
The Journal of **Histochemistry**
and
Cytochemistry

JANUARY 1988
Volume 36.

Rapid Communication

Cellular Distribution and Amount of Chromogranin A in Bovine Endocrine Pancreas¹

MONIKA EHRHART, ANNE JÖRNS, DIETRICH GRUBE, and MANFRED GRATZL²

*Abteilung Anatomie und Zellbiologie der Universität Ulm, D-7900 Ulm, Bundesrepublik Deutschland (ME, MG), and
Abteilung Anatomie 1 der Med. Hochschule, D-3000 Hannover, Bundesrepublik Deutschland (AJ, DG).*

Received for publication November 13, 1987; accepted November 20, 1987 (7C1202).

We determined the cellular distribution and the amount of chromogranin A in endocrine cells of bovine pancreas using a polyclonal antibody against bovine adrenomedullary chromogranin A. The relative amounts of chromogranin A in the different cells of the endocrine pancreas were determined by computer-assisted analyses of the optical densities of the immunoreactivities in the stained sections. More than 80% of the immunoreactive chromogranin A was located in the pancreatic B-cells. In immunoblots of acid tissue extracts, only

one chromogranin A band (MW 74 kD) was observed. Quantification of the immunoblots revealed that 3 µg of chromogranin A and 918 µg of insulin were present per gram pancreas (wet weight), equivalent to a molar ratio of 460 µmol chromogranin A per mol insulin. (*J Histochem Cytochem* 36:467-472, 1988)

KEY WORDS: Chromogranin A; Pancreatic islet (bovine); B-cells; Immunohistochemistry; Immunoblots; Quantification.

Introduction

Chromogranin A is the main component of the family of highly acidic proteins that are co-stored and co-released with the catecholamines from adrenomedullary chromaffin cells (Winkler et al., 1986; Blaschko et al., 1967). Recent investigations have shown that chromogranin A is also widely distributed in other endocrine tissues, such as the pancreatic islet cells (cf. Grube et al., 1986; Cohn et al., 1984; Wilson and Lloyd, 1984; O'Connor, 1983). Immunohistochemical studies of bovine endocrine pancreas have demonstrated that chromogranin A is present in cells producing insulin (B-cells), glucagon (A-cells), and somatostatin (D-cells) and in a few cells containing pancreatic polypeptide (PP-cells) (Yoshie et al., 1987; Ehrhart et al., 1986). Chromogranin A is co-stored in the same secretory granules as the established hormones (Ehrhart et al., 1986).

A hormonal role for chromogranin A has yet to be established. Within the adrenomedullary chromaffin secretory granules, chromogranin A is presumably involved in osmotic pressure regulation (Helle et al., 1985) and in the binding of Ca²⁺ (Reiffen and Gratzl, 1986a, 1986b; Bulenda and Gratzl, 1985). More recently, chromogranin A has been shown to contain a sequence of amino acid residues (Eiden, 1987; Huttner and Benedum, 1987; Benedum et al., 1986; Iacangelo et al., 1986) that appears homologous to pancreastatin,

which inhibits glucose-stimulated insulin secretion in rat pancreas (Tatemoto et al., 1986). Therefore, a role for chromogranin A in the regulation of hormone secretion is likely.

For further elucidation of the functional aspects of the pancreatic chromogranin A, it is essential to quantify this protein relative to the well-established hormones. In the present study we report on the amounts of chromogranin A relative to the insulin content of the pancreatic islet and on the localization of chromogranin A primarily to the insulin-storing B-cells.

Materials and Methods

Immunohistochemistry. The immunohistochemical stainings were performed in serial semi-thin sections cut at 0.25 µm and 0.5 µm from tissue blocks that had been freeze-dried, fixed by vapor-phase *p*-formaldehyde, and embedded in epoxy resin (Araldite). Details of the methods applied have been published previously (Ehrhart et al., 1986; Grube and Kusumoto, 1986). The antisera used included anti-insulin (A 564; Dakopatts, Hamburg, FRG) diluted 1:2000, anti-glucagon (A 565; Dakopatts) diluted 1:3000, anti-somatostatin (kindly provided by Dr. Etzrodt, Ulm, FRG) diluted 1:4000, anti-bovine pancreatic polypeptide (bPP; from Dr. Chance, Indianapolis, IN) diluted 1:8000, and anti-chromogranin A (CGA; provided by Dr. D. Aunis, Strasbourg, France) diluted 1:16,000. The serial semi-thin sections were sequentially immunostained for chromogranin A and the established pancreatic hormones by the peroxidase-antiperoxidase (PAP) technique of Sternberger (1986).

Quantitative Immunocytochemistry. Taking into account the basic prerequisites for quantification in immunocytochemistry (see Sternberger, 1986), we developed a technique for quantitative determinations of optical densities in immunoperoxidase-stained semi-thin sections by computer-assisted analyses. The principles of the present method have been pub-

¹ Supported by Deutsche Forschungsgemeinschaft (Gr 681) and by Forschungsschwerpunkt No. 24 of the State of Bad-Württemberg.

² Correspondence to: Prof. Dr. M. Gratzl, Abteilung Anatomie und Zellbiologie der Universität Ulm, Postfach 4066, D-7900 Ulm, FRG.

lished previously (Grube and Kusumoto, 1986), and details will be published elsewhere (Grube et al., in preparation). Briefly for quantification we found the most useful sections to be semi-thin sections, cut at 0.25 μm , which were immunostained by highly diluted antisera using a standardized immunohistochemical protocol. Optical density measurements were performed with the "Interactive Image Analysis System IBAS" (Zeiss-Kontron, FRG) equipped with a Photomicroscope II (Zeiss, FRG) and a video camera. The regions or cells to be measured were entered into the system at a final magnification of 1:1600. The corresponding images were processed by a 15-step measuring program; optical densities were given as differential histograms in arbitrary units (0 = black, 255 = white; see Figure 2). The densities of chromogranin A immunoreactivities were determined in 174 endocrine cells which, in appropriately immunostained adjacent semi-thin sections, had been identified as 103 B-cells, 47 A-cells, and 24 D-cells.

Immunochemical Analyses. Fresh pancreatic tissues from calves were obtained at the local slaughterhouse in Ulm. The tissues were placed in an ice-cold medium (20 mM Mops, 0.5 mM EGTA, 340 mM sucrose, 1 mM PMSF, pH 7.3) and transported to the laboratory. Chromogranin extraction was carried out in acidic medium to prevent proteolysis. The tissue was homogenized at approximately pH 1 (10 g tissue in 5 ml medium + 20 ml 1 N HCl) using an Ultra-Turrax (Janke and Kunkel KG; Stetten, FRG) and was centrifuged at $100,000 \times g_{av}$ for 30 min at 4°C. The supernatant was used for SDS-PAGE.

Soluble proteins of chromaffin granules were isolated as described previously (Reiffen and Gratzl, 1986a). For quantification of pancreatic chromogranin A, chromaffin vesicle content was used as reference; 40% of its protein is chromogranin A (see Winkler and Westhead, 1980). A calibration curve was constructed using dilutions containing 1.2 $\mu\text{g}/\text{ml}$ –15 $\mu\text{g}/\text{ml}$ of protein. The reference probes and the pancreatic extracts were dissolved in extraction medium containing 5% mercaptoethanol, 2% SDS, 0.001% bromphenol blue and 10% sucrose in 62.5 mM Tris-HCl, pH 6.8. Twenty μl of each sample were subjected to SDS-PAGE (Laemmli, 1970) in 11% acrylamide gels. Immunoblotting was carried out following the method of Burnette (1981): The proteins were transferred to nitrocellulose (Schleicher and Schüll; Dassel, FRG) in the electrode buffer containing 25 mM Tris, 192

mM glycine, 20% methanol, pH 8.3, at 15 V for 16–18 hr. The nitrocellulose sheets were incubated in TBS-Tween (150 mM NaCl, 50 mM Tris, 0.05% Tween-20, pH 7.6) containing 2.5% lipid-free instant milk instead of 5% BSA as blocking reagent, four times for 5 min at room temperature, and were washed for 5 min in TBS-Tween containing 0.5% instant milk. The sheets were then incubated overnight at 4°C with the rabbit anti-bovine chromogranin A antiserum diluted 1:5000 in TBS-Tween. The antiserum, characterized in a previous investigation (Ehrhart et al., 1986), was kindly provided by M.F. Bader and D. Aunis (Centre de Neurochimie, Strasbourg). The nitrocellulose was washed four times for 15 min in TBS-Tween and was then immersed in TBS-Tween containing 1 $\mu\text{Ci}/\text{ml}$ of iodinated protein A for 1 hr (prepared according to Greenwood et al., 1963, using ^{125}I). After washing in TBS-Tween three times for 1 hr, the nitrocellulose was dried and exposed to Kodak X Omat AR film overnight. For quantification, the labeled bands were cut out of the nitrocellulose and counted for radioactivity in a gamma counter. The values obtained for the pancreatic extracts were compared with the calibration curve constructed using bovine chromaffin vesicle contents.

Insulin extraction was carried out in acidified ethanol (Romans et al., 1940): 5 g of pancreatic tissue were homogenized in 50 ml of acid/alcohol solution (80% ethanol, 1.5% conc. HCl (v/v), kept for 1 hr on ice, sonicated for 5 min, and centrifuged for 30 min at $100,000 \times g_{av}$. The insulin content of the supernatant was determined using a radioimmunoassay (Isotopen-Diagnostik; Dreieich, FRG). Protein was determined by the method of Lowry (Lowry et al., 1951), using BSA as standard.

Results

Confirming our previous findings (Yoshie et al., 1987; Ehrhart et al., 1986), chromogranin A immunoreactivities were regularly found in B-, A-, and D-cells, whereas only a minority of PP-cells showed less dense immunostaining. In sections immunostained by highly diluted chromogranin A antiserum (1:16,000), it was obvious that most of the B-cells were heavily immunoreactive and that A-cells

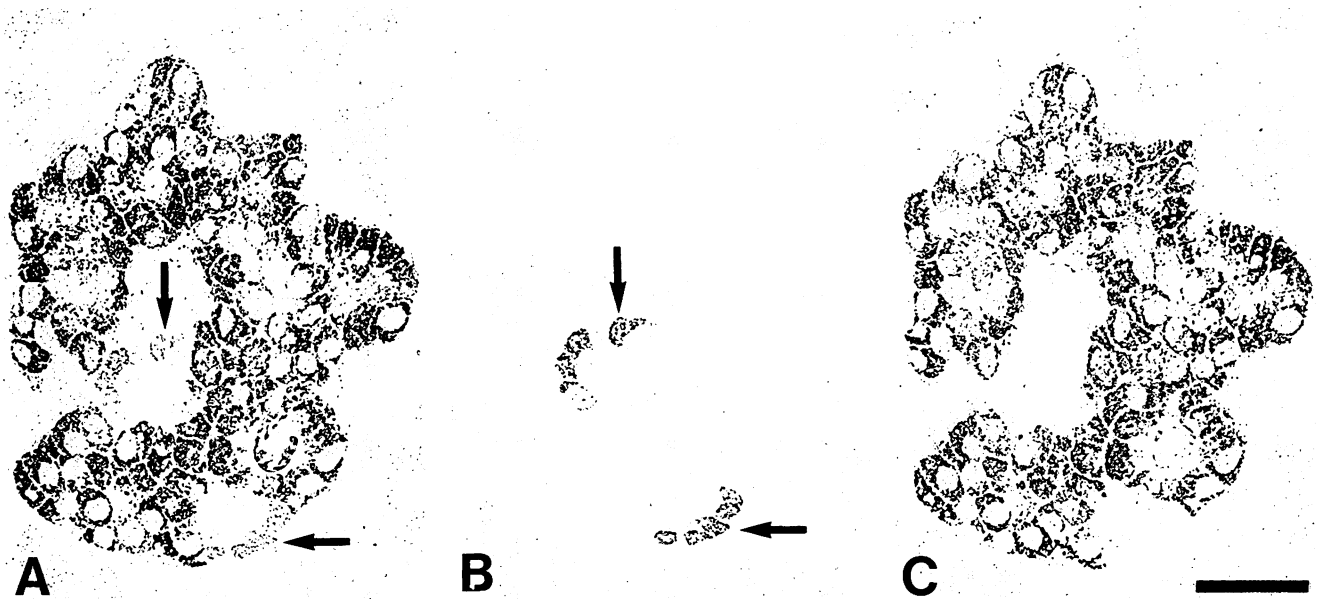


Figure 1. Three serial semi-thin (0.25 μm) sections through a bovine pancreatic islet. Sections were immunostained for chromogranin A (A), glucagon (B), and insulin (C). All glucagon and insulin cells contain chromogranin A immunoreactivities. Glucagon cells (arrows in A and B) are less densely stained than insulin cells. Phase-contrast microscopy. Original magnification $\times 495$. Bar = 30 μm .

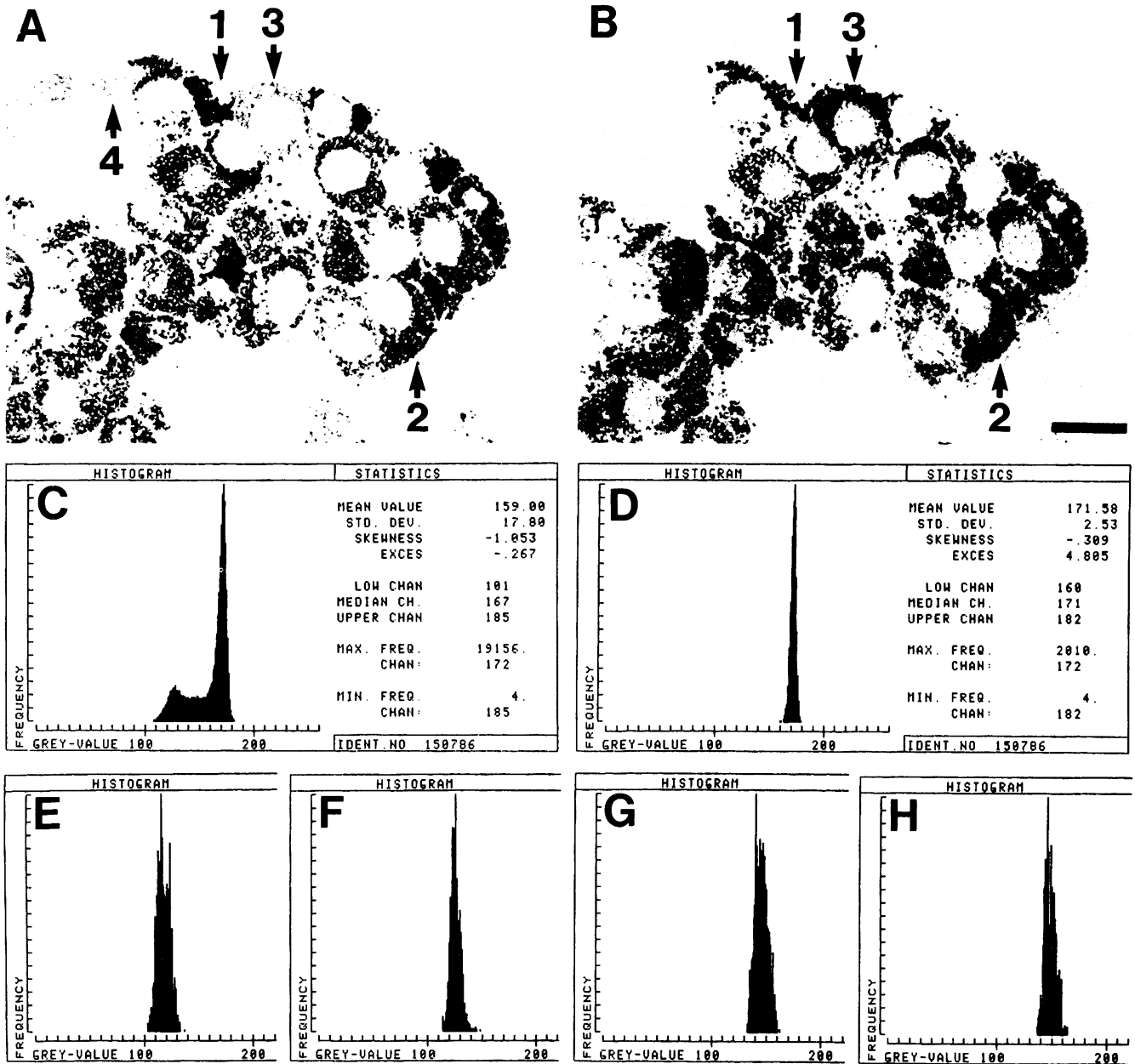


Figure 2. Computer-assisted analyses of optical density of chromogranin A immunoreactivities in bovine pancreatic insulin and glucagon cells. Two serial semi-thin (0.25 μ m) sections were immunostained for chromogranin A (A) and for insulin (B). The histogram in (C) corresponds to the whole visual field as shown in (A). The histogram in (D) depicts the optical densities in non-immunoreactive areas (= background staining). Optical densities were measured in four endocrine cells, indicated by 1, 2, 3, and 4 (A). Cells 1, 2, and 3 correspond to insulin cells, as shown in (B); cell 4 is a glucagon cell, as identified in an adjacent semi-thin section immunostained for glucagon (not shown). Among the insulin cells, the densities of chromogranin A immunoreactivities are heterogeneous. (E) Histogram of cell 1, mean value of the optical density 118 (arbitrary units). (F) Histogram of cell 2, mean value 126. (G) Histogram of cell 3, mean value 145. (H) Histogram of cell 4, mean value 149. (A, B): brightfield illumination. Original magnification \times 1125. Bar = 10 μ m.

were less densely stained (Figure 1). Likewise, D-cells were faintly stained (not shown).

At higher magnifications (Figures 2A and 2B), B-cells turned out to be heterogeneous in their densities of chromogranin A immunoreactivities (Figures 2E-2G, Figure 3). Chromogranin A immunoreactivities in A- and D-cells were weaker and more uniform

(Figures 2A and 2H, Figure 3). Therefore, as observed immunohistochemically and quantitatively, chromogranin A in the pancreas is mainly localized to B-cells.

With the antibody raised against bovine chromogranin A, a strong reaction was obtained in the immunoblots. The main immunoreactive bands of the adrenomedullary vesicle content and

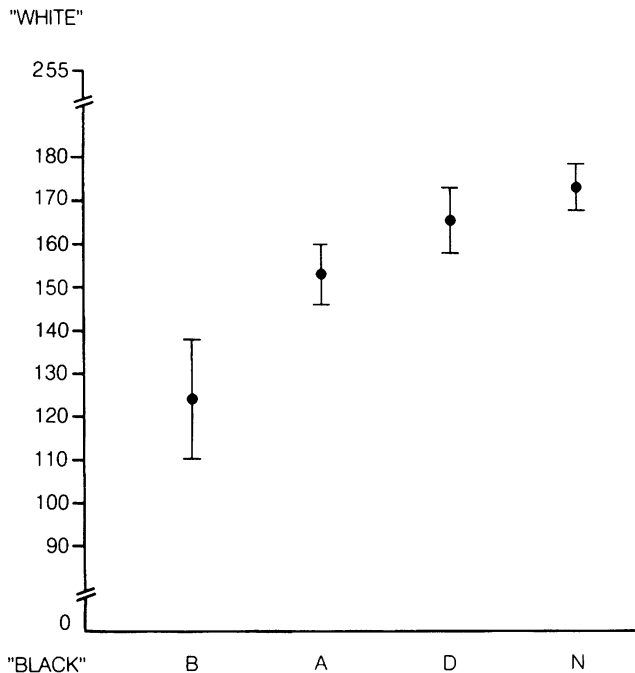


Figure 3. Optical densities of chromogranin A immunoreactivities in bovine pancreatic insulin (B), glucagon (A), and somatostatin (D) cells, analyzed according to the method as illustrated in Figure 2. Mean values \pm SEM, given in arbitrary units, for the endocrine cell types were: 124.2 (14.4) for B-cells ($n = 103$); 153.4 (7.1) for A-cells ($n = 47$); 165.2 (7.3) for D-cells ($n = 24$). Background staining (N = "noise"), 173.1 (5.4).

of pancreatic extracts moved identically. Both have an apparent molecular weight of 74 KD. In the adrenal medulla, a band of about 60 KD is also present. This band is not prominent in the pancreas (Figure 4).

For quantification, nitrocellulose strips containing the two major chromogranin A bands (74 KD and 60 KD) were cut out (Figure 5) and counted. The radioactive labeling was logarithmically proportional to the bovine chromaffin granule protein, in the range of 2.5 μ g protein/ml to 15 μ g protein/ml (Figure 2).

Using this standard curve, 96.7 ng chromogranin A/mg protein (± 24.1 ; $n = 12$) was found in the pancreatic extracts. Per gram wet weight, the pancreas contained 3.2 μ g chromogranin A (± 1.3 μ g; $n = 12$) and 918 μ g insulin (± 65.9 μ g; $n = 12$). Therefore, the bovine pancreas contained 3.5 μ g chromogranin A per mg of insulin (Table 1).

Chromogranin A has an apparent molecular weight of 74 KD in SDS-PAGE; however, an actual molecular weight of 48 KD has been obtained from sequence analyses of the cDNA encoding bovine chromogranin A (Benedum et al., 1986; Iacangelo et al., 1986). Therefore, on the molar level the endocrine pancreas contains 460 μ mol chromogranin A per mol insulin.

Discussion

Our analysis of the chromogranin A immunoreactivities of pancreatic endocrine cells indicates that chromogranin A is mainly localized in the insulin-containing B-cells. Taking into account that about

70–80% of the islet cells in bovine pancreas are accounted for by B-cells (Falkmer et al., 1984), it seems reasonable to assume that most of the pancreatic chromogranin A is co-stored with insulin in the secretory granules of the B-cells. Our quantifications indicate a ratio of chromogranin A:insulin of 0.35% by weight for the whole tissue, i.e., the B-cells account for about 3.0 μ g chromogranin A per mg of stored insulin. From the work of Hutton (1984), it is known that the secretory granules in rat B-cells contain about 500 μ g insulin per mg of protein, suggestive of a chromogranin A concentration of 0.15% by weight of granule protein. Since rat insulin-containing secretory granules contain about 46 nmol Ca^{2+} per mg of protein (Andersson et al., 1982), this would give a ratio of Ca^{2+} :chromogranin A of 33 μ mol/mg. Such a ratio is more than

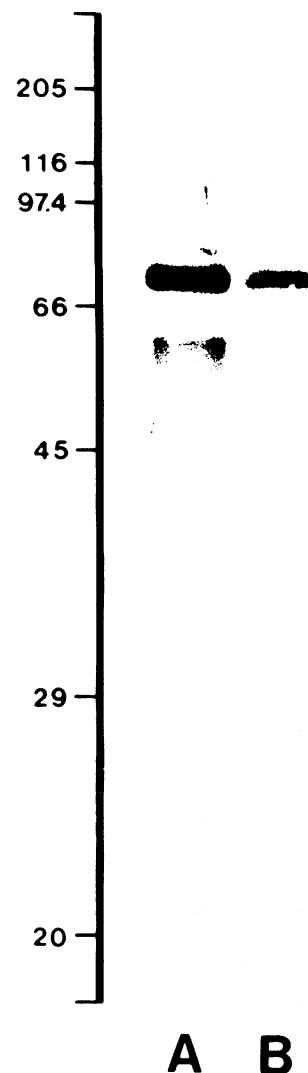


Figure 4. Immunoblotting of soluble proteins of bovine chromaffin vesicles and of an extract of bovine pancreas. Samples were subjected to PAGE (11% acrylamide) followed by blotting and immunostaining with the chromogranin A antiserum. (A) Chromaffin vesicle content, 0.29 μ g protein; (B) pancreatic extract, 100 μ g protein.

100-fold greater than the maximal binding of Ca²⁺ to adrenomedullary chromogranin A (Reiffen and Gratzl, 1986a, 1986b), which indicates that calcium binding proteins other than chromogranin A may also be present in the periphery of the protein core of the insulin-storing granules to account for the peripheral concentration of Ca²⁺ (Ehrhart et al., 1986; Lenzen and Klöppel, 1984; Ravazola et al., 1976).

The glandular content of insulin presently obtained (918 µg per g wet weight of pancreatic tissue) is in accordance with the value reported by Burgermeister et al. (1975). The islets represent about 5% of the total tissue weight (Bonner-Weir and Like, 1980). Consequently, the average concentration of insulin in the islet would be approximately 3 mmol/liter. Analogously, the content of chromogranin A (3.2 µg per g wet weight; Table 1) would correspond to 1.4 µmol/liter, assuming a MW of 48 KD (Benedum et al., 1986; Iacangelo et al., 1986). Tatemoto et al. (1986) reported on the inhibitory effects of pancreastatin on glucose-induced insulin secretion at peptide concentrations of 10 nmol/liter. Hence, if no more than 1% of pancreatic chromogranin A was released, processed to yield its pancreastatin-like sequence, and distributed throughout

Table 1. Concentration of chromogranin A (74 KD) and insulin in bovine pancreas^a

Chromogranin A per gram wet weight (n = 12)	Insulin per gram wet weight (n = 12)	Chromogranin A per mg insulin	µmol chromogranin A per mol insulin
3.2 µg/g (± 1.3)	22.03 U/g (± 1.64)	3.5 µg/mg	460 µmol/mol
	918 µg/g		

^a Data are expressed as the mean ± SEM of the number (n) of experiments.

the whole islet tissue, its concentration would be in the range reported to inhibit insulin secretion.

With the present anti-bovine chromogranin A serum, the immunoreactivity was associated with the 74 KD molecular form of pancreatic chromogranin A (see Results). Other groups have found chromogranin A-immunoreactive proteins of about 20 KD in extracts of bovine and rat pancreatic tissue (Hutton et al., 1985, 1987a, 1987b; Nolan et al., 1985), with little or no unprocessed forms present. However, when proteolysis is avoided by addition of an inhibitor of proteolysis and homogenization in acid medium (see Methods), the unprocessed 74 KD chromogranin A form dominates. Synthesis and storage of chromogranin A in its high molecular weight form (74 KD) by the pancreatic endocrine cells have also been found with another chromogranin A antiserum (Yoshie et al., 1987). In the latter study, possible proteolytic breakdown by endogenous proteases was avoided by immediate heating of the extracts. Despite these precautions, a processed form of secretogranin II (chromogranin C) has been detected in the same samples. This indicates that another type of chromogranin, which is present mainly in the pancreatic polypeptide-containing cells of the pancreatic islet, is effectively processed (Yoshie et al., 1987).

Further analysis of the cellular distribution and processing of the chromogranin/secretogranin family in the pancreatic islet cells may provide a molecular basis for understanding of their role in endocrine secretory processes.

Acknowledgments

For expert technical assistance we thank Ms Meyer, Ms Peesel, and Mr Gudat. We also wish to thank Dr M.C. Aletsee-Ufrecht for preparation of the adrenomedullary vesicle content and Ms B. Mader for typing the manuscript.

Literature Cited

Andersson T, Berggren P, Gylee E, Hellmann B (1982): Amounts and distribution of intracellular magnesium and calcium in pancreatic β-cells. *Acta Physiol Scand* 114:235

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

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

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

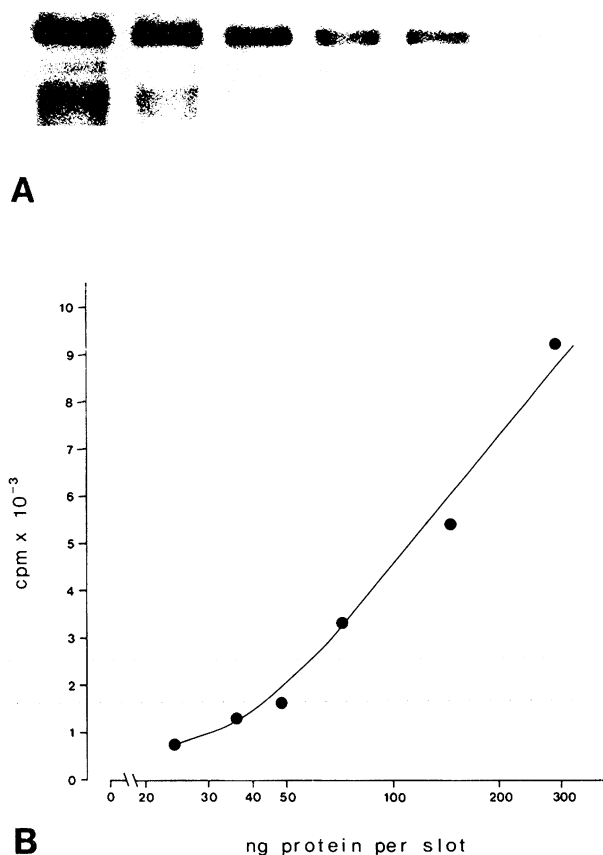


Figure 5. Quantification of chromogranin A. (A) Immunoblotting of the two main bands of chromaffin vesicle content. Samples contained 290 ng, 145 ng, 73 ng, 48 ng, 36 ng, 22 ng protein/slot. They were subjected to PAGE (11% acrylamide) followed by blotting and immunostaining with the chromogranin A antiserum. (B) Calibration curve for chromogranin A. The labeled bands were cut out of the nitrocellulose and counted in a gamma counter.

- Bulenda D, Gratzl M (1985): Matrix free Ca^{2+} in isolated chromaffin vesicles. *Biochemistry* 24:7760
- Burgermeister W, Enzmann F, Schöne HH (1975): The isolation of insulin from the pancreas. In Hasselblat A, von Bruchhausen F, eds. *Handbook of experimental pharmacology* 32/2. Berlin, Heidelberg, New York, Springer, 715
- 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
- Cohn DV, Elting JJ, Frick M, Elde R (1984): Selective localization of the parathyroid secretory protein-I/adrenal medulla chromogranin A protein family in a wide variety of endocrine cells of the rat. *Endocrinology* 114:1963
- 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
- Eiden LE (1987): Is chromogranin a prohormone? *Nature* 325:301
- Falkmer S, El-Salhy M, Titlbach M (1984): Evolution of the neuro-endocrine system in vertebrates. A review with special reference to the phylogeny and postnatal maturation of the islet parenchyma. In Falkmer S, Hakanson R, Sundler F, eds. *Evolution and tumour pathology of the neuro-endocrine system*. Amsterdam, New York, Oxford, Elsevier, 59
- Greenwood FC, Hunter WM, Glover JS (1963): The preparation of ^{131}I -labelled human growth hormone of high specific reactivity. *Biochem J* 89:114
- 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
- Grube D, Kusumoto Y (1986): Serial semithin sections in immunohistochemistry: techniques and applications. *Arch Histol Jpn* 49:391
- 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
- Huttner WB, Benedum UM (1987): Chromogranin A and pancreastatin. *Nature* 325:305
- Hutton JC (1984): Secretory granules. *Experientia* 40:1091
- Hutton JC, Davidson HW, Grimaldi KA, Peshavaria M (1987a): Biosynthesis of betagranin in pancreatic β -cells. *Biochem J* 244:449
- Hutton JC, Davidson HW, Peshavaria M (1987b): Proteolytic processing of chromogranin A in purified insulin granules. *Biochem J* 244:457
- 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
- 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
- Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680
- 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
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265
- 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
- O'Connor DT (1983): Chromogranin: widespread immunoreactivity in polypeptide hormone producing tissues and in serum. *Regul Pept* 6:263
- 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
- Reiffen FU, Gratzl M (1986a): Chromogranins, widespread in endocrine and nervous tissue, bind Ca^{2+} . *FEBS Lett* 195:327
- Reiffen FU, Gratzl M (1986b): Ca^{2+} binding to chromaffin vesicle matrix proteins: effect of pH, Mg^{2+} , and ionic strength. *Biochemistry* 25:4402
- Romans RG, Scott DA, Fisher AM (1940): Preparation of crystalline insulin. *Industr Engin Chem* 32:908
- Sternberger LA (1986): *Immunocytochemistry*, 3rd ed. New York, John Wiley & Sons
- Tatemoto K, Efenđić S, Mutt V, Makk G, Feistner GJ, Barchas JD (1986): Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. *Nature* 324:476
- Wilson BS, Lloyd RV (1984): Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. *Am J Pathol* 115:458
- Winkler H, Apps DK, Fischer-Colbric R (1986): The molecular function of adrenal chromaffin granules: established facts and unresolved topics. *Neuroscience* 18:261
- Winkler H, Westhead E (1980): The molecular organization of adrenal chromaffin granules. *Neuroscience* 5:1803
- Yoshie S, Hagn C, Ehrhart M, Fischer-Colbric R, Grube D, Winkler H, Gratzl M (1987): Immunological characterization of chromogranins A and B and secretogranin II in the bovine pancreatic islet. *Histochemistry* 87:99

Referees for the Journal of Histochemistry and Cytochemistry

The editorial policy of the Journal of Histochemistry and Cytochemistry stipulates that each manuscript be reviewed by at least two referees selected primarily on the basis of their demonstrated competence in the subject of the manuscript. The reviewing process is one of the most important requirements for establishing and maintaining high standards of scientific publication.

The Journal frequently requests critiques from experts in a variety of scientific specialties. Considerable time and thought is given by these referees each year and the Editorial Board gratefully acknowledges their valuable contribution to the Journal.

During 1987 manuscripts were reviewed by the following referees:

- | | | | |
|-----------------------|------------------------|-------------------------|----------------------|
| Dale R. Abrahamson | Normand Briere | Arline Deitch | R. Gebhardt |
| G. Adolph Ackerman | David J. Brigati | I.S. De La Landa | William D. Geoghegan |
| Judith Aggeler | Robert C. Briggs | Ronald DeLellis | Camejo German |
| Henry C. Aldrich | Richard Broadwell | Jan De Mey | Hans J. Geuze |
| Richard T. Alexander | Jerome S. Brody | Hans P. Dienes | Ian Gibbins |
| Richard A. Altschuler | Mary Anne Brock | Rolf Dierichs | J.S. Gillespie |
| Giuseppe A. Andres | D. Brown | Alexandra Dimitriv-Bona | Charles W. Goff |
| Efrain C. Azmitia | D.L.B. Brown | Michael T. Dimuzio | Sidney Goldfischer |
| Barbara Baird | Dennis Brown | Stephen B. Doty | G. Goldstein |
| Tibor Barka | R.D. Brown | Ursula Drager | Ronald E. Gordon |
| Kay Barnard | G. Brugal | D. Drenckham | Fred S. Gorelick |
| J. Michael Barrett | Clayton A. Buck | W.A.L. Duijndam | Reinhart Gossrau |
| J. Bartles | Mary Bartlett Bunge | Bonnie S. Dunbar | Victor E. Gould |
| A. Basbaum | Ann H. Bunt-Milam | William A. Dunn | Alba Greco |
| Denis G. Baskin | Brian Burke | Ann Dvorak | Phillip Greenspan |
| Nathan M. Bass | William T. Butler | Harold F. Dvorak | Philip R. Greipp |
| Margaret Beard | Bridget H. Butterworth | D. Dwyer | E.W. Gresik |
| Jay H. Beckstead | Ross G. Cameron | M. Ehrlich | O. Hayes Griffith |
| David C. Beebe | B. Carlson | Reuben Eisenstein | M.S. Grisolia |
| Donald H. Beezhold | Iris Charvat | Lawrence F. Eng | Gary Groves |
| C. Bell | Richard Cathcart | Sandor L. Erdo | R. Grzanna |
| David I. Beller | Colette Charpin | Stephen A. Ernst | Marek Haftek |
| Moise Bendayan | Edward G. Cleary | H. Dariush Fahimi | J.L. Hall |
| H. Stanley Bennett | Charles V. Clevenger | John Fallon | Nicholas S. Halmi |
| Robert H. Benno | Domenico Cocchia | Tullio Faraggiana | W.W. Hancock |
| Eric G. Berger | Philippe Cochard | Emmanuel Farber | Dean A. Handley |
| George R. Bernard | Adolph I. Cohen | Gary Felsenfeld | B.L. Hansen |
| Atul K. Bhan | Arthur Cohen | Jeffrey R. Felton | Fay Hansen-Smith |
| P. Bianco | Richard A. Coleman | V. Ferrans | Clifford Harding |
| Loran Bieber | John Condeelis | Edward G. Fey | T. Hardingham |
| A. Bignami | J. Conscience | Albert S. Fine | John Hartwig |
| Marion Birmingham | J.L. Cordell | Connie L. Finstad | Elizabeth D. Hay |
| G. Bruce Birrell | Neal Cornell | J.A. Firth | Dennis P. Healey |
| Lucille Bitensky | Pierre J. Courtoy | R. Fischer-Colbric | Stephen A. Hearn |
| Janet C. Blanks | Ronald R. Cowden | Bernard E. Flucher | Klaus Hedman |
| Bertrand Bloch | Stuart Craig | Cecil H. Fox | Ellen J. Henderson |
| F.J. Bollum | Stewart F. Cramer | Samuel French | Randolph A. Hennigar |
| J. Boniver | H.A. Crissman | Harold M. Fullmer | Damon C. Herbert |
| E. Bonucci | A.C. Cuello | Leo T. Furcht | Avram Hershko |
| Paul Boor | J. Dainty | Ferenc Gallyas | Robert Hikida |
| FT. Bosman | Mathew P. Daniels | Charles E. Ganote | Clive S. Holgate |
| Robert M. Bowker | Z. Darzynkiewicz | Philius Garant | Morley D. Hollemberg |
| Scott Brady | Jeffrey Davidson | Robert Garrone | H. Holthofer |
| Michel Brahic | E. de Harven | | Eric Holtzman |
| R. Bravo | | | Gary E.R. Hook |

- Marc Horisberger
Elizabeth Houssaint
Jerry L. Hudson
- Harunori Ishikawa
Susumu Ito
- Elaine S. Jaffe
Ronald Jaffee
Phillips M. James
D.M. Jensen
A.J. Jesaitis
T. Joh
David A. Johnson
Dennis A. Johnston
William P. Jollie
Albert L. Jones
Elizabeth W. Jones
B.R. Juhl
James E. Jumblatt
- Yashpal S. Kanwar
Donald Keefer
Robert O. Kelley
June Z. Kendall
A. John Kenny
John Kenny
Sidney P. Kent
James Kimura
Edward J. King
Joan King
Richard L. Klein
Hynda Kleinman
James K. Koehler
E. Kohen
Kenneth S. Kosik
Elena M. Kozak
J.P. Kraehenbuhl
Timothy Kute
- Paul E. Lacy
Regina Lamberts
Michael E. Lamm
Samuel A. Latt
Gordon W. Laurie
Jennifer H. LaVail
A.V. Lebouton
J. Lechago
M.W. Leigh
Larry F. Lemanski
Csaba Leranth
Charles S. Lieber
Ronald Lindahl
Charles D. Little
Gary Login
Michael R. Loken
J.B. Longley
John B. Lowe
Oliver Lowry
J.M. Lucocq
Elizabeth Luna
- H. Robson MacDonald
J.E. Madl
Guido Majno
Thomas H. Maren
- Sandy J. Marks
John B. Matthews
Frederick R. Maxfield
Joseph Mazurkiewicz
John A. McDonald
Robert C. McEvoy
Peter McLaughlin
Paul J. McMillan
Alton Meister
Donna L. Mendrick
Marek-Marsel Mesulam
Edwin M. Meyer
John S. Meyer
Marku Miettinen
Gary Miller
John W. Mills
Jun Miyauchi
U.M. Moll
G. Monga
A.J. Moody
R.D. Moore
Debra J. Morley
J.I. Morrell
Randal E. Morris
David C. Morrison
Deane F. Mosher
Mary Pat Moyer
Brian R. Mullin
H. Mullink
- Norman J. Nadler
D.H. Napper
Geoffrey R. Newman
J. Newport
Barbara A. Nichols
R.A. Niedermann
Stig Nordling
Sigurd J. Normann
- Deborah A. O'Brien
Paul J. O'Brien
W.D. O'Brien Jr.
Robert K. Ockner
Theodore Oegema
K. Ohlsson
Constance Oliver
Lelio Orci
Paul Ordronneau
L. Ornstein
Leonard Ornstein
Mary Osborn
Andre J. Ouellette
- David S. Papermaster
Jonathan A. Parsons
Richard T. Parmley
Earl L. Parr
V. Parthe
Pedro Pasik
H. Benjamin Peng
Virginia M. Pickel
Christof Pilgrim
Carlin A. Pinkstaff
Richard M. Pino
J. Platt
Charles G. Plopper
- A. Robin Poole
Donna Pozeplorka
Michael F. Press
Holde Puchtler
E. Puvion
A.K. Raap
Immo Rantala
A. Ratcliffe
J.A. Raven
J.J. Reid
M.A. Reidy
Anton Reiner
Roy Rhodes
Dan A. Riley
Michael Rindler
Michael Risley
James Roberts
Lee Roberts
John M. Robinson
Juan Rosai
Douglas L. Rosene
L.C. Rosenberg
J. Rosenbloom
Jurgen Roth
Robert V. Rouse
Jose Russo
Una S. Ryan
- S.I. Said
Miriam M. Salpeter
Philip Sannes
Lawrence Schwarz
Robert Schimke
William W. Schlaepfer
Louis H. Schliselfeld
Thomas M. Schmid
J. Schrevel
Bradley A. Schulte
C.S. Scott
U.S. Seybold
Howard M. Shapiro
Kissu Shcin
Ross Shiman
George Siegel
Antoine Simard
K. Simons
Gurmukh Singh
R.J. Sklarew
Ehud Skutelsky
Jan W. Slot
P.S. Smith
J. Sodek
Herman W. Spater
David Spector
Odie T. Stallcup
Charles A. Stanley
Urs P. Steinbrecher
Ludwig A. Sternberger
Werner Straus
Walter E. Stumpf
Thomas Sudhof
Tung-Tien Sun
Yasunosuke Suzuki
Robert C. Switzer
Ann-Christine Syvanen
Clara M. Szego
- H.W. Taesch
J.M. Tager
Prudence Talbot
Nobuo Tanako
R.E. Tashian
Suresh Tate
Alan N. Taylor
Alvin Telsler
Virginia M. Tennyson
Louis Terracio
Robert Terry
H.F. Thomas
Ann Thor
C.C. Tisher
K.T. Tokuyasu
S.U. Toverud
H. Towbin
Frank Traganos
Richard N. Trelease
John Q. Trojanowski
- Rolf Uddman
Derek Van der Kooy
M. van der Ploeg
Gary van Hoesen
Cornelis J.F. Van Noorden
Jeannie Vassy
Jack C. Vaughn
A.A.J. Verhofstad
Giuseppe Viale
Agnes Vignery
S.R. Vincent
Bruno Voss
- Alan S. Waggoner
Robert C. Wagner
Philip P. Wang
M.J. Warhol
Roger Warnke
Martin Weesendorf
Joel M. Weinberg
Helmut Wekerle
Michael Welch
Karin N. Westlund
Ulla Wewer
William Whitson
Jeffrey Whitsett
Christopher C. Widnell
Mary C. Williams
Mark C. Willingham
Cheryl L. Willman
Barbara Winkler
Patricia L. Witt
John J. Wolosewick
M.K.K. Wong
Gary S. Wood
Robert Wordinger
- Lung T. Yam
Kazuyori Yamada
Masaki Yanagishita
Shu-Hui Yen
- Dorethea Zucker-Franklin
A. Zweibaum