1008–1016

Fischer-Colbrie R, Lassmann H, Hagn C, Winkler H (1985) Immunological studies on the distribution of chromogranin A and B in endocrine and nervous tissue. Neuroscience 16:547–555

Fischer-Colbrie R, Hagn C, Kilpatrick L, Winkler H (1986) Chromogranin C: A third component of the acidic proteins in chromaffin granules. J Neurochem 47:318–321

Grube D (1980) Immunoreactivities of gastrin (G-) cells. II. Nonspecific binding of immunoglobulins to G-cells by ionic interactions. Histochemistry 66:149–167

Grube D, Eckert I, Speck PT, Wagner H-J (1983) Immunohistochemistry and microanatomy of the islets of Langerhans. Biomed Res (Suppl) 4:25–36

Grube D, Aunis D, Bader MF, Cetin Y, Jörns A, Yoshie S (1986) Chromogranin A (CGA) in gastro-entero-pancreatic (GEP) endocrine system. I. CGA in the mammalian endocrine pancreas. Histochemistry 85:441–452

Hagn C, Schmid KW, Fischer-Colbrie R, Winkler H (1986) Chromogranin A, B, and C in human adrenal medulla and endocrine tissues. Lab Invest 55:405–411

Helle KB (1966) Some chemical and physical properties of the soluble protein fraction of bovine adrenal chromaffin granules. Mol Pharmacol 2:298–310

Helle KB, Reed RK, Pihl KE, Serck-Hanssen G (1985) Osmotic properties of the chromogranins and relation to osmotic pressure in catecholamine storage granules. Acta Physiol Scand 123:21–33

Huttner WB, Benedum UM (1987) Chromogranin A and pancreastatin. Nature 325:305

Hutton JC, Hansen F, Peshavaria M (1985) β-granins: 21 kDa co-secreted peptides of the insulin granule closely related to adrenal medullary chromogranin. FEBS Lett 188:336–340

Iacangelo A, Affolter HU, Eiden LE, Herbert E, Grimes M (1986) Bovine chromogranin A sequence and distribution of its messenger RNA in endocrine tissues. Nature 323:82–86

Kilpatrick L, Gavine F, Apps D, Phillips J (1983) Biosynthetic relationship between the major matrix proteins of adrenal chromaffin granules. FEBS Lett 164:383–388

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lassmann H, Hagn C, Fischer-Colbrie R, Winkler H (1986) Presence of chromogranin A, B and C in bovine endocrine and nervous tissues: A comparative immunohistochemical study. Histochem J 18:380–386

Lenzen S, Klöppel G (1984) Intracellular localization of calcium in pancreatic B-cells in relation to insulin secretion by the perfused ob/ob mouse pancreas. Endocrinology 114:1012–1020

Lloyd RV, Mervak T, Schmidt K, Warner TFCS, Wilson BS (1984) Immunohistochemical detection of chromogranin and neuron-specific enolase in pancreatic endocrine neoplasms. Am J Surg Pathol 8:607–614

Mayor HD, Hampton JC, Rosario B (1961) A simple method for removing the resin from epoxy-embedded tissue. J Cell Biol 9:909–910

Nolan JA, Trojanowski JQ, Hogue-Angeletti R (1985) Neurons and neuroendocrine cells contain chromogranin: Detection of the molecule in normal bovine tissues by immunochemical and immunohistochemical methods. J Histochem Cytochem 33:791–798

O'Connor DT (1983) Chromogranin: Widespread immunoreactivity in polypeptide hormone producing tissues and in serum. Regul Pept 6:263–280

O'Connor DT, Burton D, Deftos LJ (1983) Chromogranin A: Immunohistology reveals its universal occurrence in normal polypeptide hormone producing endocrine glands. Life Sci 33:1657–1663

O'Farell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

Ravazzola M, Malaisse-Lagae F, Amherdt M, Perrelet A, Malaisse WJ, Orci L (1976) Patterns of calcium localization in pancreatic endocrine cells. J Cell Sci 27:107–117

Reiffen FU, Gratzl M (1986a) Chromogranins, widespread in endocrine and nervous tissue, bind Ca^{2+}. FEBS Lett 195:327–330

Reiffen FU, Gratzl M (1986b) Ca^{2+} binding to chromaffin vesicles matrix proteins: Effect of pH, Mg^{2+}, and ionic strength. Biochemistry 25:4402–4406

Rindi G, Buffa R, Sessa F, Tortora O, Solcia E (1986) Chromogranin A, B and C immunoreactivities of mammalian endocrine cells. Distribution, distinction from costored hormones/prohormones and relationship with argyrophil component of secretory granules. Histochemistry 85:19–28

Rosa P, Hille A, Lee RWH, Zanini A, De Camilli P, Huttner WB (1985) Secretogranins I and II: Two tyrosine-sulfated secretory proteins common to a variety of cells secreting peptides by the regulated pathway. J Cell Biol 101:1999–2011

Smith AD, Winkler H (1967) Purification and properties of an acidic protein from chromaffin granules of bovine adrenal medulla. Biochem J 103:483–492

Smith WJ, Kirshner N (1967) A specific soluble protein from the catecholamine storage vesicles of bovine adrenal medulla. Mol Pharmacol 3:52–62

Sternberger LA (1979) Immunocytochemistry, 2nd edn. J Wiley, New York

Tatemoto K, Efendić S, Mutt V, Makk G, Feistner GJ, Barchas JD (1986) Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. Nature 324:476–478

Varndell IM, Lloyd RV, Wilson BS, Polak JM (1985) Ultrastructural localization of chromogranin: A potential marker for the electron microscopical recognition of endocrine cell secretory granules. Histochem J 17:981–992

Wilson BS, Lloyd RV (1984) Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. Am J Pathol 115:458–468

Winkler H, Apps DK, Fischer-Colbrie R (1986) The molecular function of adrenal chromaffin granules: Established facts and unresolved topics. Neuroscience 18:261–290