論文審査の要旨及び担当者

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（論文審査の要旨）

論文題名：Intravital Two-photon Imaging of Ca^{2+} signaling in Secretory Organs of Yellow Cameleon Transgenic Mice
（イエローカメレオントランスジェニックマウスを用いた外分泌器官における生体内Ca^{2+}イメージング）

In this research, we established an effective intravital imaging system for specific cell types of secretory organs to monitor the changes of intracellular calcium ($[Ca^{2+}]i$) signaling using mouse line expressing genetically encoded Ca^{2+} indicator Yellow Cameleon 3.60 (YC3.60).$[Ca^{2+}]i$ dynamics could be monitored in specific cell types of secretory organs, especially the myoepithelial cells (MEC) of lacrimal gland (LG), during pharmacological stimulations ex vivo and intravitally. In pathological conditions of postganglionic denervation, a marked attenuation of LG $[Ca^{2+}]i$ response to cholinergic stimulation was found. Also, long-time cholinergic exposure to LG and capsaicin eye drop stimulation on ocular surface showed different tear secretion pattern and different $[Ca^{2+}]i$ dynamics in LG MEC.

During the dissertation defense, there are two key questions that are needed to be clarified. One is the principle of fluorescence resonance energy transfer (FRET) on basis of which YC3.60 probe was designed. The other is physiological two-phase tear secretion under intravital exposure of acetylcholine (ACh). FRET is a physical phenomenon in which one light-sensitive fluorophore (donor fluorophore) in its excited state transfers its excitation energy non-radiatively to a nearby fluorophore (acceptor fluorophore), thus the acceptor fluorophore is excited and emits its own fluorescence. The occurrence of FRET requires the excitation spectra of acceptor fluorophore overlap with the emission spectra of the donor fluorophore. The efficiency of FRET is highly dependent on the distance between donor fluorophore and acceptor fluorophore them within 10nm. In applications of FRET like Yellow Cameleon (YC) probe, there are calmodulin and calmodulin-binding domain of myosin light chain kinase (M13) between the donor fluorophore (CFP) and acceptor fluorophore (YFP), increase in intracellular free calcium concentration triggers the binding of M13 to calmodulin, thus close the distance of CFP and YFP and make their orientations parallel, to prepare the FRET from CFP to YFP. If we observe the YC-expressing organs through 2-photon microscope, two low-energy photons (830nm) cooperate to produce a higher-energy electronic transition in one fluorophore, and if intracellular calcium increases, FRET is evoked to occur, the intensity increase of acceptor emission is simultaneous with the intensity decrease of donor emission. The ratio change of YFP/CFP is dependent on intracellular calcium change, thus the results acquired from YC indicators are ratiometric and linear.

Tear film is composed of water, electrolytes, proteins, lipids and mucins. The secretion of proteins, electrolytes and water are stimulated by neural control. Parasympathetic nerves projected from pterygopalatine ganglion are found predominately important in maintaining the morphology and controlling the secretory functions of lacrimal gland. ACh is the major type of neurotransmitter released by postganglionic parasympathetic nerve terminals. In order to investigate the secretory dynamics of lacrimal gland under various types of physiological status, we performed ACh exposure as pharmacological approach in our intravital experiments to mimic a correlating physiological condition. Results showed that increase in tear flow rate during ACh exposure (10 minutes) to LG can be recorded 2 phases. Atropine is an antagonist of muscarinic receptor located in both acinar cells and myoepithelial cells. When LG was exposure with atropine and acetylcholine, both 2 phases did not occur. To investigate the effect of myoepithelial contraction on secretory dynamics of lacrimal gland, we performed exposure of 2,3-Butanedione monoxime (BDM), which is a myosin ATPase inhibitor used to block the contractile process of cells. When LG is under exposure of BDM and acetylcholine, the muscarinic receptors are activated but contractile function is inhibited, therefore the synthesis and secretion of secretory components are considered normal but myoepithelial cells were unable to expel out the secreted tear components. Results showed that phase 1 of the increase in tear secretion nearly disappeared and phase 2 still occurred, suggesting myoepithelial contraction highly contributes to the phase 1 increase of tear secretion during ACh exposure. Reflex tearing is a quick self-protective mechanism that a lacrimation with relatively high tear flow rate happens to rinse out the noxious stimuli when sensory afferents of ocular surface detected noxious and irritative stimulations. In our experiments, stimulation with capsaicin eyedrop was recorded only 1 phase, it was not able to form a typical second phase like 10-mintute acetylcholine exposure. When LG was immersed with BDM or tetrodotoxin (TTX) which is used to block the release of neurotransmitters from nerve endings, tear flow rate after capsaicin eye drop stimulation was very low and appeared no obvious difference compared to atropine immersion. These data indicate that a typical two-phase tear secretion is likely to be specific to long-time ACh stimulation, under which the functions of both acinar cells and myoepithelial cells were activated. And in capsaicin-induced reflex tearing, the myoepithelial contraction appears to be the major cause.

In conclusion, this research intravitally monitored $[Ca^{2+}]i$ dynamics in LG MEC of normal and pathological YC3.60 mice under pharmacological stimulations mimicking different types of physiological conditions. Although some limitations still existed, utilization of this intravital system will deepen our understanding of the functional roles of LG MEC in tear secretory mechanisms, which is meaningful and promising for innovative therapeutic improvement of relating diseases.