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With contributions by

H. Abrahamsson
A. Andersson
T. Andersson
S. J. H. Ashcroft
I. Atwater
P.-O. Berggren
G. Bommer
K. Capito
G. Dahl
C. M. Dawson
P. Donatsch
G. Eddlestone
R. Ekerdt
P. Flatt
B. Formby
M. Gratzl
V. Grill
E. Gylfe
H.-J. Hahn
N. Harnie
R. E. Hauser
C. J. Hedeskov
C. Hellerström
B. Hellman
J. C. Henquin
A. Herchuelz
L. Heylen
S. Holze
S. L. Howell
J. C. Hutton
L.-Å. Idahl
M. Kikuchi
G. Klöppel
V. Leclercq-Meyer
S. Lenzen
Å. Lernmark
D. A. Lowe
W. J. Malaisse
P. D. Marley
E. K. Matthews
P. Meda
H. P. Meissner
W. Montague
D. A. Nielsen
M. D. L. O'Connor
L. Orci
U. Panten
E. N. Parkin
A. Ü. Parman
A. Perrelet
D. G. Pipeleers
M. Preissler
A. E. Renold
B. P. Richardson
E. Rojas
A. Scott
A. Sener
J. Sehlin
G. W. G. Sharp
E. G. Siegel
D. F. Steiner
M. C. Sugden
I.-B. Täljedal
M. Tyhurst
I. Valverde
M. Welsh
G. Wauters
I. H. Williams
C. B. Wollheim

124 Figures, 51 Tables

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Table of Contents

The Role of Ca^{2+} as a Trigger for Membrane Fusion

M. Gratzl, R. Ekerdt, G. Dahl, Dept. of Physiological Chemistry and Physiology, University of Saarland, Homburg/Saar, FRG

Summary

As revealed by freeze-fracturing, secretory vesicles isolated from pancreatic islet cells fuse when incubated with low concentration of Ca^{2+}. The properties of this process are described and were found to be similar to those investigated with isolated secretory vesicles from different tissue origin. Secretory vesicle fusion is compared mainly with the ionic requirements of insulin secretion by the pancreatic B-cell and exocytosis by other secretory cells.

Introduction

During secretion by exocytosis secretory vesicles fuse with the cell membrane and discharge their contents into the extracellular space. The secretory vesicle membranes become inserted into the cell membrane. In this way, receptors present on the inner surface of secretory vesicle membranes become accessible as targets for circulating hormones (Bergeron et al 1973). An analogous situation is seen with 5'-nucleotidase, which is widely used as a marker enzyme for cell membranes but is also present in secretory vesicle membranes (Farquhar et al 1974). A similar relationship between secretory vesicles and the cell membrane was described for adenylate cyclase which is present on the cytoplasmic surface of the secretory vesicle membrane and which, following fusion, is found on the cytoplasmic surface of the cell membrane (Cheng and Farquhar 1976a, b) (Fig. 1).

The factors responsible for membrane fusion during exocytosis are present in the cell membrane as well as in secretory vesicle membranes. This can be concluded from the observation that, in stimulated cells, secretory vesicles fuse together as well as with the cell membrane ("compound exocytosis") (Fig. 1). Compound exocytósis occurs in mast cells (Röhlich et al 1971) in salivary glands (Amsterdam et al 1969; Hand 1970), in exocrine and endocrine pancreatic cells (Ekholm et al 1962; Berger et al 1975), in adrenal

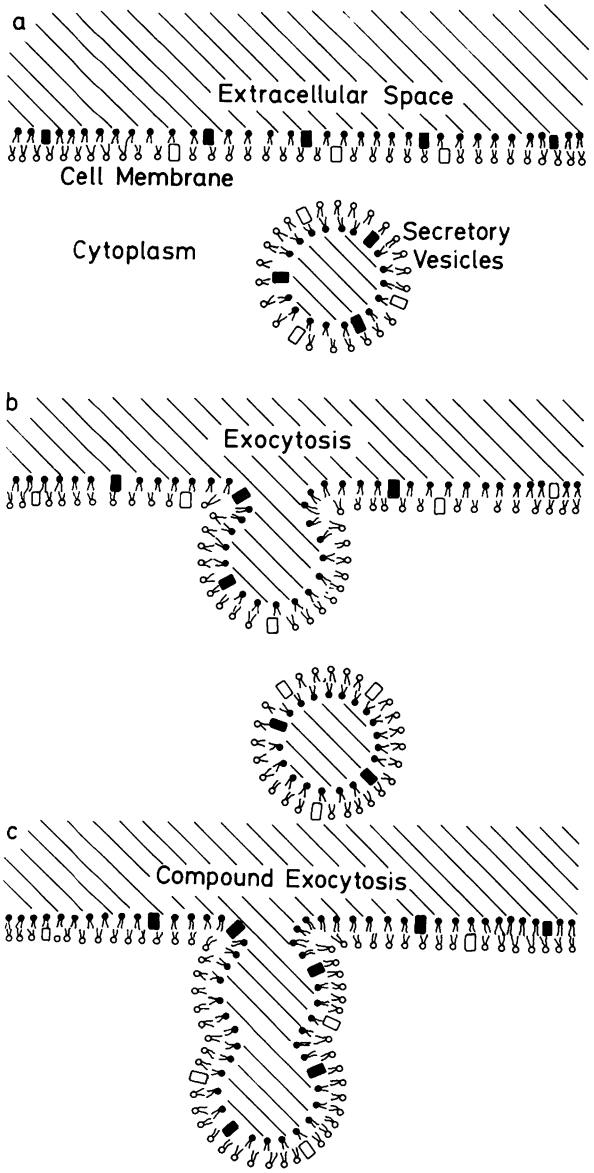

Fig. 1 (a) In the cell membrane, as well as in secretory vesicle membranes, two surfaces can be distinguished, the cytoplasmatic surfaces (the proteins or lipids of which are marked with open symbols) and the surfaces adjacent to the extracellular fluid as well as the intravesicular space (filled symbols).
(b) During exocytosis secretory vesicles fuse with the cell membrane and discharge their content into the extracellular space. The inner surface of the secretory vesicle membrane becomes the outer surface of the cell membrane.
(c) Compound exocytosis is characterized by fusion secretory vesicles among each other and with the cell membrane

medullary cells (Fenwick et al 1978; Aunis et al 1979), in adenohypophyseal and neurophyseal cells (DeVirgilis et al 1968; Gratzl et al 1977) as well as in other neurosecretory cells (Normann 1970; Andrew and Shivers 1976).

In this contribution the properties of intervesicular fusion of secretory vesicles from pancreatic islet cells (Dahl and Gratzl 1976) as well as from liver (Gratzl and Dahl 1976, 1978), neurohypophysis (Gratzl et al 1977) and adrenal medulla (Dahl et al 1979; Gratzl et al 1979; Dahl et al 1977) will be described and compared with the properties of secretion by the pancreatic B-cell as well as other secretory cells.

Intervesicular Fusion

In freeze-fracture electron micrographs secretory vesicles isolated from pancreatic islet cells are indistinguishable from those in intact cells. After addition of Ca^{2+}, fused vesicles can be observed, which are characterized by a continuous cleavage plane in both membrane faces. This is shown for isolated secretory vesicles from pancreatic islet cells in Figure 2. Fusion of secretory vesicles by low concentrations of calcium has also been observed with secretory vesicles from liver (Gratzl and Dahl 1976; 1978), neurohyophysis (Gratzl et al 1977) and adrenal medulla (Gratzl et al 1979; Dahl et al 1979). Fusion of isolated secretory vesicles has also been followed by thin section electron microscopy (Dahl et al 1979) and biochemical methods (Quinn and Judah 1978). To quantify membrane fusion the percentage of fused vesicles ("twinned vesicles") was determined after incubation with different Ca^{2+} concentrations (Fig. 3). Intervesicular fusion increases from 10^{-7} to 10^{-4} M Ca^{2+} and is half maximum around 10^{-6} M, at which point around 50% of the fusion obtained with 10^{-4} M Ca^{2+} has occurred. Such sigmoidal curves are also obtained with secretory vesicles from liver (Gratzl and Dahl 1978), neurohypophysis (Gratzl et al 1977) and adrenal medulla (Dahl et al 1979; Gratzl et al 1979). These vesicles, as well as the vesicles isolated from pancreatic islet cells, do not fuse when incubated with any other divalent cations in concentrations up to 10^{-3} M. Mg^{2+} added with Ca^{2+} to secretory vesicles inhibits Ca^{2+}-induced fusion in a concentration-dependent manner.

The ionic requirements for fusion of secretory vesicle membranes differ from that described for pure phospholipid membranes (for review see Papahadjopoulos 1978) and phospholipid membranes prepared from the lipid of secretory vesicle membranes (Dahl et al 1977; Gratzl et al 1979). Briefly, these membranes fuse if Ca^{2+} at 10^{-4} or higher concentrations is added; in most cases Mg^{2+} can replace Ca^{2+}, and addition of Ca^{2+} as well as Mg^{2+} has an additive effect.

With concentration of Ca^{2+} greater then 2.5 mM a second type of fusion of secretory vesicles has been detected. The properties of this type of reaction with respect to influence of divalent cations closely resembles that of the fusion of phospholipid membranes. Furthermore, fusion of secretory vesicles with high concentrations of divalent cations is not affected by treatment attacking membrane proteins such as

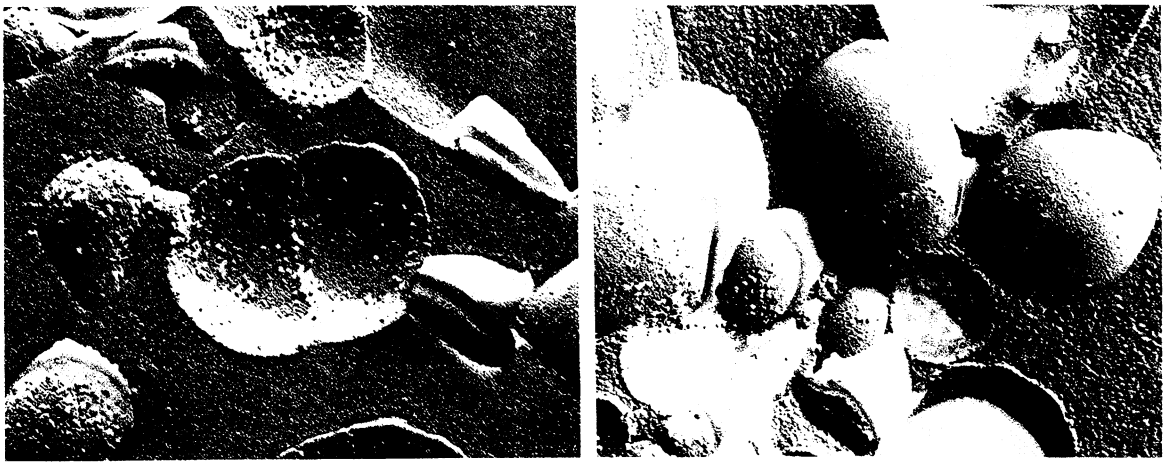

Fig. 2 Freeze-fracture electron micrograph of secretory vesicles from pancreatic B-cells fused in vitro by incubation in an isotonic medium containing 10^{-5}M Ca^{2+}. "Twinned vesicle "with continuous cleavage plane in membrane PF-faces (left) and membrane EF-faces (right). Nomenclature of fracture faces: Branton et al (1975) (From Dahl and Gratzl 1976). Magnification: x 80.000

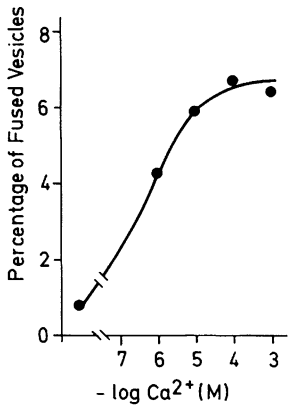

Fig. 3 Fusion of isolated secretory vesicles from pancreatic islet cells as a function of the free Ca^{2+} concentration

proteases, glutaraldehyde or neuraminidase, which abolish fusion of secretory vesicles by low concentrations of Ca^{2+} (Gratzl and Dahl 1978; Gratzl et al 1979; Dahl et al 1979). Two types of membranes fusion, a Ca^{2+}-specific one at lower concentrations and a type of fusion elicited by divalent cations at higher concentrations, have also been found by using isolated cell membranes from myoblasts in culture (Schudt et al 1976; Dahl et al 1978). As described for isolated secretory vesicles, the Ca^{2+}-specific fusion of myoblast cell membranes is affected by proteases, glutaraldehyde, neuraminidase and inhibitors of protein biosynthesis. Obviously proteinaceous membrane components participate in the Ca^{2+}-specific fusion of biological membranes.

Ionic Requirements of Secretion by Intact Cells

Insulin secretion by pancreatic B-cells stimulated by glucose or other secretagogues requires the presence of Ca^{2+} in the extracellular fluid. With the exception of Sr^{2+} and Ba^{2+}, Ca^{2+} cannot be replaced by other divalent cations, such as Be^{2+}, Cd^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+} and Ni^{2+} (for review see Lambert 1976, Hedeskov 1979).

Stimulation of pancreatic B-cells is paralled by in increase of Ca^{2+} influx into the cells, suggesting that insulin release occurs when Ca^{2+} accumulates in the cytoplasm. Divalent cations, which cannot replace extracellular Ca^{2+} in stimulus-evoked secretion, have been found to interfere with Ca^{2+} entry into pancreatic B-cells and other secretory cells. This might be the reason why these divalent cations depress glucose-stimulated insulin release in media containing physiological concentrations of Ca^{2+}. The functional importance of stimulant-induced Ca^{2+} influx is also shown by the inhibition of glucose-stimulated insulin release by organic Ca^{2+} antagonists (for review see Lambert 1976; Hedeskov 1979).

Ca^{2+} injected into the presynaptic nerve terminal in the giant synapse of the squid evoked transmitter release, whereas similar doses of Mg^{2+} and Mn^{2+} were ineffective. Injection of Sr^{2+} into the presynaptic axon had effects similar to those of Ca^{2+}. Mg^{2+} and Mn^{2+} led to a slight reduction in the amount of transmitter released by injection of Ca^{2+} (Miledi 1973). Similarly, injection of Ca^{2+}, but not Mg^{2+}, into mast cells elicited extrusion of secretory granules (Kanno et al 1973). Also microinjection of Ca^{2+} into oocytes resulted in cortical granule breakdown. Mg^{2+} did not induce this reaction (Hollinger and Schuetz 1976).

Transmitter release produced by membrane depolarization is directly correlated with the free intracellular Ca^{2+} concentration. This was shown by injection of aequorin, a protein that emits light in the presence of Ca^{2+}, into the presynaptic terminal of the squid giant

synapse (Llinás and Nicholson 1975). Information on the concentration of free Ca^{2+} within a secretory cell is also given from experiments with squid axons. The determinations set a range for free Ca^{2+} of 10^{-7}M or less which increases during stimulation (Baker et al 1971; DiPolo et al 1976). Aequorin-injected eggs of a fresh water fish show an explosive rise in intracellular free Ca^{2+} concentrations during fertilization from 10^{-7}M to about 30μM. This increase precedes cortical vesicle exocytosis (Gilkey et al 1978).

Parallelism between Intervesicular Fusion and Exocytosis

Fusion of secretory vesicles parallels the major findings observed with intact cells. It is low at Ca^{2+} concentrations found in resting cells and increases at concentrations found in stimulated cells. Thirdly, it is specific for Ca^{2+}-ions, and fourthly, it is inhibited by divalent cations which cannot replace Ca^{2+}. Concerning the pancreatic B-cells, there are two exceptions to this rule. Replacement of extracellular Ca^{2+} by Sr^{2+} or Ba^{2+} allows a glucose-stimulated insulin release from pancreatic B-cells (Hales 1970; Malaisse et al 1970). The situation with Ba^{2+} seems to be rather complicated, since Ba^{2+} causes considerable insulin release without stimulating concentrations of glucose. In this context, experiments with monolayer cultures of the endocrine pancreas are of interest, where extracellular Ca^{2+} with the aid of an ionophore was able to trigger insulin release, but Ba^{2+} (at nonstimulating concentrations) and Sr^{2+} were ineffective (Wollheim et al 1975).

In experiments with intact cells, the extracellular concentration of Ca^{2+} can easily be changed. The intracellular Ca^{2+} pools, surrounded by membranes, are more difficult to influence in a controlled manner. The low concentration of Ca^{2+} in the intracellular fluid in pancreatic B-cells as well as in other secretory cells is maintained by several energy-dependent sequestering systems located in the mitochondria reticulum and in secretory vesicles. The increase of intracellular free Ca^{2+} during stimulation probably makes use of the high extracellular Ca^{2+} concentrations by changes of the ion fluxes across the cell membrane. Co^{2+} or Ni^{2+} interfere with Ca^{2+} fluxes in the pancreatic B-cells (Dormer et al 1974; Henquin and Lambert 1975) Sr^{2+}, Ba^{2+} and Co^{2+} affect Ca^{2+} fluxes in squid axons (Blaustein and Santiago 1977; DiPolo 1979). ATPase activated by low concentrations of Ca^{2+} have been found in islet subcellular fractions (Formby et al 1976). However, the importance of these ATPases for the regulation of the free intracellular Ca^{2+} concentration can be judged only if activation by Ca^{2+} occurs similarly in the presence of millimolar concentrations of Mg^{2+}, present in the intracellular fluid (Brinley et al 1977). Also, the specificity of these systems has not been investigated, but it is known from mitochondria isolated from other secretory cells that Sr^{2+} and other divalent cations are taken up by an energy-dependent mechanism (Vainio et al 1970) and that Sr^{2+} shares the same site as Ca^{2+} and competes with this ion (Carafoli 1975). If the systems responsible for the regulation of low intracellular free Ca^{2+} concentrations are challenged, for example by injection of Sr^{2+} (Miledi 1973), this divalent cation could easily influence the transport and flux systems in such a way that the free concentration of intracellular Ca^{2+} increases and triggers transmitter release.

From the comparison of the properties of membrane fusion of subcellular membranes with the properties of secretion of insulin by the pancreatic B-cell,as well as other secretory cells, it can be concluded that both processes can occur under similar conditions. We have thus proposed that Ca^{2+} acts as the final intracellular messenger, triggering fusion of secretory vesicles with each other as well as with the cell membrane.

From studies on secretory vesicle fusion, several predictions for experiments with intact cells can be made. For example, from the knowledge that fusion of secretory vesicles decreases with temperature but can still be detected at 2°C (Gratzl and Dahl 1978), it can be concluded that exocytotic membrane fusion should be able to take place at temperatures as low as 2°C. This has recently been shown for pancreatic B-cells, where low temperature, even in the absence of an external stimulus, evokes exocytotic release of insulin. Furthermore, under these conditions intervesicular fusion can be observed throughout the cell, presumably triggered by a general rise in the intracellular free Ca^{2+} concentration (Dahl and Henquin 1978). Cold-induced hormone release is also known to occur in the neurohypophysis (Douglas and Ishida 1965) and both biochemical (Hong and Poisner 1974) and morphological findings (Dreifuss et al 1974, Gratzl et al 1977) support an exocytotic mechanism.

The molecular mechanism, whereby Ca^{2+} interacts with membrane proteins or lipids to allow the membranes to be fused, is completely unknown. Clearly, one of the first interactions of Ca^{2+} with membranes to be fused is binding. Ca^{2+} ion binding to secretory vesicles from pancreatic islet cells as well as other secretory cells has been studied by measurements of electrophoretic mobility (Dean 1974, 1975; Dean and Matthews 1975). From these investigations equilibrium constants for Ca^{2+}-binding have been calculated to be in the millimolar range. Ca^{2+} binding to secretory vesicles from adrenal medulla was recently reinvestigated using Ca^{2+}-sensitive indicator substances. It has turned out that, in addition to the low affinity sites already detected by electrophoretic mobility measurements, high affinity sites with a dissociation constant of 5×10^{-6}M are present at the secretory vesicle membrane from adrenal medulla (Dahl et al 1979). These sites may actually represent the button which is pressed to initiate the fusion mechanism.

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