Ca^{2+}-INDUCED FUSION OF GOLGI-DERIVED SECRETORY VESICLES ISOLATED FROM RAT LIVER

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1. Introduction

During the transport of plasma proteins from the cytoplasma of hepatocytes to the extracellular fluid small vesicles may act as shuttles between the Golgi complex and the plasma membrane. This type of intracellular transfer is well established for various secretory cells and may be adopted also for the hepatocyte. Recent investigations have shown that secretory vesicles fuse with each other during secretion in mast cells [4] exocrine [5,6] and endocrine pancreatic tissue [7]. The intervesicular fusion provides a tool for studies on membrane fusion, since Golgi-derived vesicles can be isolated from the hepatocyte and their interaction with various agents, suggested to trigger membrane fusion, can be monitored by freeze-cleaving.

2. Materials and methods

Golgi fractions were isolated from livers of female Sprague–Dawley rats as described [1,2,3]. The method used is based on the overloading of Golgi elements with very low density lipoprotein particles produced in vivo by ethanol treatment. As modifications we buffered all sucrose solutions with 0.01 M cacodylate at pH 7.4 and supplemented them with 1 mM EGTA. In this investigation only the most homogenous Golgi fractions (called GF_1 in the publications cited above) isolated from a discontinous sucrose density gradient were used. Protein was determined according to Lowry et al. [13]. All chemicals were of the purest grade commercially available.

10 μl of the suspension of Golgi-vesicles ($\approx$ 1.5 mg protein/ml) were mixed with 10 μl of CSE-medium (0.01 M cacodylate, pH 7.4, 0.25 M sucrose and 1 mM EGTA) containing different concentrations of Ca^{2+} (the concentration of which was adjusted as described [10]), Mg^{2+}, Ba^{2+}, Sr^{2+}, or La^{3+}. After 5 min of incubation (37°C) the suspension was fixed by the addition of 10 μl of 2% glutaraldehyde in CSE, supplemented with the corresponding concentrations of ions used in the experiment, and 5 min later 10 μl glycerol were added for cryoprotection. The suspensions were kept at room temperature for 10 min and then small droplets ($\approx$ 0.5 μl) were frozen in Freon 22 cooled by liquid nitrogen. Freeze-cleaving [11] was performed in a Balzers freeze-etch unit BAF 300. Replicas were examined in a Siemens-Elmiscop 101 at 100 kV. Photographs were taken as positives (platinum deposition: black). Fracture faces are designated according to the new nomenclature introduced recently [12]: The membrane faces are denominated P and E, where P corresponds to the earlier A-face and E to the earlier B-face.

3. Results and discussion

A representative fracture through the population of isolated Golgi-vesicles is shown in fig.1a. The vesicles exhibit an apparent diameter of 0.2 μm and membrane associated particles (MAPs) stick more to the the concave P-face than to the convex E-face. This distribution of MAPs is consistent with that observed in secretory vesicles of different cells [7,14]. After raising the Ca^{2+}-concentration in the incubation

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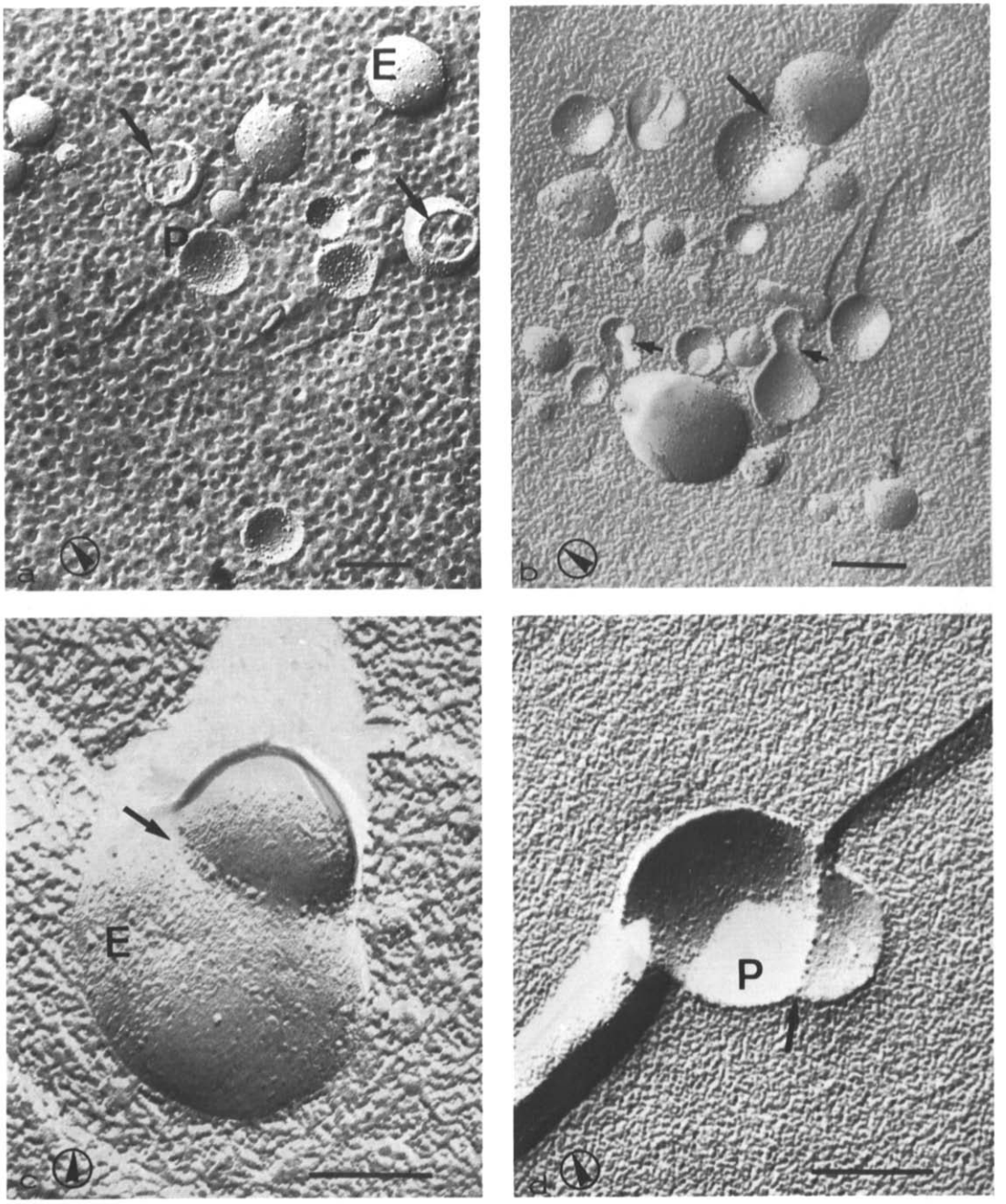

Fig.1. Electronmicrographs of freeze-fractured isolated Golgi-derived secretory vesicles from rat liver. (a) Secretory vesicles incubated in a solution with low Ca^{2+} concentration ($< 10^{-8}$ M). Vesicles are not aggregated. MAPs stick more to the concave P-face (P) than to the E-face (E) and are randomly distributed. Two vesicles are fractured partly through the interior of the vesicles exposing their lipoprotein content (arrows). (b) After incubation in high Ca^{2+} concentration ($2 \cdot 10^{-5}$ M) attached vesicles become visible. In the zone of contact MAPs are aggregated (arrow). Twin vesicles with a waist are also observed (small arrows). (c) E-face of twin vesicles with a ring-like aggregation of MAPs (arrow). The cleavage plane is continuous indicating fused vesicles. (d) P-face of fused vesicles with a ring-like aggregation of MAPs (arrow). Encircled arrowheads indicate direction of shadowing. Magnification (a) + (b): 54 000. (c) + (d): 90 000. Scale 0.2 μm.

medium vesicles become attached to each other and siamese twin vesicles are now visible (fig. 1b). These structures represent fused secretory vesicles with a common lumen since the cleavage plane is continuous in membrane P-face (fig.1c) as well as in membrane E-face (fig.1d). The number of fused vesicles depends upon the Ca^{2+}-concentration in the medium as shown in table 1. Half maximal percentage of fused vesicles was observed between 10^{-6} and 10^{-7} M Ca^{2+}. Incubation of isolated secretory vesicles with other cations (Mg^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+} and La^{3+}) in 10^{-4} M concentration results also in an attachment of vesicles, the fraction of fused vesicles however was very low and comparable with that obtained in Ca^{2+}-concentrations of less than 10^{-8} M (table 1).

The ability to fuse among each other was found to be restricted to isolated Golgi-derived vesicles. Preparations of rat liver microsomes were unable to fuse when our conditions are applied.

In this study cryoprotection for freeze-cleaving was performed with glycerol, a substance suggested to be a fusogenic agent for mitochondria [15] and erythrocytes [16]. From the following reasons, however, it seems unlikely that the fusion of Golgi-derived vesicles reported here can be attributed to glycerol. Cryoprotection was performed after fixation with glutaraledehyde and secondly, fused vesicles could be observed in freeze-fractured samples where fixation and addition of glycerol were omitted tolerating the appearance of ice crystals.

Table 1

Tab. I: Ion-dependence of the fusion of isolated Golgi-derived vesicles.

Cation	Concentration (M)	Fusion (%)
Ca^{2+}	$< 10^{-8}$	1.3
	10^{-7}	3.2
	10^{-6}	8.3
	10^{-5}	9.3
	10^{-4}	10.2
Mg^{2+}	10^{-4}	1.5
Sr^{2+}	10^{-4}	1.8
Ba^{2+}	10^{-4}	1.7
La^{3+}	10^{-4}	1.4
Mn^{2+}	10^{-4}	2.6

The figures were evaluated by counting 400 vesicles for each cation concentration in a scanned replica.

The fusion of secretory vesicles isolated from rat liver exhibit a similar dependence on Ca^{2+}-concentration as those from pancreatic endocrine tissue [8]. Furthermore in both systems other cations were ineffective to replace for Ca^{2+} as trigger of fusion. The other cations tested only led to aggregation of vesicles.

During the fusion of isolated pancreatic endocrine secretory vesicles [8] and the fusion of plasma membranes isolated from myoblasts in culture [9] membrane associated particles (MAPs) play an important role. They aggregate in the zone of contact of two vesicles and remnants of these aggregations can often be observed in the waist of vesicles undergoing fusion. The same morphological features were also observed in secretory vesicles from rat liver after exposure to Ca^{2+} (fig.1c, d).

The most interesting result in this investigation is presumably the observation that fusion of secretory vesicles increases in the range of normal intracellular concentration of free Ca^{2+} [17]. Therefore, an increase of free Ca^{2+} could act as trigger to cause exocytosis of lipids and proteins from the liver by initiating membrane fusion. This has been made probable for glucose induced insulin secretion where Ca^{2+} is regarded as the final intracellular trigger in stimulus–secretion coupling [8].

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