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Cytochemical Markers of Neural and Endocrine Cells

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Mit 129 Abbildungen und 34 Tabellen

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Chromogranin A in the endocrine pancreas: Extracellular or intracellular function?

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With 3 figures

Chromogranin A belongs to a family of highly acidic proteins, the chromogranins/secretogranins. These proteins are widely distributed in endocrine cells storing hormones or amines in dense cored vesicles. Chromogranin A has first been detected in the adrenal medulla, were it is costored and coreleased together with the catecholamines (Banks and Helle, 1965; Blaschko et al., 1967). Both intracellular and extracellular functions of chromogranin A are currently discussed: Within the chromaffin granules of the adrenal medulla chromogranin A is probably involved in the regulation of the osmotic pressure (Helle et al., 1985). In addition, chromogranin A is part of the Ca^{2+}storage complex in the matrix of the chromaffin vesicles (Reiffen and Gratzl, 1985a, b; Bulenda and Gratzl, 1985; Gratzl, 1987, 1988). Chromogranin A derived peptides have been shown to inhibit nicotine induced catecholamine secretion from adrenal medullary chromaffin cells (Simon et al., 1988). Thus, in the adrenal medulla beside the established intracellular functions also an extracellular function is likely.

No functional role has yet been described for chromogranin A in other endocrine cells. However, it was shown recently that chromogranin A contains the amino acid sequence of the putative hormone pancreastatin (Eiden, 1987; Huttner and Benedum, 1987; Iacangelo et al., 1988). Porcine pancreastatin inhibits the first phase of glucose induced insulin secretion of rat pancreas (Tatemoto et al., 1986; Efendic et al., 1987). Thus, pancreatic chromogranin A has a potential function as a prohormone. Therefore, the cellular and subcellular distribution in the endocrine pancreas, the molecular form of pancreatic chromogranin A as well as its amount in relation to the hormones are of special interest.

Methods

Immunocytochemistry: Small pieces of bovine pancreas were fixed with Acrolein (5% Acrolein, 0.1 M phosphate buffer, 2% sucrose) for 1 h and embedded in epoxy resin. For lightmicroscopical investigations semithin sections were immunostained for chromogranin A using the peroxidase-anti-peroxidase method according to Sternberger (1986). For immunoelectron microscopy, ultrathin sections were immunostained using the protein A gold technique (Ehrhart et al., 1986). For lightmicroscopical investigations, the antiserum was diluted 1:5,000, for electronmicroscopy it was diluted 1:10,000.

Immunochemical analyses: The extraction of chromogranin A of calf pancreatic tissues was carried out in 1 N HCl to prevent proteolysis. Soluble proteins of chromaffin granules from the adrenal medulla were isolated as described previously (Reiffen and Gratzl, 1986a). The extracts were subjected to SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose and immunostained for chromogranin A using ^{125}I-protein A (Burnette, 1981).

The immunocytochemical and the immunochemical studies were carried out using a polyclonal antiserum raised against bovine adrenal medullary chromogranin A kindly provided by M. F. Bader and D. Aunis (Centre de Neurochimie, Strasbourg, France).

Results and discussion

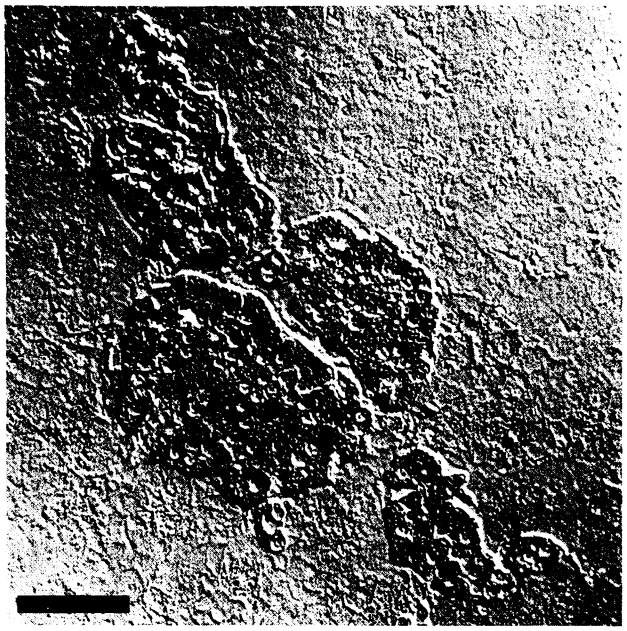

Fig. 1. Semithin section (0.5 µm) of an islet of bovine pancreas. The section is immunostained for chromogranin A. Bar = 200 µm

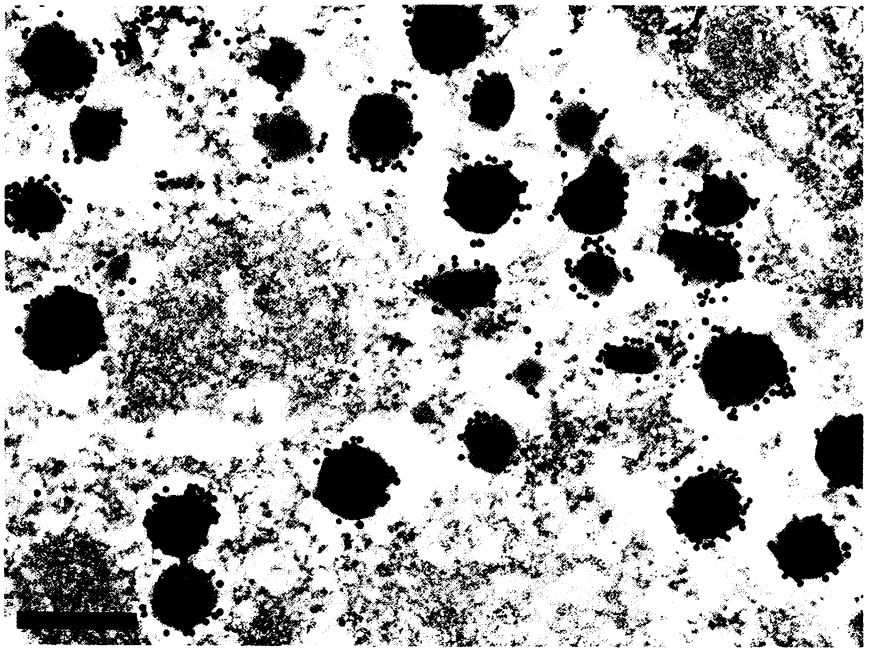

Fig. 2. Photomicrograph of an ultrathin section of B-cell vesicles. The section is immunostained for chromogranin A. Bar = 0.5 µm.

Within the bovine pancreas, the islets of Langerhans were heavily stained for chromogranin A (Fig. 1). The comparison of chromogranin A-immunoreactivity with that of the hormones showed that the insulin-, glucagon-, and somatostatin-containing cells reacted intensively with the chromogranin A-antiserum (Ehrhart et al., 1986). The chromogranin A-immunoreactivity is confined exclusively to the hormone containing vesicles (Fig. 2).

The immunocytochemical studies showed that chromogranin A in the endocrine pancreas is stored together with the hormones in the same vesicles. Consequently, chromogranin A can be secreted together with the hormones of the endocrine pancreas. To elucidate the function of pancreatic chromogranin A it is essential to know, whether or not it is processed within the cells of the endocrine pancreas. Using immunoblots, the molecular weight of pancreatic chromogranin A was compared with that of chromogranin A of the adrenal medulla. When proteolysis of chromogranin A was inhibited during extraction either by heating (Yoshie et al., 1987) or acidification of the extracts mainly the unprocessed form of pancreatic chromogranin A was detected (Fig. 3). It has the same apparent molecular weight of 74 kD as the main component of adrenal medullary chromogranin A (Fig. 3). Using immunoblotting as a quantitative method, it turned out that the endocrine pancreas contains on a molar basis 2,000 times more insulin than chromogranin A (460 µmol/mol insulin; Ehrhart et al., 1988).

The wide distribution of chromogranin A in endocrine cells indicates an universal function of this protein. For the adrenal medulla, intravesicular functions as well as ex-

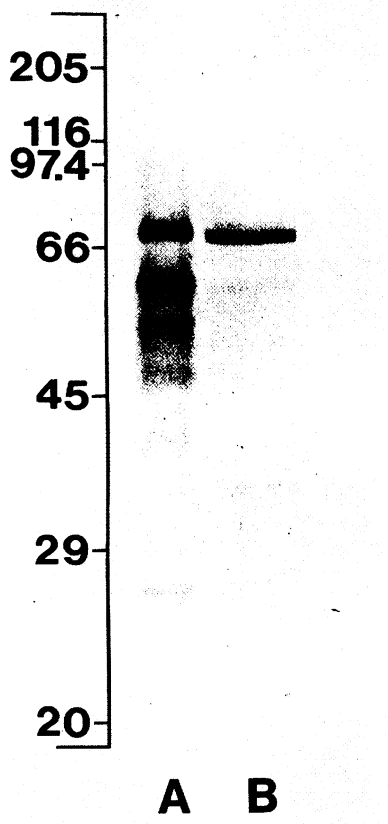

Fig. 3. Immunoblotting of soluble proteins of bovine chromaffin vesicles and of an extract of bovine pancreas. Samples were subjected to polyacrylamide gel electrophoresis (11%) followed by blotting and immunostaining for chromogranin A. (A) chromaffin vesicle content; (B) pancreatic extract.

tracellular functions of chromogranin A are known (see above). Within the endocrine pancreas, the estimated amount of chromogranin A is more than 100 times less than the amount of chromogranin A that would be necessary to bind the intravesicular calcium (Ehrhart et al., 1988). Thus, a functional role of chromogranin A in the storage of Ca^{2+} within the secretory vesicles of the endocrine pancreas is not likely.

No regulatory effect on hormone secretion has so long been shown for chromogranin A itself. However, the aminoacid sequence of porcine chromogranin A includes the sequence of the putative hormone pancreastatin (Iacangelo et al., 1988). Recently, it has been described that pancreastatin is released together with insulin from the porcine pancreas (Östenson et al., 1988). A sequence homologous to pancreastatin is also contained in bovine, human and rat chromogranin A (cf. Iacangelo et al., 1988). However, within the bovine endocrine pancreas, chromogranin A is stored in its unprocessed form. Thus chromogranin A has to be degraded shortly before or after secretion to yield pancreastatin which subsequently could inhibit the secretion of insulin.

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References

Banks, P., Helle, K.B., and Mayor, D.: Evidence for the presence of a chromogranin-like protein in bovine splenic nerve granules. Molec. Pharmac. **5**, 210–212 (1968).

Blaschko, H., Comline, R.S., Schneider, F.H., Silver, M., and Smith, A.D.: Secretion of a chromaffin granule protein, chromogranin, form the adrenal gland after splanchnic stimulation. Nature **215**, 58–59 (1967).

Bulenda, D., and Gratzl, M.: Matrix free Ca^{2+} in isolated chromaffin vesicles. Biochemistry **24**, 7760–7765 (1985).

Burnette, W.N.: "Western Blotting": elektrophoretic 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 (1981).

Efendic, S., Tatemoto, K., Mutt, V., Quan, C., Chang, D., and Östenson, C.G.: Pancreastatin and islet hormon release. Proc. Natl. Acad. Sci. USA **84**, 7257–7260 (1987).

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

Ehrhart, M., Jörns, A., Grube, D., and Gratzl, M.: Cellular distribution and amount of chromogranin A in the bovine endocrine pancreas. J. Histochem. Cytochem. **36**, 467–472 (1988).

Eiden, L.E.: Is chromogranin a prohormone? Nature **325**, 301 (1987).

Gratzl, M.: Uptake and release of Ca^{2+} by chromaffin vesicles. In: Rosenheck, K., and Lelkes, P.I. (eds.): Stimulus-secretion coupling in chromaffin cells. CRC Press Inc., Boca Raton, 111–123 (1987).

Gratzl, M.: Metabolism and function of Ca^{2+} in secretory cells. In: Thorn, N.A., Treiman, M., Peterson, O.H., and Thaysen, J.H. (eds.): Molecular mechanisms in secretion. Munksgaard, Copenhagen, 364–376 (1988).

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

Huttner, W.B., and Benedum, U.M.: Chromogranin A and pancreastatin. Nature **325**, 305 (1987).

Iacangelo, A.L., Fischer-Colbrie, R., Koller, K.J., Brownstein, M.J., and Eiden, L.E.: The sequence of porcine chromogranin A messenger RNA demonstrates chromogranin A can serve as the precursor for the biologically active hormone, pancreastatin. Endocrinology **122**, 2339–2341 (1988).

Östenson, C.G., Holst, J.J., and Efendic, S.: Pancreastatin and insulin are released in parallel from the perfused porcine pancreas. Diabetologia, in press (1988).

Reiffen, F. U., and Gratzl, M.: Chromogranins, widespread in endocrine and nervous tissue, bind Ca^{2+}. FEBS Lett. **195**, 327–330 (1986a).
Reiffen, F. U., and Gratzl, M.: Ca^{2+} binding to chromaffin vesicles matrix proteins: effect of pH, Mg^{2+}, and ionic strength. Biochemistry **25**, 4402–4406 (1986b).
Simon, J. P., Bader, M. F., and Aunis, D.: Secretion from chromaffin cells is controlled by chromogranin A-derived peptides. Proc. Natl. Acad. Sci. USA **85**, 1712–1716 (1988).
Sternberger, L. A.: Immunocytochemistry. 3rd ed. J. Wiley, New York 1986.
Tatemoto, K., Efendic S., Mutt, V., Makk, G., Feistner, G. J., and Barchas, J. D.: Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. Nature **324**, 476–478 (1986).
Yoshie, S., Hagn, C., Ehrhart, M., Fischer-Colbrie, R., Grube, D., Winkler, H., and Gratzl, M.: Immunological characterization of chromogranins A and B and secretogranin II in the bovine pancreatic islet. Histochemistry **87**, 99–106 (1987).

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