

# Characterization of secretory mechanisms in lacrimal and salivary glands

Akademisk avhandling

Som för avläggande av farmaceutisk doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet kommer att offentlig försvaras i rum 2119, Arvid Wallgrens backe, Hus 2, Hälsovetarbacken, torsdagen den 15 april 2021, klockan 13.00

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Avhandlingen baseras på följande delarbeten

- I. **Johnsson M.**, Winder M., Zawia H., Lödöen I., Tobin G. & Götrick B.  
In vivo studies of effects of antidepressants on parotid salivary secretion in the rat. *J Arch Oral Biol.* 2016 67:54-60.
- II. **Dankis M.**, Aydogdu Ö., Tobin G. & Winder M.  
Inhibitory effects of antidepressants on lacrimal gland secretion in the anaesthetized rat. *Submitted*
- III. **Dankis M.**, Carlsson T., Aronsson P., Tobin G. & Winder M.  
Novel insights into the function of muscarinic and purinergic receptors in primary cultures of rat lacrimal gland myoepithelial cells. *Submitted*
- IV. **Dankis M.**, Aronsson P., Carlsson T., Tobin G. & Winder M.  
Functional muscarinic and purinergic responses in primary co-cultures of rat lacrimal gland myoepithelial and acinar cells. *Manuscript*

# Characterization of secretory mechanisms in lacrimal and salivary glands

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## Abstract

Dry mouth and dry eyes are multifactorial morbidities that can lead to a severely reduced quality of life. Approximately 20% of the population suffers from ocular or oral dryness. In the pursuit of pharmacological treatments of these troublesome symptoms, we sought to identify new targets and characterize underlying mechanisms that modulate lacrimal and salivary secretion.

Xerogenic and xerophthalmic effects of antidepressants were examined in a rat *in vivo* model. Centrally mediated secretion was stimulated by applying citric acid to the tongue or by administration of menthol to the surface of the eye, and peripherally induced secretion was mediated by *i.v.* injection of the muscarinic agonist methacholine. The xerogenic effects of the tricyclic antidepressant clomipramine, the selective serotonin reuptake inhibitor citalopram, and serotonin-noradrenaline reuptake inhibitor venlafaxine were shown to be centrally mediated. In contrast, only clomipramine attenuated the peripherally stimulated response, while it was ameliorated by citalopram and venlafaxine. Likewise, the xerophthalmic effects of clomipramine and citalopram were centrally mediated. Further, similar to what was displayed in the salivary investigation, clomipramine attenuated peripherally stimulated lacrimation. However, citalopram exhibited no peripheral hyposecretory effects. In conclusion, in contrast with the common perception, modern antidepressant compounds such as selective serotonin reuptake inhibitors do not feature peripherally mediated anticholinergic properties. These findings verify the more suitable therapeutic profile of modern antidepressants and support the use of local parasympathomimetic treatment of drug induced dry mouth and dry eyes.

Lacrimal gland secretory mechanisms and the effects of cholinergic and purinergic mediators were studied in primary monocultures and co-cultures of rat lacrimal gland cells. The primary culture isolation procedure was validated by monitoring the cultures immunochemically. After four weeks, a monoculture of myoepithelial cells was established which was shown to be sustained throughout the six-week isolation process. Prior to this, at 2-3 weeks, a co-culture of acinar and myoepithelial cells was evident. In conjunction, lacrimal gland tissue and primary cell cultures were studied morphologically for identification of cholinergic receptors. Immunohistochemical investigation of both myoepithelial cells and lacrimal gland tissues showed expression of a heterogeneous muscarinic receptor population, indicating a multifaceted presence of functional receptors. However, no alterations in intracellular calcium were observed in myoepithelial cells, following stimulation with cholinergic modulators. This finding indicated a functional cholinergic dependence on intercellular interactions with acinar cells and an alternative cholinergic signal transduction pathway that excludes calcium. Based on the monoculture results, we next established a primary co-culture of rat lacrimal gland acinar and myoepithelial cells. In these studies, myoepithelial cells displayed a latent calcium response to cholinergic stimuli. This response was attributed to purinergic intercellular interactions, likely via ATP released from acini cells.

In conclusion, the current findings show that antidepressant-induced hyposecretion is mainly centrally mediated. We established and validated sustainable isolation procedures for monocultures of primary myoepithelial cells, in which co-cultures of acinar and myoepithelial cells arise midway. Furthermore, we showed that lacrimal gland secretion can be multifaceted, highlighting the importance of investigating effects of selective muscarinic and purinergic modulatory compounds in the efforts of developing new treatments for dry eyes and dry mouth.

**Keywords:** *dry mouth, dry eyes, antidepressant, muscarinic receptor, lacrimal gland, primary cell culture*