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Rab3a ablation related changes in morphology of secretory vesicles in major endocrine pancreatic cells, pituitary melanotroph cells and adrenal gland chromaffin cells in mice

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ABSTRACT

In this work we have compared the ultrastructural characteristics of major pancreatic endocrine cells, pituitary melanotrophs and adrenal chromaffin cells in the normal mouse strain (wild type, WT) and mice with a known secretory deficit, the Rab3a knockout strain (Rab3a KO). For this purpose, pancreata, pituitary glands and adrenal glands from the Rab3a KO and from the WT mice were analysed, using conventional transmission electron microscopy (TEM). In order to assess the significance of the presence of Rab3a proteins in the relevant cells, we focused primarily on their secretory vesicle morphology and distribution. Our results showed a comparable general morphology in Rab3a KO and WT in all assessed endocrine cell types. In all studied cell types, the distribution of secretory granules along the plasma membrane (number of docked and almost-docked vesicles) was comparable between Rab3a KO and WT mice. Specific differences were found in the diameters of their secretory vesicles, diameters of their electron-dense cores and the presence of autophagic structures in the cells of Rab3A KO mice only. Occasionally, individual electron-dense round vesicles were present inside autophagosome-like structures; these were possibly secretory vesicles or their remnants. The differences found in the diameters of the secretory vesicles confirm the key role of Rab3a proteins in controlling the balance between secretory vesicle biogenesis and degradation, and suggest that the ablation of this protein probably changes the nature of the reservoir of secretory vesicles available for regulated exocytosis.

1. Introduction

The secretory vesicles of endocrine cells are spherical organelles with diameters of few hundred nanometers. They are responsible for the regulated release of hormones, like peptide hormones (e.g., glucagon, insulin, α-MSH, β-endorphin), or biogenic amines (e.g., epinephrine, serotonin). Structurally, secretory vesicles or granules can be distinguished by their ultrastructural appearance and immunoreactivity for cell-type-specific hormonal content (Borgonovo et al., 2006). In classical ultrastructural analysis, within the pancreas, different types of cells can be readily distinguished by the electron density of the crystalline core and the fit of the halo (Cabrera et al., 2006; Caramia et al., 1965; de Carvalho et al., 2006; Elayat et al., 1995; El-Naggar, 2000; Etayo et al., 2000; Lee et al., 2003). Intermediate lobe cells or melanotrophs in the pituitary gland are endocrine cells that mainly produce the α-melanocyte stimulating hormone (αMSH) and β-endorphin, both of which are cleavage products of proopiomelanocortin (POMC). The chromaffin cells in the adrenal medulla possess typical ultrastructural features, most notably large chromaffin granules, containing one of the catecholamines, epinephrine or norepinephrine (Coupland and Tomlinson, 1989).

Secretory vesicles that have freshly budded from the trans-Golgi network are still immature (Borgonovo et al., 2006). Further processing and packaging of the vesicle cargo leads to progressive condensation of the vesicle's electron-dense cores and their conversion into mature secretory vesicles (Glombik and Gerdes, 2000). Condensation of the vesicle material could reduce the size of the vesicle and later on reduce the rate of the hormone dissolving process in the extracellular space after exocytosis (Borgonovo et al., 2006). The specificity of docking of the secretory vesicle and its fusion to the plasma membrane is critical to preserving secretory vesicle identity as an organelle and the proper flow of cargo within the cell (Zerial and McBride, 2001). Hence, the secretory vesicles are a heterogeneous vesicular population within a cell and they can differ in size as well as in their release properties (Grabner et al., 2005; Michael et al., 2006; Perrais et al., 2004).

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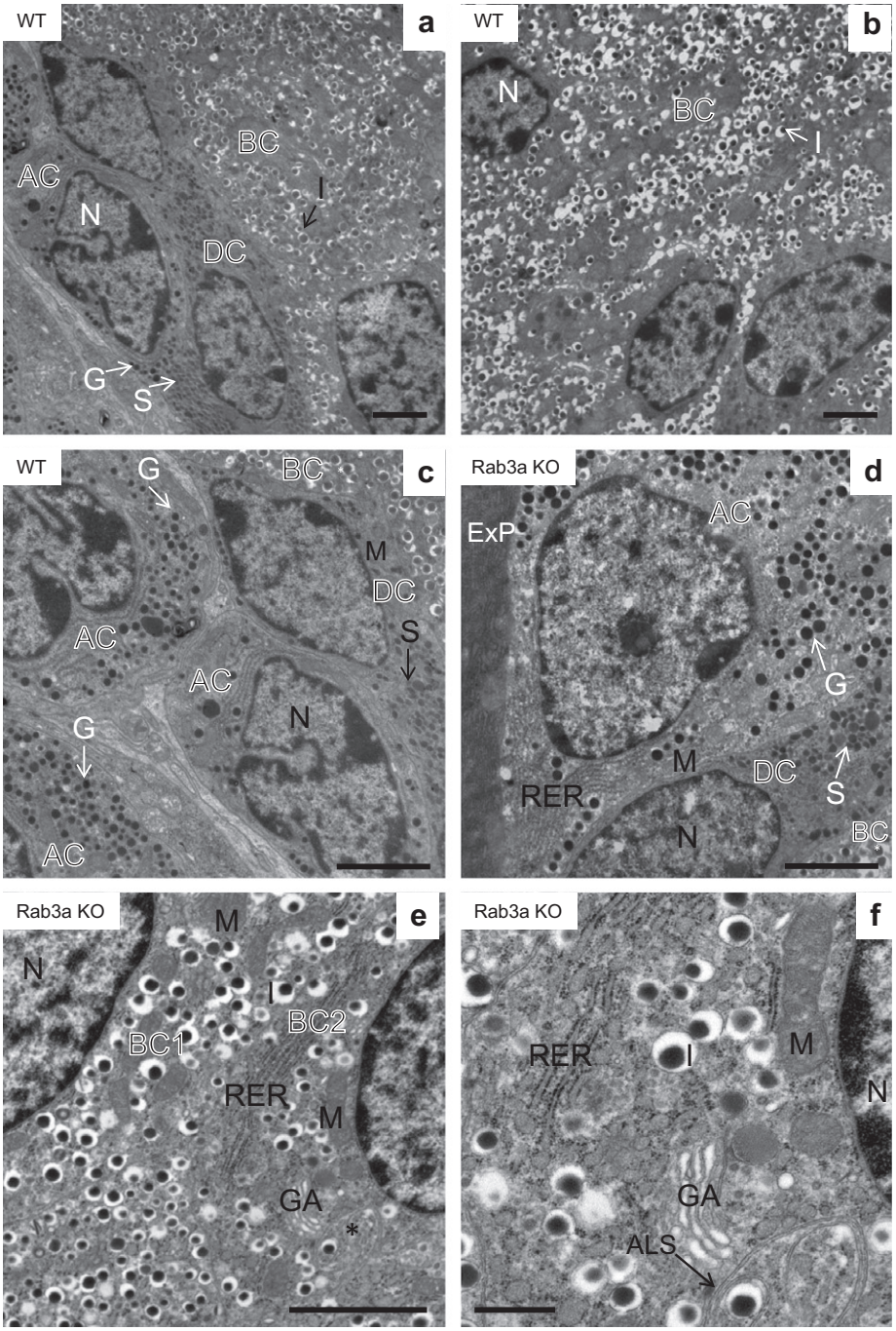

Fig. 1. Ultra-thin sections of the mouse pancreas: a, b: 3000x; c, d: 4400x; e: 7000x; f: 12000x. (a) Periphery of the islet was composed of alpha (AC) and delta cells (DC). In the region toward the center, beta cells (BC) were seen (WT mice). (b) The inner part of the islet was composed of beta cells (BC) (WT mice). (c) The endocrine part of the islet in WT mice and in Rab3a KO mice (d). Endocrine pancreatic cells had comparable ultrastructure in WT mice and Rab3a KO mice. Alpha cells (AC) had a centrally located nucleus, a clearly visible rough endoplasmic reticulum (RER) and many glucagon granules (G) and mitochondria (M).In the cytoplasm of beta cells (BC), the most prominent structures were insulin granules (∗) composed of an electron-dense core, a salient lucent halo and the membrane. Delta cells (DC) had a polygonal or oval nucleus (N), and electron-dense cytoplasm with numerous somatostatin granules (S). (e) Ultra-thin section of an insulin containing beta cells (Rab3a KO mice). The beta cell on the left side (BC1) was filled with numerous insulin granules, but it had little RER and few Golgi complexes. In contrast, the beta cell on the right (BC2) contained abundant RER, a clearly visible Golgi complex (GA), and many mitochondria (M), but it contained only a few insulin granules. (f) Enlarged region from Fig. e. In the cytoplasm of beta cell 2 (BC2), an autophagosome-like structure is seen at higher resolution. It is composed of the cytoplasm containing electron-lucent material and electron-dense granules, enveloped by the membranes. ALS, autophagosome-like structure; ExP, exocrine part of the pancreas; G, glucagon granule; I, insulin granule; N, nucleus; S, somatostatin granule. Scale bar: 2 μm (a–e); 500 nm (f).

Recent studies on knockout mice lacking individual granule specific proteins have challenged the hypothesis that an "essential" protein for the assembly of secretory granules exists (Borgonovo et al., 2006). The essential protein candidates include Rab proteins, which are monomeric GTPases; their regulatory principle lies in their ability to function as molecular switches that cycle between the active GTP- and the inactive GDP-bound conformations (Darchen et al., 1990; Gonzales and Scheller, 1999; Südhof, 1997; Zerial and McBride, 2001). The functions of Rab proteins have been described to be numerous and diverse. In all eukariyotes, different Rab proteins have been described as playing essential roles in the transport process of secretory granules, since they are located in