

Extracellular matrix proteins, integrins and regulated secretion in lacrimal acinar cells

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It has recently been shown that interactions between a component of a basement membrane extract and cultured rat lacrimal gland acinar cells are essential for an efficient stimulus-secretion coupling of both the cholinergic and VIP-ergic pathways (1). Removal of the component results in a 50% decrease of the regulated secretion, but re-addition of it completely restores the response within 2 hrs. Since integrins are known to play critical roles in interactions between the extracellular matrix and the cell by directing a number of functions, e.g. cell adhesion, cytoskeletal organization and signal transduction, it is our hypothesis *i.* that integrins are involved in modulating the regulated secretion of the lacrimal gland acinar cell, either by direct interaction with intracellular signalling cascades or through the cytoskeletal organization, and *ii.* that the hormonal changes brought about by the onset of menopause affects the integrin expression and, thus, the responsiveness of the lacrimal gland. In the present study, we have examined the presence of several integrin subunits in cells kept in primary culture, and we have also examined the effects of culturing rabbit acinar cells in the presence of different matrix proteins on secretion stimulated by different secretagogues.

Methods. Single cells were prepared from lacrimal glands from female NZW rabbits and cultured for 40 hrs, allowing for re-establishment of acinus-like structures. Harvested cells were washed and collected on microscope-slides using a cyto-spin technique. The cells were fixed and analyzed for the presence of integrin subunits using primary antibodies directed against integrin subunits. Binding was detected using an alkaline phosphatase:anti-alkaline phosphatase (APAAP) three-layer method and a Fast Red substrate system for color development. For secretory studies, single cells were isolated as above and cultured on an Engelbreth-Holm-Swarm basement membrane extract (BMS) or purified matrix proteins. Cells were then rinsed and incubated at 37°C for 60 min w./w.o. different secretagogues. Media were collected and analyzed for α -hexosaminidase catalytic activity as a measure of secretion.

Results. Cultured acinar cells from rabbit lacrimal gland stained positive for the α_2 , α_V , α_1 and α_4 subunits, whereas only weak staining was observed for α_6 subunit. Stimulation by VIP, PACAP, phenylephrine or isoproterenol resulted in a 2-fold increase of secretion, whereas carbachol, an acetylcholine analogue, resulted in a more than 6-fold increase over basal in cells cultured with BMS. The latter can be compared with earlier results obtained from freshly isolated cells (1.7-fold) (2) and cells cultured w.o. BMS (<2.5-fold) (3). Laminin, fibronectin and vitronectin had similar effects as BMS on regulated secretion, whereas collagen IV was less potent.

Conclusion. Our results indicate an important role for ECM in modulating regulated secretion by rabbit lacrimal acinar cells, and further studies will hopefully identify the integrin(s) involved and the mechanism of action.

References

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