한국전자현미경학회지 제 26 권 제 1 호 1996
Korean J. Electron Microscopy 26(1), 67~77(1996)

Immunoelectron Microscopic Study on the Endocrine Pancreas of the Native Korean Goat

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한국재래산양 췌장내분비세포의 면역전자현미경적 연구

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(Received January 6, 1996)

요 약

한국재래산양의 췌장내분비세포에 대해 몇종의 항혈청을 이용하여 면역전자현미경적으로 관찰하여 다음과 같은 결론을 얻었다.

췌도에서는 glucagon (A), insulin (B), somatostatin (D) 및 pancreatic polypeptides (PP-I과 PP-II) 등 5종의 세포를 동정하였다. 이 중 A, B 및 D세포의 형태학적 특징은 다른포유동물의 그것과 유사하였고 D세포는 serotonin 면역성이 인정되었다. PP세포는 과립의 형태학적 특징으로 보아 두가지 형태가 인정되었으며, 제I형은 원형인 동질성의 과립(220~440 nm)을 가지며, 과립내용물과 한계막 사이에는 얇은 halo를 보였으나 제II형은 과립이 다형태성을 보이며(240~440 nm major axis, 150~200 nm minor axis) 과립내용물과 한계막 사이는 거의 밀착되어 있었다. 이상의 결과로 한국재래산양의 췌도에는 A, B, D, PP-I 및 PP-II 등 5형의 세포로 구성되어 있으며, 이 중 PP-I세포는 다른 포유류의 PP세포에, PP-II세포는 enterochromaffin cell에 상당할 것으로 생각되었다.

Key words : Pancreatic islet, Endocrine cell, Immunoelectron microscopy, native Korean goat

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* This paper was supported by NON DIRECTED RESEARCH FUND, Korea Reserch Foundation, 1993.

INTRODUCTION

It has long been generally accepted that the endocrine pancreas in a wide variety of mammals is composed of four types of cells, glucagon (A), insulin (B), somatostatin (D) and pancreatic polypeptide (PP) cells, on the basis of granular morphologies and immunocytochemical characteristics. Although the ultrastructure of normal pancreatic islet cells has been described for many vertebrates, from fish (Stefan and Falkmer, 1980) to man (Like, 1967; Shibasaki and Ito, 1969; Munger, 1970; Deconinck *et al.*, 1971; Gepts, 1977; Beaten *et al.*, 1977; Pelletier, 1977; Bergstrom *et al.*, 1977; Larsson *et al.*, 1975; Buchan and Polak, 1980), little attention has been addressed to the endocrine pancreas of the domestic ruminants. Several works have dealt with the ultrastructure in the sheep (Larsson *et al.*, 1976; Hitaka *et al.*, 1979; Titlbach *et al.*, 1985) and bovine (Galabova and Petkov, 1975; Bonner-Weir and Like, 1980) endocrine pancreas; however, no detailed morphological description of the pancreatic endocrine cells in the goat has hitherto been recorded.

Therefore, we carried out an immunocytochemical investigation on the pancreatic islet cell types of the native Korean goat with electron microscopy in order to base identification of these cells on immunocytochemical criteria.

MATERIALS AND METHODS

Tissue Preparation: Three native Korean goats weighing 13～14 kg were used. The animals were given an overdose of pentobarbital sodium and sacrificed. The tissue obtained from the right pancreatic lobe was cut into blocks and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3, for 4 hr at 4°C. The tissues were then postfixed in osmium tetroxide for 2hr, dehydrated and embedded in commercial epoxy resin. Some nonosmicated tissues fixed with a 2% paraformaldehyde and 2.5% glutaraldehyde mixture were embedded in a Lowicryl K4M resin (Chemische Werke Lowi, Germany). The polymerization of Lowicryl K4M resin was carried out at −35°C for 24hr and later at room temperature for 3～4 days with an ultraviolet polymerizer (Dosaka EM Co Ltd., Kyoto, Japan). Ultrathin sections obtained from epoxy and K4M embedding resin were cut with an ultramicrotome (Ultacut S, Reichert-Nissei), mounted on a nickel grid and dried at 37°C.

Immunoelectron Microscopic Procedures: Ultrathin sections of the Lowicryl K4M embedded materials were used in following procedures. In immunocytochemistry, a humidity chamber was used during all steps. Briefly, the sections were rinsed with phosphate buffer (pH 7.3) saline (PBS) and treated with 1% bovine serum albumin (BSA) for 30 minutes at room temperature. They were transferred onto a drop of anti-glucagon serum (1:500, CRB Ltd.), anti-insulin serum (1:500, Incstar Corp.), anti-somatostatin serum (1:500, CRB Ltd.), anti-serotonin serum (1:200, INC) or anti-bovine pancreatic polypeptide (1:5,000, UCB bioproducts), and incubated overnight at 4°C. They were then rinsed with PBS three times and incubated with colloidal gold (15 nm) labeled protein A complex (E-Y Laboratories, Inc., USA) at room temperature for 1 hr. They were rinsed with PBS, then with distilled water and finally stained with saturated aqueous uranyl acetate and lead citrate, and observed under an electron microscope (JEOL 1210) at 100 kV. Control sections were incubated with nonimmunized rabbit serum or PBS instead of

primary antisera. In a preliminary study (paraffin sections), all antisera showed positive immunoreactivity in the islets of goat pancreas.

RESULTS

In this study, five different types of pancreatic endocrine cells were detected by immunoelectron microscopy. These five types of cells reacted with each anti-serum, glucagon, insulin, somatostatin and bovine pancreatic polypeptide, respectively. They also had different ultrastructures (osmicated specimens), sizes and profiles of secretory granules.

The A cells (glucagon-containing cells) were readily identified by their typical secretory granules. These cells contained large numbers of secretory granules. Their granules measured about 200~440 nm and were spherical with an electron dense core and a narrow halo between the dense core and limiting membrane. Some granules occasionally showed a wide halo between the dense core and limiting membrane (Figs. 1a and inset). At the immunoelectron microscopical level, the dense cores of almost immunoreactive glucagon secretory granules were densely labeled with colloidal particles (Figs. 1a and b). No specific staining for glucagon was detected in B or D cells (Fig. 1a).

The B cells (insulin-containing cells) also contained large numbers of secretory granules. Their granules were spherical with a moderately electron dense core and a wide halo between the dense core and the limiting membrane. These granules varied from 180 to 450 nm in diameter, and no crystalloid shaped cores were observed (Figs. 2a and inset). The localization of insulin showed a positive reaction in the typical secretory granules of these cells (Figs. 2a and b). The reaction products were mainly over the dense cores of the secretory granules (Fig. 2a). No positive reactivity for insulin was shown in the other cells.

The D cells (somatostatin-containing cells) showed ultrastructural profiles of secretory granules similar to those in other mammals. Their secretory granules measured 130~280 nm in diameter and were spherical with a moderately dense core and closely fitting limiting membrane (Figs. 3a and inset). Somatostatin immunoreactivity could be observed in the dense core of the secretory granules (Figs. 3a and b). However, less immunoreactivity was shown than in the other cell types (Fig. 3a). An interesting observation was that immunoreactivity for serotonin was detected in the secretory granules of the D cells. The A and B cells were not stained by the anti-serotonin serum (Fig. 4).

On the basis of the morphological and immunocytochemical characteristics of secretory granules, two types of PP cells, PP-I and PP-II cells, immunostained by bovine pancreatic polypeptide, were identified in this study (Figs. 5, 6a, 6b, 7a and 7b). The PP-I cell had round (200~400 nm in diameter), homogeneous and electron dense granules with a narrow halo between the dense core and limiting membrane, and the PP-II cells was pleomorphic (240~440 nm on the major axis, 150~200 nm on the minor axis) with dense cored granules having a closely fitting membrane (Figs. 7b and inset). In the section immunostained with anti-bovine pancreatic polypeptide, the distribution of gold particles localized homogeneously in the dense cores of the granules in the same manner in both PP cell types (Figs. 6b and 7b). The A, B and D cells were not stained by the anti-bovine pancreatic polypeptide serum (Figs. 5, 6a and 7a).

DISCUSSION

The present morphological and immunoelectron microscopical identification of A, B and D cells was generally in agreement with previous reports on domestic ruminants other than the goat family (Galabova and Petkov, 1975; Hitaka *et al.*, 1979; Bonner-Weir and Like, 1980; Titlbach *et al.*, 1985). In the present study, the diameters of A, B and D cell granules (osmicated specimens) were 200~400 nm, 180~450 nm and 130~280 nm, respectively. Similar sizes are observed in cattle (Galabova and Petkov, 1975; Titlbach *et al.*, 1985), pigs (Capella and Solcia, 1972), dogs (Munger *et al.*, 1965), guinea pigs (Caramia *et al.*, 1965; Baskin *et al.*, 1984), and baboons (Wolfe-Coote and Toit, 1987), whereas in the human the diameter of D cell granules is smaller (Shibasaki and Ito, 1969) or larger (Deconinck *et al.*, 1971; Beaten *et al.*, 1977) than those in the A and B cell granules. Additionally, it has been reported by Bonner-Weir and Like (1980) that the granule sizes of A, B and D cells are similar to those of cattle, or somewhat larger than those of cattle (Galabova and Petkov, 1975) and sheep (Hitaka *et al.*, 1979; Titlbach *et al.*, 1985). Differences in the granule profiles of the B cells in various mammals have also been reported. The crystalloid structure of B granules was shown in man (Like, 1967; Shibasaki and Ito, 1969; Munger, 1970; Deconinck *et al.*, 1971; Pelletier, 1977; Buchan and Polak, 1980), baboons (Wolfe-Coote and Toit, 1987), dogs (Munger *et al.*, 1970), pigs (Capella and Solcia, 1972) and fish (Kobayashi and Takahashi, 1970), but not in guinea pig (Caramia *et al.*, 1965; Baskin *et al.*, 1984), golden hamster (Petkov *et al.*, 1970), rabbit and opossum (Munger *et al.*, 1965). In the ruminants, no crystalloid structure of B granules was found in cattle (Galabova and Petkov, 1975; Bonner-Weir and Like, 1980), whereas in sheep there was a discrepancy between the report of Titlbach *et al.* (1985) of crystalloid structures and that of Hitaka *et al.*(1979) who observed a homogeneous one. Although Larsson *et al.*(1976) suggested that the reason for the different granule sizes of the endocrine pancreas is mainly species variation, Wolfe-Coote and Toit (1987) reported little variation between species. The reasons for the discrepancies in the granule sizes and profiles found in this study are unknown, but they are probably due to the difference of species, experimental methods and cell activities.

Recently, the presence of serotonin-immunoreactive cells in the endocrine pancreas has been reported in ruminants (Yamada *et al.*, 1986; Nakajima *et al.*, 1988; Grube and Yoshie, 1989; Cetin, 1992; Lee and Lee, 1992; Mota *et al.*, 1992). Furthermore, the appearance of these cells was also confirmed in the guinea pig (Cetin and Grube, 1991), pig (Capella and Solcia, 1972), rat (Goldsmith *et al.*, 1975) and sheep (Hitaka *et al.*, 1979) at the ultrastructural level. Serotonin immunoreactivity was found in the glucagon- and BPP-immunoreactive cells of the rat, *Caiman latirostris* and in bovine pancreatic islets (Kaung, 1985; Yamada *et al.*, 1986; Nakajima *et al.*, 1988), but was not colocalized in the same cell. In this study, however, immunoreactivity for serotonin was mainly restricted to the D cell granules, whereas no immunoreactivity for serotonin was shown in the A and B cell granules. This finding suggests that, in the goat pancreas, somatostatin cells also contain small amounts of serotonin as described by previous investigators (Larsson *et al.*, 1976; Nakajima *et al.*, 1988; Cetin, 1992). Further studies using double staining at ultrastructural level should be designed to

identify whether both somatostatin and serotonin colocalize in the same cells.

The presence of PP cells in the pancreatic islets has been observed in the bovine and sheep by immunohistochemistry and at the ultrastructural level (Larsson *et al.*, 1976; Hitaka *et al.*, 1979; Bonner-Weir and Like, 1980; Reddy and Elliott, 1985; Titlbach *et al.*, 1985; Nakajima *et al.*, 1988), while previous ultrastructural studies have not described enterochromaffin (EC) cells in the bovine (Galabovo and Petkov, 1975; Bonner-Weir and Like, 1980) and sheep (Reddy and Elliott, 1985; Titlbach *et al.*, 1985). However, two types of PP cells were described in the rat pancreatic islets (Beaten *et al.*, 1977; Kaung, 1985). Kaung (1985) reported that the glucagon and pancreatic polypeptide-containing granules were morphologically distinct from glucagon granules but similar to pancreatic polypeptide granules and somatostatin granules. Moreover, the colocalization of pancreatic polypeptide immunoreactivity with glucagon immunoreactivity has been reported previously in the rat (Kaung, 1985), human (Grube and Bohn, 1983) and baboon (Wolfe-Coote and Toit, 1987), while glucagon and pancreatic polypeptide immunoreactivity does not colocalize in the same cell but shows serotonin immunoreactivity (Nakajima *et al.*, 1988). EC cells generally show serotonin immunoreactivity and have characteristic granules in the gastroenteropancreatic system (Buchan and Polak, 1980). Therefore, these cells could be readily distinguished from the other pancreatic endocrine cells.

Although the granules of PP-I and PP-II cell types in this study were morphologically similar to those of A and EC cell granules, whether these cell types are identical to the PP and EC cells described by previous investigators (Parrillar *et al.*, 1969; Capella and Solcia, 1972; Larsson *et al.*, 1976; Beaten *et al.*, 1977; Hitaka *et al.*, 1979; Kaung, 1985; Reddy and Elliott, 1985; Titlbach *et al.*, 1985; Grube and Yoshie, 1989; Cetin and Grube, 1991) or whether there is colocalization of glucagon and serotonin in the PP-I and PP-II cell granules remains unclear. Recently, Hashimoto *et al.*,(1988) reported that, in the fetal rat, coexpression of glucagon and insulin was observed in some endocrine cells of the developing pancreas. Saulinie-Michel *et al.* (1992) also reported that the somatostatin cells formed two populations: one of cells containing only somatostatin and the other containing both insulin and somatostatin, using the RW cell line containing the highest level of insulin mRNA.

They suggested that this presence might be closely related to the regulation of the expression of insulin and somatostatin genes and the differentiation pathways of the two respective cell types. From this viewpoint, it could be suggested that pancreatic endocrine cells in the goat are differentiated from four types of cells, insulin, somatostatin/serotonin, PP-I type/glucagon and PP-II type/serotonin cells at an early stage.

ABSTRACT

Pancreases obtained from native Korean goats were used, and examined by immunoelectron microscopy using several antisera. Five types cells, glucagon (A), insulin (B), somatostatin (D), and pancreatic polypeptide (PP-I and PP-II) cells, were identified in the pancreatic islets. The morphologies of A, B, and D cells corresponded to the typical charateristics described in previous reports on other mammals. Serotonin immunoreactivity was observed in the D cells on the basis of the granular profiles. Two types of PP cells could be distinguished on the basis of the granular profile: the first type was formed by round, homogeneous secretory granules (220~

400 nm) having a narrow halo between the dense core and limiting membrane, while the other type consisted of cells whose secretory granules (240~440 nm in the major axis, 150~200 nm in the minor axis) were pleomorphic, having a dense core and a closely fitting limiting membrane.

From these results, we suggest that the pancreatic islets of the native Korean goat consist of five types of endocrine cells, A, B, D, PP-I and PP-II cells. Among these, PP-I type cells may correspond to the classical PP of other mammalian pancreases, while PP-II type cells may correspond to the enterochromaffin cells in other species.

REFFERENCES

Baskin DG, Gorray KC, Fujimoto WY, 1984. Immunocytochemical identification of cells containing insulin, glucagon, somatostatin, and pancreatic polypeptide in the islets of Langerhans of the guinea pig pancreas with light and electron microscopy, Anat. Rec. 208, 567-578

Beaten D, De Mey J, Gepts W, 1977. Immunohistochemical and ultrastructural identification of the pancreatic polypeptide-producing cell (PP-cell) in the human pancreas, Cell Tissue Res. 186, 239-246

Bergstrom BH, Loo S, Hirsch HJ, Schutzengel D, Gabby KH, 1977. Ultrastructural localization of pancreatic polypeptide in human pancreas, J. Clin. Endocrinol. Metab. 44, 795-798

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

Buchan AM, Polak JM, 1980. The classification of the human gastroentero-pancreatic endocrine cells, Invest. Cell Pathol. 3, 51-71

Capella C, Solcia E, 1972. The endocrine cells of pig gastrointestinal mucosa and pancreas, Arch. Histol. Jap. 35, 1-29

Caramia F, Munger BL, Lacy PE, 1965. The ultrastructural basis for the identification of cell types in the pancreatic islets I. Guinea pig, Z. Zellforsch. 67, 533-546

Cetin Y, 1992. Chromogranin A immunoreactivity and Grimelius argyrophilia, Anat. Embryol. 185, 207-215

Cetin Y, Grube D, 1991. Topology of chromogranins in secretory granules of endocrine cells, Histochemistry 96, 301-310

Deconinck JF, Potvliege PR, Gepts W, 1971. The ultrastructure of the human pancreatic islets I. The islets of adult, Diabetologia 7, 266-282

Galabova R, Petkov P, 1975. Electron microscopy of the endocrine pancreas of cattle (*Bos taurus* L.), Acta Anat. 92, 560-569

Gepts W, 1977. Endokrines Zellsystem des Pankreas, Verh. Dtsch. Ges. Pathol. 61, 55-66

Goldsmith PC, Rose JC, Arimura A, Ganong WF, 1975. Ultrastructural localization of somatostatin in pancreatic islets of the rat, Endocrinology 97, 1061-1064

Grube D, Bohn R, 1983. The microanatomy of human islets of Langerhans, with special reference to somatostatin (D-) cells, Arch. Histol. Jap. 46, 327-353

Grube D, Yoshie S, 1989. Immunohistochemistry of chromogranin A and B, secretogranin II in the canine endocrine pancreas, Arch. Histol. Cytol. 52, 287-298

Hashimoto T, Kawano H, Daikoku S, Shima K, Taniguchi H, Bada S, 1988. Transient coappearance of glucagon and insulin in the progenitor cells of the rat pancreatic islets, Anat. Embryol. 178, 489-497

Hitaka S, Tamate H, Hikosaka K, 1979. The fine structure of endocrine cells in the sheep pancreas, Tohoku J. Agri. Res. 30, 66-75

Kaung HC, 1985. Electron microscopic immunocytochemical localization of glucagon and pancreatic polypeptide in rat pancreas: characterization of a population of islet cells containing both peptides, Anat. Rec. 212, 292-300

Kobayashi K, Takahashi Y, 1970. Light and electron microscopic observations on the islets of Langerhans in *Carassius carassius longsdorfii*.

Arch. Histol. Jap. 31, 433–454

Larsson L-I, Sundler F, Håkanson R, 1975. Immunohistochemical localization of human pancreatic polypeptide (HPP) to a population of islet cells, Cell Tissue Res. 156, 167–171

Larsson L-I, Sundler F, H kanson R, 1976. Pancreatic polypeptide A postulated new hormone: Identification of its cellular storage site by light and electron microscopic immunocytochemistry, Diabetologia 12, 211–226

Lee J-H, Lee H-S, 1992. Immunohistochemical studies of the pancreatic endocrine cells of various animals, Korean J. Vet. Res. 32, 497–510

Like AA, 1967. The ultrastructure of the secretory cells of the islets of Langerhans in man, Lab. Invest. 16, 937-951

Mota DL, Yamada J, Gerge LL, Pinheiro PPN, 1992. An immunohisto-chemical study on the pancreatic endocrine cells of the three-toed sloth, *Bradypus variegatus*, Arch. Histol. Cytol. 55, 203–209

Munger BL, 1970. The ultrastructural charaterization of four cell types in human and other primate pancreatic islets, Anat. Res. 166, 352

Munger BL, Caramia F, Lacy PE, 1965. The ultrastructural basis for the identification of cell types in the pancreatic islets II. Rabbit, dog and opossum, Z. Zellforsch. 67, 776–798

Nakajima S, Kitamura N, Yamada J, Yamashita T, Watanabe T, 1988. Immunohistochemical study on the endocrine pancreas of cattle with special reference to coexistence of serotonin and glucagon or bovine pancreatic polypeptide, Acta Anat. 131, 235–240

Parrilla P, Gomea-Acebo J, R-Condela JL, 1969. Ultrastructural evidence for the presence of enterochromaffin type II cells in the pancreatic islets of the rabbit, J. Ultrast. Res. 26, 1–7

Pelletier G, 1977. Identification of four cell types in the human endocrine pancreas by immunoelectron microscopy, Diabetes 26, 749–756

Petkov P, Galabova R, St. Manolov, 1970. Electron microscopic investigations on the Langerhans islets of the golden hamster, Arch. Histol. Jap. 32, 229–239

Reddy SN, Elliott RB, 1985. Ontogeny of cells containing insulin, glucagon, pancreatic polypeptide hormone and somatostatin in the bovine pancreas, Aust. J. Biol. Sci. 38, 237–243

Saulnier-Michel C, Fromont-Racine M, Pictet R, 1992. Co-expression of insulin and somatostatin genes in pancreatic endocrine cells selected for their high level of insulin gene transcription, J. Cell Sci. 101, 795–799

Shibasaki S, Ito T, 1969. Electron microscopic study on the human pancreatic islets, Arch. Histol. Jap. 31, 119–154

Stefan Y, Falkmer S, 1980. Identification of four endocrine cell types in the pancreas of *Cottus scorpius* (Teleostei) by immunofluorescence and electron microscopy, Gen. Comp. Endocrinol. 42, 171–178

Titlbach M, Falt K, Falkmer S, 1985. Postnatal maturation of the islets of Langerhans in sheep. Light microscopic, immunohistochemical, morphometric, and ultrastructural investigations with particular reference to the transient appearance of argyrophil insulin immunoreactive cells, Diab. Res. 2, 5–15

Wolfe-Coote SA, du Toit DF, 1987. Morphology and endocrine production of cells in the islets of Langerhans of the chacma baboon, Anat. Res. 218, 56–65

Yamada J, Campos VJM, Kitamura N, Pacheco AC, Yamashita T, Yanaihara N, 1986. An immunohistochemical study of endocrine cells in the pancreas of *Caiman latirostris* (Alligatorinae), with special reference to pancreatic motilin cells, Biomed. Res. 7, 199–208

FIGURE LEGENDS

Fig. 1. Low (a) and high (b) magnification immunoelectron micrographs showing glucagon immunoreactivity in goat pancreatic islet cells treated with Lowicryl resin. In the cells containing glucagon granules, colloidal particles are localized to the dense granules (A, a and b), but no colloidal particles are localized to the granules of D (D, a) cells. Inset: High magnification showing the glucagon granules with an electron dense core and narrow halo between the dense core and limiting membrane (osmicated specimens). a: Bar=1 μm, b: Bar=0.5 μm, inset: 0.2 μm.

Fig. 2. Low (a) and high (b) magnification immunoelectron micrographs of goat B cells treated with Lowicryl resin. These cells contain a number of secretory granules which are intensely labeled with colloidal gold particles. Inset: High magnification showing the typical B granules with a moderate electron dense core and wide halo between the dense core limiting membrane(osmicated specimens). a: 1 μm, b: 0.4 μm, inset: 0.2 μm.

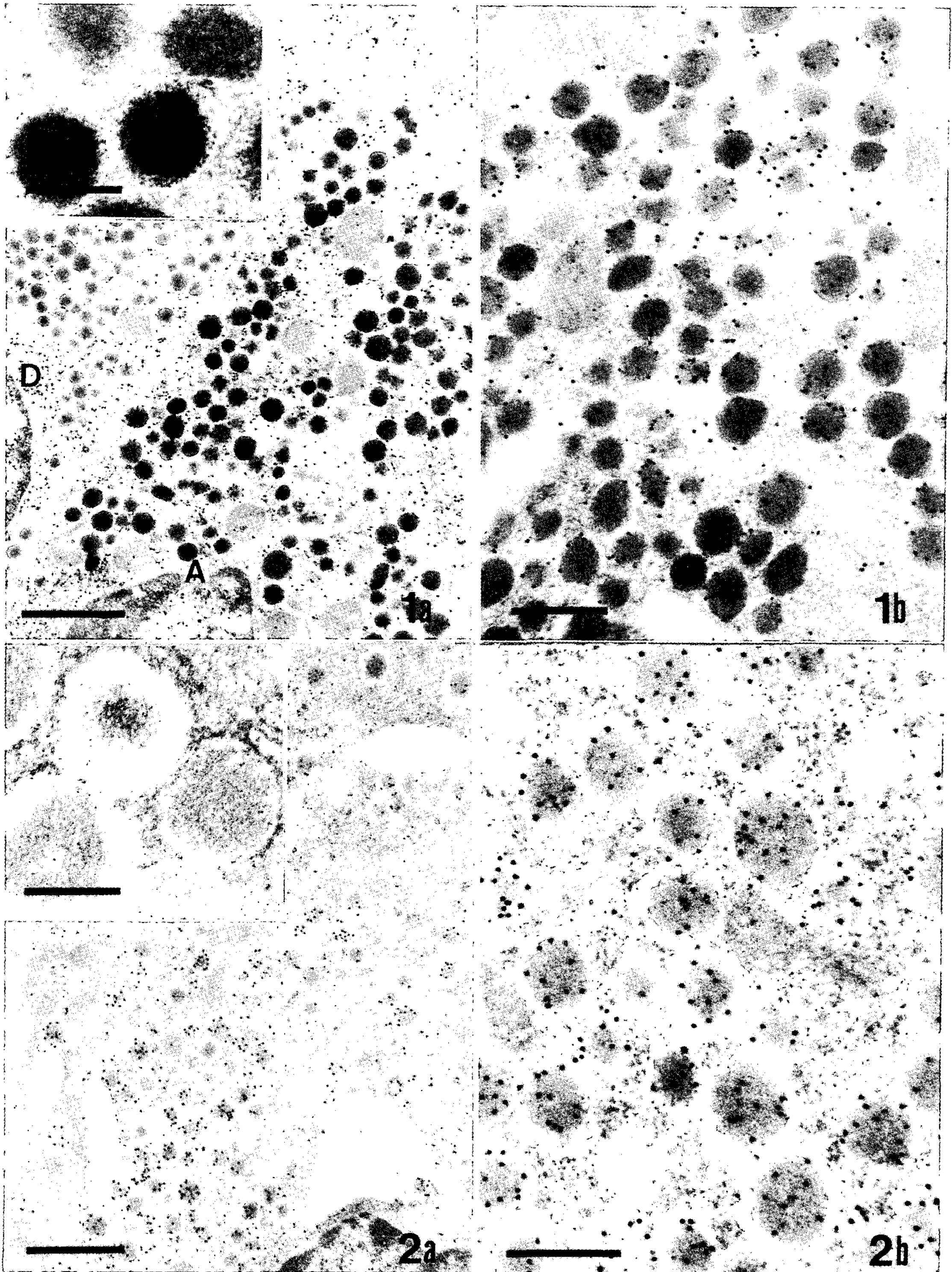
Fig. 3. Low (a) and high (b) magnification immunoelectron micrographs of goat pancreatic islet cells treated with Lowicryl resin. The D cell contains many somatostatin granules labeled with colloidal particles. Inset: High magnification showing the somatostatin granules with a moderately electron dense core and closely fitting membrane (osmicated specimens).
a: Bar=0.5 μm, b: Bar=0.4 μm, inset: 0.2 μm.

Fig. 4. Immunoelectron micrograph showing serotonin immunoreactivity in goat pancreatic islet cells treated with Lowicryl resin. Only the granules of the D cell are immunoreative to serotonin (•). No immunoreativity is seen in the granules of A (A) and B (B) cells. Bar=1 μm.

Fig. 5. Immunoelectron micrographs showing BPP immunoreativity in goat pancreatic islet cells treated with Lowicryl resin. The PP-I cell (I) contains round granules labeled for BPP with colloidal gold particles, but no colloidal particle is seen in the granules of the A cell (A). In this micrograph, you can see that the granules of the BPP-positive cell (I) are quite similar to the A cell granules (A). Bar=2 μm.

Fig. 6. Low (a) and high (b) magnification immunoelectron micrographs of goat pancreatic islet cells treated with anti-BPP and Lowicryl resin. The PP-I cell also contains round or oval granules labeled for BPP with colloidal particles (I, a and b), but no colloidal particles are seen in the granules of the B (B, a) and D (D, a) cells. a: 2 μm, b: 0.4 μm.

Fig. 7. Low (a) and high (b) magnification immunoelectron micrographs of goat pancreatic islet cells treated with Lowicryl resin. The PP-II cell (II, a and b) contains pleomorphic granules labeled for BPP with colloidal particles, but no colloidal particles are seen in the granules of A (A, a), B (B, a) and D (D, a) cells. Inset: Micrograph of osmicated specimens showing the PP-II cell granules with a pleomorphic, electron dense core and fitting limiting membrane.
a: 2 μm, b: 0.4 μm, inset: 0.5 μm.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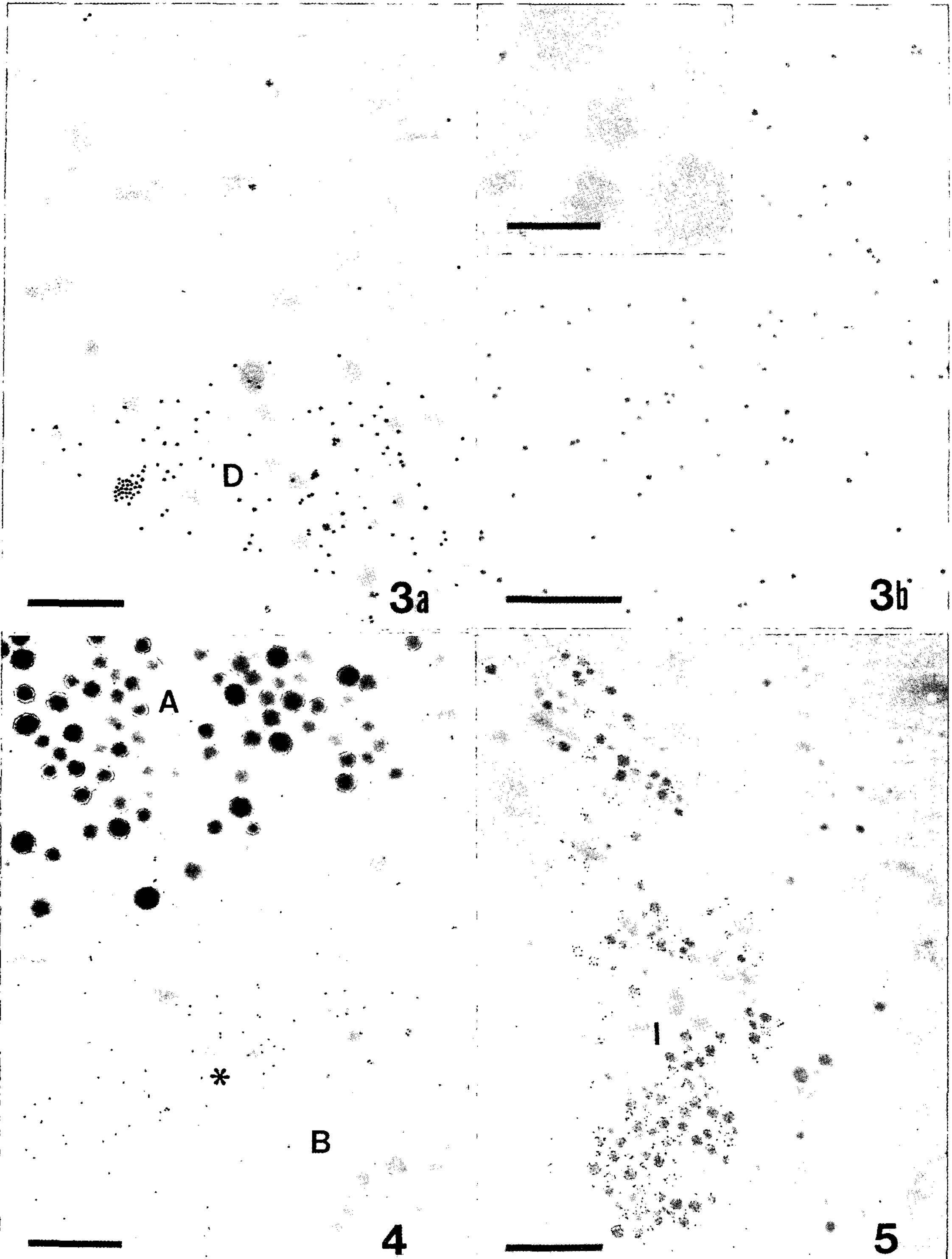
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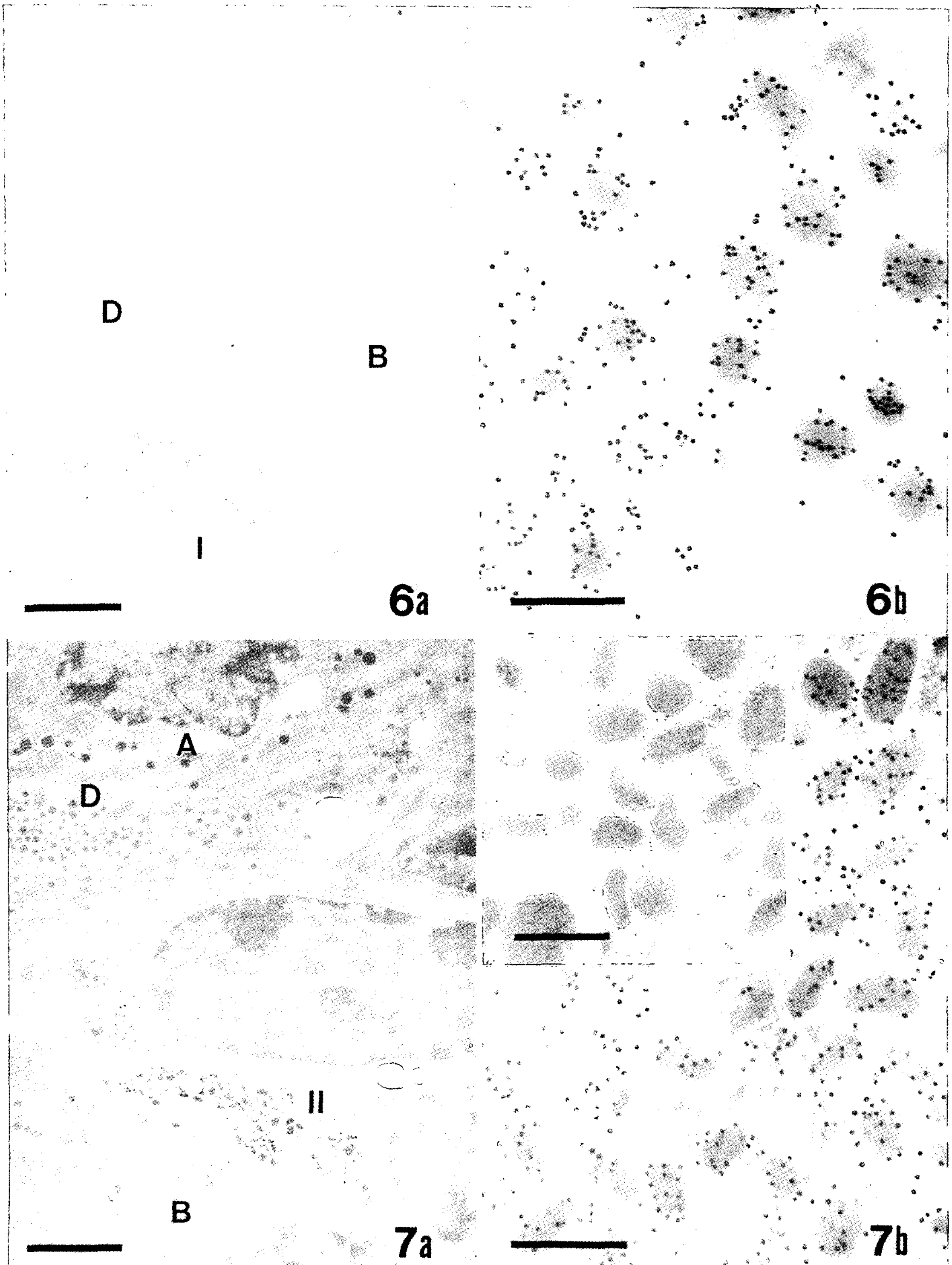
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