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Calcium signaling in lacrimal glands

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ABSTRACT

Lacrimal glands provide the important function of lubricating and protecting the ocular surface. Failure of proper lacrimal gland function results in a number of debilitating dry eye diseases. Lacrimal glands secrete lipids, mucins, proteins, salts and water and these secretions are at least partially regulated by neurotransmitter-mediated cell signaling. The predominant signaling mechanism for lacrimal secretion involves activation of phospholipase C, generation of the Ca^{2+}-mobilizing messenger, IP_3, and release of Ca^{2+} stored in the endoplasmic reticulum. The loss of Ca^{2+} from the endoplasmic reticulum then triggers a process known as store-operated Ca^{2+} entry, involving a Ca^{2+} sensor in the endoplasmic reticulum, STIM1, which activates plasma membrane store-operated channels comprised of Orai subunits. Recent studies with deletions of the channel subunit, Orai1, confirm the important role of SOCE in both fluid and protein secretion in lacrimal glands, both *in vivo* and *in vitro*.

1. Introduction

The major function of lacrimal glands is to provide water, electrolytes, proteins and mucins to lubricate and protect the environmentally exposed surfaces of the eye (cornea and conjunctiva) [1]. Mammals have a major gland associated with each eye, and a number of minor glands (*i.e.*, goblet cells, meibomian gland), which contribute to constitutive and neurogenic tears and all of which may be involved in pathological conditions when functionally impaired. An understanding of the basic mechanisms underlying lacrimal gland secretion may provide insights to the treatment of debilitating age-related dry eye diseases, as well as the more general exocrine dysfunction in Sjögren's syndrome [2]. Here we review the basic cell biology underlying the signaling pathways leading to secretion of proteins and fluid from the major lacrimal glands.

The flow of tears has long been known to be under both parasympathetic and sympathetic control [1,3,4]. Early studies demonstrated that stimulation of muscarinic–cholinergic receptors increased the discharge of granule stored protein, largely peroxidase, from rat exoribital lacrimal gland [5–7]. While not fully defined, the cholinergic-induced calcium signal likely exerts multiple effects during protein secretion to coordinate the mobilization of secretory vesicles to the lacrimal apical membrane where they fuse and release their contents. Synaptotagmin is a likely target to detect the cholinergic-induced calcium signal which works in concert with SNARE proteins (including VAMP8) to complete and open the vesicle fusion pore [8].

Ca^{2+}-dependent activation of monovalent channels (K^- and Cl^-) plays a critical role in fluid secretion, generating electrochemical and osmotic gradients to drive the movement of water and accumulation of electrolytes into the lumen of acinar clusters and the ductal system [9]. In *in vitro* preparations, muscarinic receptors increased the efflux of K^+ [7], a response thought to reflect ionic movements related to fluid secretion. Both protein secretion and K^+ efflux responses depended at least partially upon extracellular Ca^{2+}, and were associated with increased uptake of radioactive Ca^{2+} into the glands [6,7].

Subsequently, it was demonstrated that an α-adrenoceptor mechanism similarly activated both protein discharge and increased K^+ permeability [10–12], although curiously the α-adrenergic protein secretion was somewhat less sensitive to removal of extracellular Ca^{2+}. Unlike parotid salivary glands, lacrimal glands do not apparently contain β-adrenoceptors, but do contain adenylyl cyclase activating vasoactive intestinal peptide receptors [13] and melanotropin receptors [14]. Other Ca^{2+}-linked

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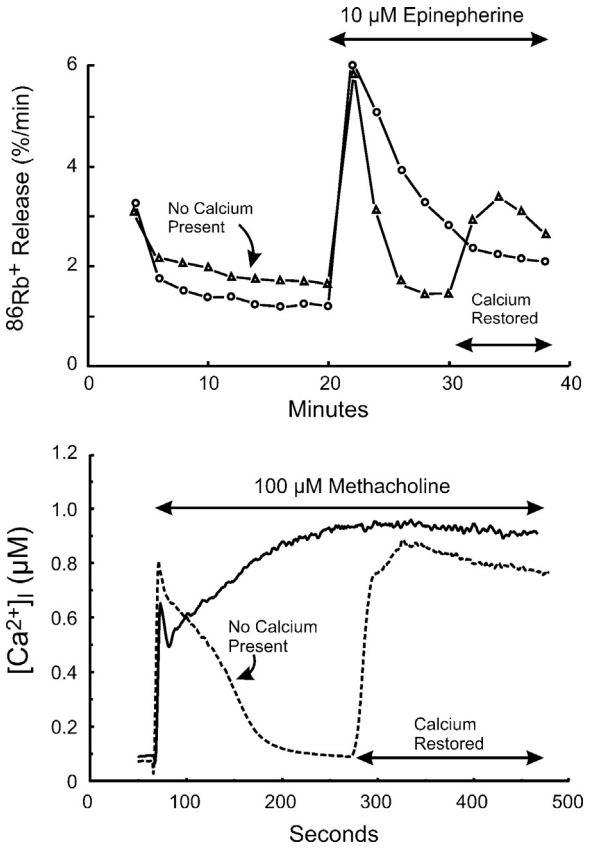

Fig. 1. Two phases of Ca^{2+} signaling in *in vitro* lacrimal gland preparations. Top: Changes in intracellular Ca^{2+} in slices of rat lacrimal gland are inferred from the efflux rate of $^{86}Rb^+$. Redrawn from data originally presented in [11]. Bottom: Changes in intracellular Ca^{2+} in mouse lacrimal acinar cells are measured with the Ca^{2+} indicator, Fura-2. In both cases, in the absence of extracellular Ca^{2+}, the response is transient, and subsequently restored by addition of Ca^{2+}.

receptor types shown to significantly modulate lacrimal secretion include multiple types of purinergic P2X and P2Y receptors [15–18], substance P, serotonin, histamine [19] and protease-activated receptors [20].

In early studies of Ca^{2+} signaling, direct measurement of intracellular Ca^{2+} with chemical or genetically encoded indicators was not available. Changes in intracellular Ca^{2+} were often inferred from the time course and magnitude of Ca^{2+}-mediated responses, and for lacrimal glands and certain other epithelial cells, Ca^{2+}-activated K^+ channels provided this link [21]. The rate of K^+ efflux from lacrimal cells was assessed by an isotope washout technique whereby slices of lacrimal gland were equilibrated with $^{86}Rb^+$, a surrogate for K^+ [22]. By stepwise transfer of the slices through a series of non-radioactive incubations, released radioactivity could be measured and time-based changes in the first order rate constant for $^{86}Rb^+$ efflux calculated. By use of an experimental sequence first described for studies in parotid gland [23,24], protocols omitting and restoring extracellular Ca^{2+} revealed a biphasic response: the initial transient increase in $^{86}Rb^+$ efflux was independent of extracellular Ca^{2+}, while the sustained efflux response depended on extracellular Ca^{2+} being present [25] (Fig. 1). The Ca^{2+} independent component of the response was thought to result from an intracellular release of Ca^{2+}, because only one such response could be obtained in the absence of external Ca^{2+}, and then an incubation in Ca^{2+}-containing medium was necessary to restore the response. This latter finding will be discussed in more detail in relation to its relevance to the mechanism of Ca^{2+} influx.

2. Release of intracellular Ca^{2+}

Ca^{2+} signaling in lacrimal acinar cells was initially seen to result from a biphasic mobilization of Ca^{2+} to the cytoplasm, an initial release of intracellular Ca^{2+} which was followed by or accompanied by an increase in Ca^{2+} entry across the plasma membrane [7]. The intracellular release mechanism was the first to be solved. From as early as the 1950s, it was known that certain receptors, including muscarinic cholinergic receptors, stimulated a turnover of inositol lipids [26]. In 1975, Bob Michell [27] published his classic review on inositol lipids in which he proposed that this turnover in some manner served to link receptor activation to Ca^{2+} signaling. In 1983, Mike Berridge demonstrated that following receptor activation, the head group of phosphatidylinositol 4,5-bisphosphate, inositol 1,4,5-trisphosphate (IP_3), rapidly appeared in fly salivary glands, and suggested that this molecule served as a second messenger for Ca^{2+} release [28]. Soon thereafter, in a collaboration between Berridge, Irene Schulz and Robin Irvine, IP_3 was shown to release Ca^{2+} from non-mitochondrial stores in a preparation of permeabilized pancreatic acinar cells [29]. Consistent with this idea, in lacrimal glands Ca^{2+}-mobilizing agonists stimulated turnover of inositol lipids and this involved degradation of phosphatidylinositol 4,5-*bis*-phosphate and formation of soluble inositol phosphates [30]. IP_3 was later shown to release intracellular Ca^{2+} in lacrimal acinar cells, by a technique involving introduction of the molecule in intact acinar cells *via* a patch pipet [31]. IP_3 has also been shown to release intracellular Ca^{2+} in permeabilized lacrimal acinar cells [32], and following microinjection into lacrimal acinar cells [33]. This release of Ca^{2+} appears to come from a relatively homogenous pool of Ca^{2+} within the endoplasmic reticulum. Thus, in permeabilized cell experiments in other exocrine glands, inhibition of mitochondrial uptake of Ca^{2+} does not impair loading of the pool sensitive to IP_3 [29]. Interestingly, spatial measurement of acetylcholine-induced Ca^{2+} signals in clusters of rat lacrimal cells demonstrate a distinct gradient of $[Ca^{2+}]_i$ that appears to be maximal at the luminal pole of the cell [34]. Thus, while the agonist-sensitive Ca^{2+} signal appears to be released from a homogeneous ER Ca^{2+} pools, the spatial characteristics of the Ca^{2+} signal may be determined by $InsP_3$ receptors localized to specific regions of the cell. This pattern of calcium release may result in differential physiological effects at luminal *versus* basolateral membranes, for example in control of lacrimal secretion.

As will be discussed below, a useful tool for studying Ca^{2+} pools is the plant toxin, thapsigargin, that inhibits the endoplasmic reticulum Ca^{2+} pump (SERCA) and specifically releases endoplasmic reticulum Ca^{2+} [35]. In permeabilized lacrimal acinar cells, prior discharge of thapsigargin-sensitive Ca^{2+} stores precluded any further release by IP_3, confirming that the source in lacrimal cells is the endoplasmic reticulum. The homogeneity of this pool was demonstrated in a study utilizing fura-2-loaded attached primary mouse lacrimal acinar cells [36]. Intracellular stores were discharged, in a Ca^{2+} depleted medium, by one of three agents: methacholine, presumed to release the IP_3-sensitive pool; thapsigargin, which would release the total endoplasmic reticulum pool; and the calcium ionophore, ionomycin, which would discharge essentially all intracellular Ca^{2+} pools. Each of these three strategies essentially prevented further release by either of the other two. For example, after Ca^{2+} release by methacholine, not further release was seen with either thapsigargin or ionomycin. However, when Ca^{2+} was elevated for a prolonged period, with a high concentration of methacholine, a pool of Ca^{2+} appeared in excess of that which could be released by thapsigargin. Loading of this pool was prevented by injection of the mitochondrial Ca^{2+} uptake inhibitor, ruthenium red. Thus, consistent with other studies, the mitochondria contain little Ca^{2+} at rest, but actively accumulate it when it is released by IP_3 [37].