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Integrin adhesion in regulation of lacrimal gland acinar cell secretion

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Abstract

The extracellular microenvironment regulates lacrimal gland acinar cell secretion. Culturing isolated rabbit lacrimal gland acinar cells on different extracellular matrix proteins revealed that laminin enhances carbachol-stimulated secretion to a greater extent than other extracellular matrix proteins investigated. Furthermore, immunofluorescence indicated that integrin subunits, potentially functioning as laminin receptors are present in acinar cells. Among these, the integrin α6 and β1 subunit mRNA expression was also confirmed by RT–PCR and sequence analysis. Secretion assays, which measured β-hexosaminidase activity released in the culture media, demonstrated that function-blocking integrin α6 and β1 monoclonal antibodies (mAbs) induce a rapid, transient and dose-dependent secretory response in cultured cells. To determine the intracellular pathways by which integrin α6 and β1 mAbs could induce secretion, selected second messenger molecules were inhibited. Although inhibitors of protein kinase C and IP_3-induced Ca^{2+} mobilization attenuated carbachol-stimulated secretion, no effect on integrin mAb-induced release was observed. In addition, protein tyrosine kinases do not appear to have a role in transducing signals arising from mAb interactions. Our data clearly demonstrate, though, that cell adhesion through integrins regulates secretion from lacrimal gland acinar cells. The fact that the integrin mAbs affect the cholinergic response differently and that the integrin β1 mAb secretion, but not the α6, was attenuated by the phosphatase inhibitor, sodium orthovanadate, suggests that each subunit utilizes separate intracellular signaling pathways to induce exocytosis. The results also indicate that the secretory response triggered by the β1 integrin mAb is generated through dephosphorylation events.

Keywords: extracellular matrix; receptor; carbachol; expression; phosphorylation; phosphatase; laminin

1. Introduction

Lacrimal gland acinar cells are responsible for the production and secretion of proteins and fluid, essential for maintaining a healthy ocular surface. The secretory response is mainly regulated by neurotransmitters of the autonomic nervous system, where release of secretory vesicles can be triggered by activation of the IP_3/DAG as well as cAMP-dependent second messenger pathways (Hodges and Dartt, 2003).

In tissue, lacrimal gland acinar cells are organized in cell clusters, with their apical membrane directed towards a central lumen, into which tear fluid secretion occur. The basolateral surface of the lacrimal gland epithelium is surrounded by a specialized extracellular matrix (ECM) substratum called the basement membrane. Structural supportive properties of the basement membrane arise from laminin and collagen IV networks linked to glycoproteins and proteoglycans (Erickson and Couchman, 2000; Streuli and Bissell, 1990). In combination with soluble molecules such as growth factors and ions, the basement membrane exerts its physiological effects by acting via integrins and other cellular receptors. Cell attachment to the underlying substratum is a process today known to influence a variety of cellular events as polarization, migration, proliferation and gene expression (Hynes, 1999; Juliano et al., 2004; Lee and Juliano, 2004; Matlin et al., 2003).

Integrins, consisting of an α- and a β-subunit, are cell adhesion glycoprotein transmembrane receptors. A large number of different α- and β-subunits have been identified, and the association of these determines ligand specificity and the biological actions of integrins. Each cell type seems to have

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a restricted and unique set of integrin heterodimers, thereby controlling specific cellular functions (Bello-DeOcampo et al., 2001; Guan et al., 2003; Menko and Philip, 1995; Wayner et al., 1993). Integrins can establish both cell-cell linkage and cell-ECM adhesion, where the latter constitute integrin binding to e.g. the ECM proteins laminin, collagen, fibronectin or vitronectin (Springer and Wang, 2004). Upon attachment and activation, integrins mediate the assembly of multiple cytoskeleton components and signaling molecules into intracellular focal adhesion complexes, capable of downstream signaling (Giancotti and Ruoslahti, 1999; Giancotti, 2000). Several of the recruited proteins present at focal adhesion sites are highly regulated by phosphorylation events. Autophosphorylation of the non-receptor tyrosine kinase FAK is believed to be the initial controlling step. Src-tyrosine kinases and other adaptor molecules are implicated in further regulation of the cellular responses observed upon cell attachment (Schaller, 2001).

The present study is aimed at exploring the role of the ECM proteins and integrins in regulation of lacrimal gland function. For this purpose, integrin subunit protein expression was determined by confocal fluorescence microscopy and presence of specific integrin subunit mRNAs were detected by reverse transcriptase-PCR. Images showed that integrins functioning as laminin receptors are present in cultured cells. It was also observed that acinar cells cultured on exogenously added laminin appear to respond better to carbachol stimulation than other ECM proteins evaluated. Further experiments demonstrated that function-blocking mAbs against integrin α6 and β1 subunits induce a strong, but transient, secretory response from acinar cells, independent from the well-established cholinergic signaling pathways. Despite many reports stating that Src-tyrosine kinase activation is important for signaling from focal adhesion sites, protein tyrosine kinase inhibitors did not affect the secretory response induced by integrin mAbs. Moreover, the general tyrosine phosphatase inhibitor sodium orthovanadate significantly reduced the carbachol and integrin β1 mAb triggered secretion but had only marginal effects on the integrin α6 induced secretory response, indicating the presence of different signaling pathways for the two integrin subunits in lacrimal gland acinar cells.

2. Methods

2.1. Materials

The mouse monoclonal antibodies (mAbs) against integrin subunit; α1 (FB12), α2 (P1E6), α3 (P1B5), α4 (P1H4), α5 (P1D6), αv (P3G8), β1 (P4G11), β2 (P4H9-A11), β3 (25E11) β4 (ASC-9), the rat monoclonal α6 integrin (NKI-GoH3) antibody and the rabbit polyclonal β5 integrin antibody, used in immunofluorescence studies, were all obtained from Chemicon International (Temecula, CA). Two rat monoclonal function-blocking antibodies were used in secretion studies, one directed against integrin subunit α6 (GoH3) was from Research Diagnostics Inc. (Flanders, NJ) and the other directed against integrin subunit β1 (mab13) (Akiyama et al., 1989) was a gift from Dr. Akiyama, National Institute of Environmental Health Sciences (Research Triangle Park, NC). The integrin α6 (GoH3) antibody has been found to be reactive specifically with rabbit epithelial cells (Gruskin-Lerner et al., 1997). Of the integrin β1 antibodies used, the P4G11 clone recognized double bands, when tested by immunoblotting of rabbit lacrimal gland homogenate, see inset in Fig. 2. The mab13 clone has not previously been characterized in rabbit, but has been used in function-blocking studies of several cell types of human origin (Akiyama et al., 1989; Beauvais et al., 2004; Bello-DeOcampo et al., 2001; Jung et al., 2000). Protein A/G Plus-agarose was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Insulin–transferrin–sodium selenite mix, hydrocortisone, linoleic acid, FITC-conjugated goat anti-mouse IgG antibody, FITC conjugated goat anti-rat IgG antibody, methylumbelliferyl *N*-acetyl-β-D-glucosaminide (4MUGlcNAc), collagen I, carbachol, sodium orthovanadate, chelerythrine chloride and genistein were obtained from Sigma Chemical (St Louis, MO). Prolong antifade mounting medium, Alexa Fluor 568 conjugated goat anti-rabbit secondary antibody and rhodamine phalloidin were from Molecular Probes (Eugene, OR). Vitronectin was obtained from Biosource International (Nivelles, Belgium). Fibronectin and the solution of penicillin, streptomycin and glutamine were purchased from Invitrogen Corp. (Carlsbad, CA). Collagen IV, laminin and BD Matrigel® basement membrane matrix was obtained from BD Bioscience (Bedford, MA). The Src-tyrosine kinase family inhibitor PP2 (pyrazolopyrimidine) and the inhibitor of IP_3 induced Ca^{2+} release, 2-APB (2-aminoethoxydiphenylborate), were from Calbiochem, (La Jolla, CA). Remaining standard reagents used were all from Sigma Chemical.

2.2. Cell isolation and culture

The procedure for lacrimal gland acinar cell isolation from female NZW rabbits weighing 1.7 ± 0.2 kg (ESF-Products, Estuna AB, Norrtälje, Sweden) essentially followed the method described earlier (Gierow et al., 1996). Animals were handled according to directions from the Ethical Committee for Animal Experiments (Linköping, Sweden) and the ARVO statement for use of animals in ophthalmic and vision research. An exception from the protocol is the enzyme concentrations used for enzymatic digestion of tissue fragments; collagenase 200 U/ml (Invitrogen), hyaluronidase 350 U/ml (Worthington, Freehold, NJ) and deoxyribonuclease (DNase) I 53 U/ml (Calbiochem). Purified single acinar cells were placed at a cell density of 6.6×10^5 cells/cm^2 in 6-well plates, 6.3×10^5 cells/cm^2 in 12-well plates and 8.5×10^5 cells/cm^2 in 48-well plates in a final volume of 1 ml, 0.5 ml and 0.15 ml, respectively. Cells were cultured in a serum-free medium (PCM-2), made from a 1:1 mixture of Ham's F-12 (Invitrogen) and DMEM (low glucose Dulbecco's modified Eagle's medium, Invitrogen) supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, 2 mM glutamine, 0.1 mM sodium citrate, a mix of insulin, transferrin and sodium selenite (5 μg/ml/5 μg/ml/5 ng/ml), 2 mM sodium butyrate, 5 nM