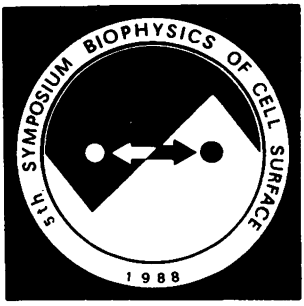

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CELL CONTACT AND MEMBRANE FUSION IN ENDOCRINE CELLS

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Summary

Cell contacts involving specialized cell adhesion molecules and information transfer by hormones over long distances are general features of neurones and endocrine cells.

NCAMs (neural cell adhesion molecules), three closely related polypeptides, which were first detected in nerve cells, are also expressed in endocrine cells. Their extracellular domains are either attached via a phospholipid (PI) anchor (NCAM-120) to the plasma membrane or linked via a membrane spanning segment to an intracellular domain of different length (NCAM-140 and NCAM-180). In adult rat endocrine tissue (neuro- and adenohypophysis, adrenal medulla, pancreatic islets) only NCAM-140 has been observed (with the exception of the neurohypophysis which expresses all three NCAM variants). In contrast, the corresponding tumour cell lines (pheochromocytoma, insulinoma) in addition contain NCAM-180 suggesting that cell contact in tumour cells may be different on the molecular level compared to non-tumourous tissue.

Hormone release (by exocytosis) from endocrine cells involves contact between the membrane of the hormone containing secretory vesicles and the inner surface of the plasma membrane. In permeabilized cells the regulation of the fusion of these membranes can be investigated directly. These preparations permit investigations of the calcium triggered step and its modulation by GTP-binding proteins, protein kinase C or other regulatory systems. Endocrine tumour cells share with their non neoplastic counterparts the molecular requirements for exocytotic membrane fusion.

Introduction

Cell-cell interaction either by direct contact or via secretory products such as hormones or transmitters is important for the formation of cell collectives and for their communication. Recent investigations have demonstrated that the cell surface of endocrine cells contains the same adhesion molecules which are found in neurones (NCAMs). The release of secretory products by exocytosis is also a feature common to neural and endocrine cells. The mechanism of fundamental processes such as cell adhesion and secretion can be expected to be very similar in both cell types.

Neural cell adhesion molecules (NCAMs) of endocrine cells

Neural and endocrine cells share the expression of NCAM on their cell surface /8, 15, 16/. Using a polyclonal anti-NCAM antiserum an intense specific staining of the cell surface of all endocrine cells so far examined has been observed by immunocytochemistry at light and electron microscope levels. The endocrine cells investigated included those from both parts of the hypophysis, adrenal medulla and pancreatic islets. Also the tumour counterparts of the latter two, pheochromocytoma (PC12) and insulinoma (RIN) cells, exhibited an intense specific staining over the entire cell surface. No appreciable level of intracellular staining was noticed in any of the cells studied.

In adult brain three different NCAMs with molecular weights of 180 KD, 140 KD and 120 KD are expressed (c.f. /11/) and the extracellular domain of each form is homologous in sequence. The different NCAM polypeptides differ in their membrane-associated and cytoplasmic domains. As revealed by immunoblotting mature endocrine cells express NCAM-140 (FIG. 1). The 120 KD determinant could be found only in trace amounts in neurohypophyseal extracts. While NCAM-180 is the most prominent component in the neurohypophysis, other endocrine tissues such as the adenohypophsis, adrenal medulla or pancreatic islets were not found to express NCAM-180. However the pattern observed in the tumour counterparts of the latter two, pheochromocytoma and insulinoma cells indicates that malignant tranformation results in the expression of an additional NCAM-180 polypeptide (FIG. 1).

Studies of NCAMs in endocrine cells and their corresponding tumours will increase the information on cell surface molecule expression during tissue formation and malignant transformation.

FIG. 1 Immunological identification of NCAM in cerebellum and different endocrine tissues and cell lines: (1) cerebellum, (2) neurohypophysis, (3) adenohypophysis, (4) adrenal medulla, (5) PC12 cells, (6) pancreatic islet cells, (7) RIN cells.
Molecular masses (KD) of NCAM polypeptides are indicated

Exocytosis studied from within endocrine cells

Permeabilization of the plasma membrane by appropriate techniques permits access to and control over the cytoplasm. However, membrane permeabilization must be restricted to the plasma membrane leaving the exocytotic machinery unchanged.

Pore forming bacterial proteins such as alpha-toxin from Staphylococcus aureus and streptolysin O (SLO) from beta-hemolytic streptococci /10/ permeabilize endocrine cells for small molecules up to 1 KD (e.g. ions, nucleotides) or for large proteins like lactate dehydrogenase and immunoglobulins, respectively /1-7, 9, 12-14, 17/. In permeabilized adrenal medullary chromaffin cells or pheochromocytoma cells calcium in μmolar concentrations is an absolute requirement to trigger

exocytosis. In adrenal chromaffin cells, but not in pheochromocytoma cells, ATP is also required. Therefore, beside the Ca^{2+}-induced membrane fusion, an ATP dependent process, e. g. intracellular transport of vesicles, is necessary before exocytosis can take place in these cells. Ca^{2+}-stimulated exocytosis from permeabilized endocrine cells can be modulated by protein kinase C or a GTP-binding protein /3/. Phorbolesters like TPA activate the protein kinase C system and give rise to an increased Ca^{2+}-induced catecholamine release from pheochromocytoma cells. The poorly hydrolysable homologue GTPγS inhibits exocytosis from PC12 cells in a pertussis toxin sensitive manner, suggesting that a G protein of the type Go or Gi modulates exocytosis in these cells /3/.

FIG. 2 summarizes our current knowledge of the regulation and modulation of exocytosis.

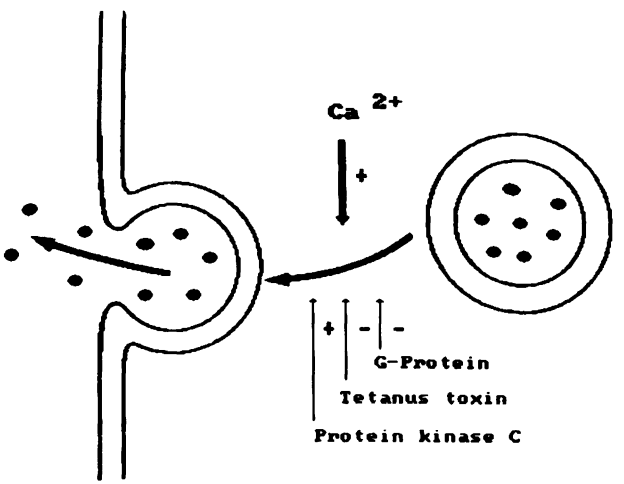

FIG. 2 Regulation and modulation of exocytosis by permeabilized pheochromocytoma cells

Using PC12 cells permeabilized with SLO, intracellular proteins involved in exocytosis can be probed with appropriate antibodies. Antibodies against calmodulin, which is involved in a variety of Ca^{2+}-sensitive processes, or synaptophysin (p38), a membrane protein present in clear synaptic and chromaffin vesicles /18/ were found not to affect exocytosis in permeabilized cells. However, tetanus toxin in the presence of a reducing agent which causes the two chains of the native toxin to separate /19/ was found to strongly inhibit Ca^{2+}-induced catecholamine secretion /4/. Such approaches should prove useful in the future for a more detailed analysis of exocytotic mechanisms and in addition enable the targets for this toxin to be elucidated.

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