THE ROLE OF INTRACELLULAR ORGANELLES IN Ca²⁺-HOMEOSTASIS IN SECRETORY CELLS OF THE LACRIMAL GLAND

A. B. Kotliarova¹,², V. V. Manko²

¹O.O. Bogomoletz Institute of Physiology, NAS of Ukraine
4, Akademik Bogomoletz St., Kyiv 01024, Ukraine
²Ivan Franko National University of Lviv, 4, Hrushevskyi St., Lviv 79005, Ukraine
e-mail: annkotliarova@gmail.com

It is known that different Ca²⁺-transport systems make distinct contribution to the formation of Ca²⁺-signal in various secretory cells. In recent years there has been a significant increase in interest in study of the lacrimal glands functioning. However, the results of study of the Ca²⁺-signalling in lacrimal gland have not yet been summarized in the literature. This review is devoted to analysis of functioning of Ca²⁺-transport system of secretory cells in the rat exorbital lacrimal gland.

IP₃Rs in exorbital lacrimal gland cells were effectively inhibited by 2-APB (10 µM) and activated by IP₃ (2 µM), as well as by cholinomimetics, carbacholine (10 µM) and purine receptor agonists, ATP (1 mM). Signaling pathways activated by P2Y-receptors in the lacrimal gland secretory cells were partially mediated by IP₃R activation. RyRs of lacrimal gland secretory cells were activated by Ca²⁺ and low concentrations of ryanodine (0.05–1 µM). Simultaneous activation of RyRs and IP₃Rs in these cells caused Ca²⁺ release from the same store. Ca²⁺ mobilization from the intracellular stores induced by carbacholine (10 µM) or thapsigargin (1 µM) caused store-operated Ca²⁺ entry in secretory cells of the studied glands, which was partially inhibited by 2-APB. SERCA of exorbital lacrimal gland cells was efficiently inhibited by eosin Y (5–10 µM) and by thapsigargin (1 µM). In contrast to permeabilized cells, the Ca²⁺ content in intact cells did not change under the influence of eosin Y (5–20 µM), indicating the inhibition of PMCA. Ca²⁺-ATPase activity of permeabilized cells of studied glands depended on the incubation time, the substrate amount and Ca²⁺ concentration in the incubation medium. Mitochondrial Ca²⁺-uniporter of lacrimal gland secretory cells was inhibited by ruthenium red (10 µM). Effects of ryanodine and ruthenium red on the Ca²⁺ content in cells were statistically significantly non-additive. Furthermore, ryanodine at concentrations of 1–3 µM caused a dose-dependent decrease in the respiration rate of the studied cells and this effect persisted after cells preincubation with ruthenium red or thapsigargin. This suggests that in addition to the endoplasmic reticulum RyRs activation, ryanodine inhibited the Ca²⁺ transport to the mitochondrial matrix, which was insensitive to the ruthenium red.

Keywords: lacrimal glands, IP₃Rs, RyRs, SOCC, SERCA, Ca²⁺-uniporter.
It is generally accepted that Ca\(^{2+}\) plays a significant role in secretory process by the exocrine glands [2, 13, 19, 40, 41, 57]. Ca\(^{2+}\) concentration in cytosol is determined by two oppositely directed Ca\(^{2+}\) flows. The inward flow (relative to cytosol) results from passive transport of Ca\(^{2+}\) from the extracellular space through the plasma membrane and/or Ca\(^{2+}\) release from intracellular stores. Opposite in direction outward flow causes the reduce of cytosolic Ca\(^{2+}\) due to its active output to the extracellular fluid or into intracellular stores [41].

It is known that different Ca\(^{2+}\)-transport systems make distinct contribution to the formation of Ca\(^{2+}\)-signal in various secretory cells. In recent years there has been a significant increase in interest in study of the lacrimal glands functioning. However, the results of study of the Ca\(^{2+}\)-signalling in lacrimal gland have not yet been summarized in the literature. Ca\(^{2+}\)-signaling in secretory cells of these glands is far less studied than in other types of glands. The number of publications that have at least indirect relation to Ca\(^{2+}\)-transport systems of lacrimal glands is 2–4 orders of magnitude less than the corresponding number of articles about organization of the Ca\(^{2+}\)-signaling in other secretory glands. In the literature there are only few data regarding the identification and properties of some Ca\(^{2+}\)-transport systems in the lacrimal gland secretory cells [5, 14, 21, 22, 25, 31, 34–38, 45, 60], while there is no systematic analysis of their functioning, features and the role in Ca\(^{2+}\)-signaling.

Understanding the mechanism involved in Ca\(^{2+}\)-signalling in lacrimal gland secretory cells is essential for understanding of the regulation of their secretory process. These results can be the basis for the development of means for pharmacological correction of the dry eye syndrome, since both fluid and protein secretion by cells of lacrimal glands are Ca\(^{2+}\)-dependent processes.

In our first article, published in Studia Biologica [35], we addessed the identification and features of SERCA and PMCA. The purpose of this review is to analyze the contribution of other intracellular Ca\(^{2+}\)-transport systems in the Ca\(^{2+}\)-homeostasis of the lacrimal gland secretory cells.

The role of endoplasmic reticulum in Ca\(^{2+}\) homeostasis of secretory cells. In 1995 it was shown that stimulation of the lacrimal acinar cells of rat by acetylcholine (10 \(\mu\)M) or ATP (2 mM) induces rapid generation of inositol 1,4,5-trisphosphate (IP\(_3\)) [22]. IP\(_3\) is a second messenger, which activates IP\(_3\)R in the endoplasmic reticulum, causing Ca\(^{2+}\) release to the cytosol from IP\(_3\)-sensitive stores [12, 16, 21, 22, 23, 36], and this, in turn, stimulates fluid and protein secretion by the lacrimal glands [14, 43].

Under the influence of carbacholine (1 \(\mu\)M) – M-cholinergic receptors agonist, the Ca\(^{2+}\) content in the intact cells of studied glands decreased significantly due to the Ca\(^{2+}\) release from the endoplasmic reticulum. IP\(_3\)R inhibitor – 2-APB [42, 46, 56, 58, 59] in concentration of 10 \(\mu\)M completely prevented carbacholine-induced reduction of Ca\(^{2+}\) content in cells [36].

In the lacrimal gland secretory cells IP\(_3\)R retain their functional activity in the dygiti-tonin-permeabilized cells. The reduction of stored Ca\(^{2+}\) under the influence of IP\(_3\) is the direct evidence for this. In the presence of 2-APB (10 \(\mu\)M in medium, statistically significant changes under the influence of IP\(_3\) were not recorded (Fig. 1) [36]. Despite low specificity of 2-APB, it is a clear proof of IP\(_3\)R presence in the secretory cells of rat exorbital lacrimal gland.

IP\(_3\)R activation in the studied cells occurs as a result of interaction of substances that are the mediators of autonomic nervous system (acetylcholine, ATP) with appropriate
receptors on the plasma membrane [22, 43]. During research of lacrimal gland functioning irritation effects of parasympathetic and sympathetic nerve fibers are often modulated by action of various cholinergic and adrenergic agonists. But, ATP is co-mediator of many cholinergic and adrenergic synapses, the release of which could significantly modulate the effect of primary mediator [7, 15].

Extracellular ATP causes an increase in cytosolic Ca\(^{2+}\) concentration in the acinar cells of various exocrine glands by interacting with P2-type of purinoreceptors [44, 51, 55]. The mechanism of signal transduction upon activation of different P2Y-receptors may involve different signaling pathways [1]. P2Y\(_{11}\) and P2Y\(_{13}\)-receptors are expressed in the basement membrane of the lacrimal gland acinar cells of mice [52]. Other authors found that the ATP-induced responses of acinar cells of the rat lacrimal glands are implemented mainly (along with P2X-receptors activation) through the activation of P2Y\(_{1}\)-receptors and subsequent IP\(_3\)-mediated Ca\(^{2+}\) mobilization, which was shown using heparin, U73122 – an inhibitor of phospholipase C, and using RT-PCR [27]. Kamada, Saino, Oikawa et al. identified (except for P2Y\(_{1}\)) also P2Y\(_{2,4,12,14}\) receptors in the lacrimal gland secretory cells, but expression of P2Y\(_{11}\) and P2Y\(_{13}\) was not confirmed [27].

The mechanism of signal transduction of P2Y receptors in the studied cells involves IP\(_3\)R activation, since in presence of their inhibitor – 2APB (10 µM) in the nominal Ca\(^{2+}\)-free medium (that prevents P2X receptors activation), ATP-induced changes of Ca\(^{2+}\) content were less expressive. This reduction of Ca\(^{2+}\) in cells is really caused by the P2Y-receptors activation, since it was inhibited (but not completely) by suramin (100 µM) [36].

The mechanism of signal transduction in studied cells in conditions of both P2Y receptors and M-cholinergic receptors activation, at least partially, involved IP\(_3\)R activation, but there were no data with which neurotransmitter (acetylcholine or noradrenaline) the ATP is released from nerve endings surrounding the lacrimal gland.
When both M-cholinergic receptors and P2Y receptors were activated, Ca\(^{2+}\) was released from the same IP\(_3\)-sensitive store, since the effects of ATP and carbacholine at high concentrations (1 mM and 10 \(\mu\)M, respectively) on the Ca\(^{2+}\) content were non-additive [36]. This is important in view of the physiological appropriateness clarification why various agonists activate Ca\(^{2+}\) release from the same IP\(_3\)-sensitive store, through the activation of common path of signal transduction.

It was also found [22] that stimulation of the lacrimal gland cells by acetylcholine caused the Ca\(^{2+}\)-response with maximum amplitude, regardless of the time after stimulation by ATP. The authors concluded that ATP causes the release of only a small part of the available Ca\(^{2+}\), allowing acetylcholine to cause the re-release of Ca\(^{2+}\) from the same store [22].

The release of stored Ca\(^{2+}\) from the endoplasmic reticulum during the cell stimulation by agonists is due to the activation of IP\(_3\)Rs and is amplified by RyRs activation. On basis of the results obtained by Western blot analysis and immunohistochemical methods it was found that all isoforms of RyRs are expressed in the lacrimal glands secretory cells of mice [45].

The dependence of RyRs sensitivity on Ca\(^{2+}\) concentration is inherent for many types of cells [6, 9, 11, 17, 26, 39, 48]. This property was confirmed for the lacrimal gland secretory cells. Ryanodine (0,05–1 \(\mu\)M) reduces Ca\(^{2+}\) content in the secretory cells of lacrimal glands in dose-dependent manner [32] (Fig. 2). On basis of the two-factor analysis of variance it was revealed that Ca\(^{2+}\) concentration in the medium determined the Ca\(^{2+}\) content in the investigated cells by 8.6 \%, and the concentration of ryanodine – by 87.9 \%. The unaccounted factor amounted to only 3.49 \% [32].

![Fig. 2. The effect of ryanodine on Ca\(^{2+}\) content in the permeabilized secretory cells of rat exorbital lacrimal gland: tissue Ca\(^{2+}\) content in the absence of ryanodine was normalized to one; incubation time – 15 min; [K\(^+\)] = 140 mM; * – difference compared with control reliable with \(P < 0.05\), ** – with \(P < 0.01\); *** – with \(P < 0.001\); \(n = 4–5\) (according to the publication [32])](image-url)
Simultaneous activation of RyRs and IP$_3$Rs in these cells caused Ca$^{2+}$ release from the same store, since the effects of IP$_3$ (2 µM) and ryanodine (100 nM) on the total Ca$^{2+}$-content in permeabilized secretory cells were statistically significantly non-additive [37].

Agonist-induced Ca$^{2+}$ release from the intracellular stores into the cytosol (and its outflow from cells) tends to reduce the pool of stored Ca$^{2+}$ [49], which in turn activates the store-operated Ca$^{2+}$ entry (SOCE) from the extracellular space [10, 18, 36, 38].

Preincubation of the secretory cells with carbacholine (10 µM) resulted in a reduction of Ca$^{2+}$ content in them. The increase in Ca$^{2+}$ concentration in the incubation medium up to 2 mM after preincubation with carbacholine was accompanied by the restoration of Ca$^{2+}$ content in the investigated cells to baseline, obviously by SOCE (Fig. 3, A, B) [36].

Adding a specific SERCA inhibitor – thapsigargin (1 µM) [54] to the nominal Ca$^{2+}$-free medium caused a decrease in Ca$^{2+}$ content in the investigated cells [36] (Fig. 4, A, B).

SOCE caused by Ca$^{2+}$ release from the store using carbacholine (10 µM) and Ca$^{2+}$ release from the store using thapsigargin (1 µM) was partially inhibited by 2-APB (10 µM) (Fig. 3, 4) [36].

It was shown that the store-operated calcium channels in the lacrimal gland secretory cells of mouse are formed by Orai1 [60]. Lacrimal acinar cells lacking Orai1 have diminished lacrimal fluid secretion following activation of the muscarinic receptor. These
results also demonstrate the central role of SOCE in lacrimal exocrine function, since in Orai1 knockout mice calcium-dependent peroxidase secretion was eliminated [60].

Ca\(^{2+}\) which entered the cell by SOCE is transported to the endoplasmic reticulum. The only system that provides Ca\(^{2+}\) transport from the cytosol to the endoplasmic reticulum is SERCA (Ca\(^{2+}\), Mg\(^{2+}\)-ATPase of endoplasmic reticulum) [41]. Primarily, thapsigargin [54] and eosin Y [30, 35, 53] belong to the inhibitors of Ca\(^{2+}\)-pumps. It was shown that 2-APB is also capable to inhibit SERCA of the lacrimal gland secretory cells [31].

Ca\(^{2+}\) which entered the cell by SOCE is transported to the endoplasmic reticulum. The only system that provides Ca\(^{2+}\) transport from the cytosol to the endoplasmic reticulum is SERCA (Ca\(^{2+}\), Mg\(^{2+}\)-ATPase of endoplasmic reticulum) [41]. Primarily, thapsigargin [54] and eosin Y [30, 35, 53] belong to the inhibitors of Ca\(^{2+}\)-pumps. It was shown that 2-APB is also capable to inhibit SERCA of the lacrimal gland secretory cells [31].

Eosin Y reduced Ca\(^{2+}\)-ATPase activity of permeabilized cells in a dose-dependent manner. At 20 µM eosin Y in medium caused complete inhibition of Ca\(^{2+}\)-sensitive ATPase activity of permeabilized cells [34]. Ca\(^{2+}\)-ATPase activity of permeabilized cells of studied glands depended on the incubation time, the substrate amount and Ca\(^{2+}\) concentration in the incubation medium. Maximums of Ca\(^{2+}\)-sensitive and eosin Y-sensitive ATPase activities were observed at 2–3 mM of exogenous ATP in the medium. The maximum of both total and eosin Y-insensitive ATPase activities were observed at 10\(^{-6}\) M Ca\(^{2+}\) in the medium [34].

It should be noted that the results obtained by the ATPase activity measurement of permeabilized cell under the influence of these inhibitors [34] are in good agreement with the results obtained from measuring changes of the Ca\(^{2+}\) content in cells [35]. Reduction...
of Ca\(^{2+}\) content in permeabilized cells by thapsigargin (1 µM) – specific SERCA inhibitor was the same as by eosin Y (5 mM). In contrast to permeabilized cells, the Ca\(^{2+}\) content in intact cells did not change under the influence of eosin Y (5–20 µM), indicating the PMCA inhibition [35].

*The contribution of mitochondria to Ca\(^{2+}\)-homeostasis in secretory cells.* Mitochondria make a comprehensive impact on the regulation of Ca\(^{2+}\)-signals in the secretory cells of various glands [8, 50, 61]. Ca\(^{2+}\) uptake by mitochondria is realized particularly through Ca\(^{2+}\)-uniporter in their inner membrane [29]. For its inhibition ruthenium red is often used [20, 28]. Today the functional activity of mitochondrial Ca\(^{2+}\)-uniporter in the lacrimal gland secretory cells is confirmed [4] and its interaction with other Ca\(^{2+}\)-transport systems in cells was studied on basis of the estimation of additivity of the effects of appropriate inhibitors [37].

Ruthenium red (10 µM) – an inhibitor of Ca\(^{2+}\)-uniporter and thapsigargin (1 µM) – an inhibitor of SERCA reduced the Ca\(^{2+}\) content in intact and permeabilized cells of lacrimal glands. The total effect of ruthenium red and thapsigargin on Ca\(^{2+}\) content in cells was additive [37]. This suggests on the influence of these two inhibitors Ca\(^{2+}\) is passively released from different stores. Similarly, effects of IP\(_3\) (2 µM) and ruthenium red in permeabilized cells, and M-cholinergic receptors agonist carbacholine (10 µM) and ruthenium red on intact cells were additive [37]. Beside this, adding carbacholine to the polarographic chamber caused an intensification of respiration of the studied cells. Mitochondrial Ca\(^{2+}\)-uniporter inhibition, which prevents the Ca\(^{2+}\) flow in the matrix caused the levelling of carbacholine-stimulating effect, and IP\(_3\)R inhibition partially eliminated the effect of carbacholine [33]. However, the effects of ryanodine (0.1 µM) and ruthenium red on total Ca\(^{2+}\)-content in the lacrimal cells were non-additive (Fig. 5) [37].

A lack of ryanodine effect on a background of red ruthenium can be caused by several factors. First, ruthenium red may inhibit the RyRs of endoplasmic reticulum, which was previously demonstrated, in particular, for non-excitable cells [47]. But the described effects of ruthenium red on Ca\(^{2+}\) content in the tissue of lacrimal gland, in our opinion, is not related to the RyRs inhibition, as the decrease of functional activity of these channels must be accompanied by an increase (rather than decrease) in Ca\(^{2+}\) content in the tissue. The effect of ryanodine is associated not only with the Ca\(^{2+}\) release from endoplasmic reticulum, but also with activation of mitochondrial RyRs (mRyRs), which were identified, for example, in the inner mitochondrial membrane of cardiomyocytes and were inhibited by ruthenium red [3].

Furthermore, ryanodine at concentrations of 1–3 µM caused a dose-dependent decrease in the respiration rate of the studied cells (Fig. 6) and this effect persisted after cells preincubation with ruthenium red or thapsigargin. This suggested that in addition to the endoplasmic reticulum RyR activation, ryanodine inhibited the Ca\(^{2+}\) transport to the mitochondrial matrix, that was insensitive to the ruthenium red [37].
Fig. 5. The effect of ryanodine on Ca\(^{2+}\) content in permeabilized cells of the lacrimal glands in the presence of ruthenium red: \([K^+] = 140 \, \text{mM}, [\text{Ca}^{2+}] = 10^{-7} \, \text{M}\); 1 – control (K), 2 – Ca\(^{2+}\) content in presence of ryanodine (A), 3 – in presence of ruthenium red (B), 4 – for the simultaneous action of both substances ((A + B)), 5 – algebraic sum of the Ca\(^{2+}\)-content changes by the separate action of ryanodine and ruthenium red ([K – A] + [K – B]), 6 – change in Ca\(^{2+}\) content by the simultaneous action of both substances ([K – {A + B}]); Tissue Ca\(^{2+}\) content in the absence of inhibitors/agonists was normalized to one; incubation time – 15 min; ** – difference compared to control reliable with \(P < 0.01\); *** – with \(P < 0.001\); ^ – significant difference between the sum of changes in two separate agents actions and their simultaneous action (\(P_a < 0.05\)); \(n = 6\) [37]

Fig. 6. The level of cell respiration in presence of ryanodine and FCCP in medium under conditions of Ca\(^{2+}\)-transport systems inhibition: Arrow shows the addition of FCCP (1 \(\mu\text{M}\)) to a final concentration of 0.1, 1.2 or 3 \(\mu\text{M}\); \(n = 5\) [37]

Рис. 5. Вплив ріанодину на вміст Ca\(^{2+}\) у пермеабілізованих клітинах сльозових залоз за наявності рутенію червоного: стрілками показано додавання FCCP (1 мкмоль/л) до кінцевої концентрації 0,1; 1; 2 чи 3 мкмоль/л; \(n = 5\) [37]

Рис. 6. Інтенсивність клітинного дихання за наявності у середовищі ріанодину та FCCP за інгібування Ca\(^{2+}\)-транспортовальних систем: стрілками показано додавання FCCP (1 мкмоль/л), а також ріанодину до кінцевої концентрації 0,1; 1; 2 чи 3 мкмоль/л; \(n = 5\) [37]
SUMMARY

Based on our results and literature data, we suggested the following scheme of Ca^{2+}-transport systems functioning in lacrimal gland secretory cells (Fig. 7).

Fig. 7. Scheme of coordinated functioning of different Ca^{2+}-transport systems in extraorbital lacrimal gland cells of the rat:
→ – activation, ⊥ – inhibition; CUP – Ca^{2+}-uniporter, IP_{3} – inositol 1,4,5-trisphosphate, IP_{3}Rs – IP_{3}-receptors, mRyRs – mitochondrial ryanodine receptors, PLC – phospholipase C, PMCA – Ca^{2+}-pump of the plasma membrane, RyRs – ryanodine receptors, SERCA – sarco/endoplasmic reticulum calcium pump, SOCC – store-operated Ca^{2+} entry channels.

Рис. 7. Схема взаємодіючих функцій різних систем транспортування Ca^{2+} секреторних клітин зовнішньоообритального сльозового залози шурів:
→ – активація, ⊥ – інгібування; CUP – Ca^{2+}-уніпортер, IP_{3} – інозитол-1,4,5-трифосфат, IP_{3}Rs – IP_{3}-чутливі Ca^{2+}-канали, mRyRs – мітохондріальні ріанодинчутливі Ca^{2+}-канали, PLC – фосфоліпаза C, PMCA – Ca^{2+}-помпа плазматичної мембрани, RyRs – ріанодинчутливі Ca^{2+}-канали, SERCA – Ca^{2+}-помпа ендоплазматичного ретикулуму, SOCC – депокеровані Ca^{2+}-канали.
Primary mediators for lacrimal gland secretory cells are ATP – the agonist of the P2X and P2Y-purinoceptors and acetylcholine and its analogues – agonists of the identified M₃-cholinergic receptors [12, 22, 23, 36, 43].

Signalling pathways activated by the P2Y-receptors in the lacrimal gland secretory cells were partially mediated by the IP₃R activation [36]. IP₃Rs of exorbital lacrimal gland cells were effectively inhibited by 2-APB (10 µM) [36], which was previously demonstrated for other cell types [42, 46, 56, 58, 59]. When both M-cholinergic receptors and P2Y receptors were activated, Ca²⁺ was released from the same IP₃-sensitive store since the effects of ATP and carbacholine at high concentrations (1 mM and 10 µM, respectively) on the Ca²⁺ content were non-additive [36].

RyRs of endoplasmic reticulum in secretory cells of the lacrimal glands were activated by Ca²⁺ and low concentrations of ryanodine (0,05–1 µM) [32].

Ca²⁺ mobilization from the intracellular stores was induced by carbacholine (10 µM) or thapsigargin (1 µM) caused store-operated Ca²⁺ entry in secretory cells of studied glands, that was partially inhibited by 2-APB (10 µM) [36].

An increase in cytosolic Ca²⁺ concentration after the IP₃R and RyR activation is accompanied by activation of SERCA, PMCA, Ca²⁺-uniporter and mRyRs.

SERCA of exorbital lacrimal gland cells was efficiently inhibited by eosin Y (10–20 µM) and by thapsigargin (1 µM) [34]. Ca²⁺ content in intact cells, in contrast to permeabilized cells, did not change under the influence of eosin Y (5–20 µM), indicating the PMCA inhibition [35].

In addition to RyR activation in the endoplasmic reticulum, ryanodine inhibited the Ca²⁺ transport to the mitochondrial matrix, that was insensitive to the ruthenium red [37]. This effect can be explained by presence of RyRs in the mitochondrial membrane (previously demonstrated to cardiomyocytes [3]), that may be important for regulating of cell energy supply.

Adding carbacholine to polarographic chamber caused an intensification of respiration of studied cells [33]. These results are in good agreement with those obtained previously by another group [24]. This effect is associated with Ca²⁺ intake in the mitochondrial matrix and subsequent activation of the mitochondrial oxidation. Mitochondrial Ca²⁺-uniporter inhibition, which prevents the Ca²⁺ flow in the matrix caused the levelling of carbacholine-stimulating effect, and IP₃Rs inhibition partially eliminated the effect of carbacholine. It has an important general biological significance because it allows the regulation of mitochondrial respiration by a direct positive feedback, long before there would be a lack of ATP and triggers the inverse regulatory effects, homeostatic (not signaling) by their nature.

Further studies of the Ca²⁺-transport system functioning in the lacrimal gland secretory cells addresses of their functioning changes during aging and regulation by hormones.

1. Abbracchio M.P., Burnstock G., Boynaems J.-M. et al. International Union of Pharmacology. Update and subclassification of the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. Pharmacological Reviews, 2006; 58(3): 281–341.
2. Ambudkar I.S. Regulation of calcium in salivary gland secretion. Critical Reviews in Oral Biology & Medicine, 2000; 11(1): 4–25.
3. Beutner G., Sharma V.K., Giovannucci D. et al. Identification of a ryanodine in receptor in rat heart mitochondria. *The Journal of Biological Chemistry*, 2001; 276(24): 21482–21488.
4. Bird G.St.J., Obie J.F., Putney J.W., Jr. Functional homogeneity of the non-mitochondrial Ca\(^{2+}\) pool in intact mouse lacrimal acinar cells. *The Journal of Biological Chemistry*, 1992; 267(26): 18382–18386.
5. Bird G.St.J., Putney J.W., Jr. Effect of Inositol 1,3,4,5-tetrakisphosphate on inositol trisphosphate-activated Ca\(^{2+}\) signaling in mouse lacrimal acinar cells. *The Journal of Biological Chemistry*, 1996; 271(12): 6766–6770.
6. Bull R., Marengo J.J. Sarcoplasmic reticulum release channels from frog skeletal muscle display two types of calcium dependence. *FEBS Letters*, 1993; 331(3): 223–227.
7. Burnstock G. Pathophysiology and therapeutic potential of purinergic signalling. *Pharmacological Reviews*, 2006; 58(1): 58–86.
8. Bychkova S., Manko V., Klevets M. et al. Role of mitochondria in Ca\(^{2+}\)-signals in secretory cells of digestive glands. *Visnyk of Lviv University. Biological Series*, 2007; 44: С. 3–14.
9. Chen S.R., Li X., Ebisawa K. et al. Functional characterization of the recombinant type 3 Ca\(^{2+}\) release channel (ryanodine receptor) expressed in HEK293 cells. *The Journal of Biological Chemistry*, 1997; 272(39): 24234–24246.
10. Chew C.S., Petropoulos A.C. Thapsigargin potentiates histamine-stimulated HCl secretion in gastric parietal cells but does not mimic cholinergic responses. *Cell Regulation*, 1991; 2(1): 27–39.
11. Copello J.A., Barg S., Onoue H. et al. Heterogeneity of Ca-gating of skeletal muscle and cardiac ryanodine receptors. *Biophysical Journal*, 1997; 73(1): 141–156.
12. Dartt D.A. Signal transduction and control of lacrimal gland protein secretion: a review. *Current Eye Research*, 1989; 8(6): 619–636.
13. Dartt D.A., Hodges R.R. Cholinergic agonists activate P2X receptors to stimulate protein secretion by the rat lacrimal gland. *Investigative Ophthalmology & Visual Science*, 2011; 52(6): 3381–3390.
14. Dartt D.A., Rose P.E., Joshi V.M. et al. Role of calcium in cholinergic stimulation of lacrimal gland protein secretion *Current Eye Research*, 1985; 4 (4): 475–483.
15. Dubyak G.R., El-Moatassim C. Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. *American Journal of Physiology*, 1993; 265(3 Pt 1): C577–C606.
16. Ferrari D., La Sala A., Chiozzi P. et al. The P2 purinergic receptors of human dendritic cells: identification and coupling to cytokine release. *FASEB Journal*, 2000; 14(15): 2466–2476.
17. Fill M., Copello J.A. Ryanodine receptor Calcium release channels. *Physiological Reviews*, 2002; 82(4): 893–922.
18. Foskett J.K., Rolffman C.M., Wong D.J. Activation of calcium oscillations by thapsigargin in parotid acinar cells. *The Journal of Biological Chemistry*, 1991; 266 (5): 2778–2782.
19. Gerasimenko J.V., Gerasimenko O.V., Petersen O.H. The role of Ca\(^{2+}\) in the pathophysiology of pancreatitis. *The Journal of Physiology*, 2014; 592(Pt 2): 269–280.
20. Griffiths E.J. Use of ruthenium red as an inhibitor of mitochondrial Ca\(^{2+}\) uptake in single rat cardiomyocytes. *FEBS Letters*, 2000; 486(3): P. 257–260.
21. Gromada J., Jorgensen T.D., Dissing S. Cyclic ADP-ribose and inositol 1,4,5-triphosphate mobilizes Ca\(^{2+}\) from distinct intracellular pools in permeabilized lacrimal acinar cells. *FEBS Letters*, 1995; 360(3): 303–306.
22. Gromada J., Jorgensen T.D., Dissing S. Role of protein kinase C in the regulation of inositol phosphate production and Ca\(^{2+}\) mobilization evoked by ATP and acetylcholine in rat lacrimal acini. *Pflugers Archiv*, 1995; 429(4): 578–586.
23. Gromada J., Jorgensen T.D., Dissing S. The release of intracellular Ca\(^{2+}\) in lacrimal acinar cells by alpha-, beta-adrenergic and muscarinic cholinergic stimulation: the roles of inositol triphosphate and cyclic ADP-ribose. *Pflugers Archiv*, 1995; 429(6): 751–761.
24. Herzog V., Sies H., Miller F. Exocytosis in secretory cells of rat lacrimal gland peroxidase release from lobules and isolated cells upon cholinergic stimulation. The Journal of Cell Biology, 1976; 70(3): 692–706.

25. Hodges R.R., Vrouvlianis J., Shatos M.A. et al. Characterization of P2X purinergic receptors and their function in rat lacrimal gland. Investigative Ophthalmology & Visual Science, 2009; 50(12): 5681–5689.

26. Jeyakumar L.H., Copello J.A., O’Malley A.M. et al. Purification and characterization of ryanodine receptor 3 from mammalian tissue. The Journal of Biological Chemistry, 1998; 273(26): 16011–16020.

27. Kamada Y., Saino T., Oikawa M. et al. P2Y purinoceptors induce changes in intracellular calcium in acinar cells of rat lacrimal glands. Histochemistry and Cell Biology, 2012; 137(1): 97–106.

28. Kannurpatti S.S., Joshi P.G., Joshi N.B. Calcium sequestering ability of mitochondria modulates influx of calcium through glutamate receptor channel. Neurochemical Research, 2000; 25(12): 1527–1536.

29. Kirichok Y., Krapivinsky G., Clapham D.E. The mitochondrial calcium uniporter is a highly selective ion channel. Nature, 2004; 427(6972): 360–364.

30. Kosterin S.A., Bratkova N.F., Babich L.G. et al. Effect of inhibitors of energy-dependent Ca\(^{2+}\)-transporting systems on calcium pumps of a smooth muscle cell. Ukrainian Biochemical Journal, 1996; 68(6): 50–61. (In Russian).

31. Kotliarova A., Manko V. Effect of 2-APB on SERCA of lacrimal gland secretory cells. Experimental and Clinical Physiology and Biochemistry, 2013; 3: 38–43. (In Ukrainian).

32. Kotliarova A., Palamar O., Manko V.V. RyRs of endoplasmic reticulum in exorbital lacrimal gland secretory cells of rat. In: Khamar I.S. (Ed.) Youth and Progress of Biology: abstracts book of the VII International Scientific Conference of Students and PhD Students associated with academic’s Volodymyr Vernadskiy birth sesquicentennial (April 16–19, 2013, Lviv). Lviv: Ivan Franko National University of Lviv, 2013: 419–420. (In Ukrainian).

33. Kotliarova A. B., Manko B.O., Manko V.V. The effect of carbacholine on cell respiration of the rat exorbital lacrimal gland. Actual Problems of Humanitarian and the Natural Sciences, 2013; 12–3: 41–43. (In Russian).

34. Kotliarova A. B., Manko V. V. ATPase activity of isolated cells of the rat exorbital lacrimal gland under in situ condition. Visnyk of Lviv University. Biological Series, 2012; 60: 270–282. (In Ukrainian).

35. Kotliarova A. B., Manko V. V. Ca\(^{2+}\) transporting systems in secretory cells of the rat exorbital lacrimal gland I. Ca\(^{2+}\) pumps of plasma membrane and endoplasmic reticulum. Studia Biologica, 2012: 6(2): 99–114. (In Ukrainian).

36. Kotliarova A. B., Manko V. V. IP\(_3\)-sensitive Ca\(^{2+}\)-channels of endoplasmic reticulum in secretory cells of the rat exorbital lacrimal gland. Ukrainian Biochemical Journal, 2013; 85(5): 27–36. (In Ukrainian).

37. Kotliarova A. B., Merlavs’ky V. M., Dorosh O. M. et al. The role of Calcium uniporter in Calcium-homeostasis of the exorbital lacrimal gland secretory cells. Physiological Journal, 2014; 60(5): 73–81. (In Ukrainian).

38. Kwan C.Y., Takemura H., Obie J.F. et al. Effects of MeCh, thapsigargin, and La\(^{3+}\) on plasmalemmal and intracellular Ca\(^{2+}\) transport in lacrimal acinar cells. American Journal of Physiology – Cell Physiology, 1990; 258(6 Pt 1): C1006–C1015.

39. Laver D.R., Roden L.D., Ahern G.P. et al. Cytoplasmic Ca\(^{2+}\) inhibits the ryanodine receptor from cardiac muscle. The Journal of Membrane Biology, 1995; 147(1): 7–22.

40. Low J.T., Shukla A., Behrendorff N. et al. Exocytosis, dependent on Ca\(^{2+}\) release from Ca\(^{2+}\) stores, is regulated by Ca\(^{2+}\) microdomains. Journal of Cell Science, 2010; 123(18): 3201–3208.

41. Manko V. V. Ca\(^{2+}\) transporting systems in secretory cells of exocrinic glands. Lviv: Ivan Franko National University of Lviv, 2011. 271 p. (In Ukrainian).
42. Maruyama T., Kanaji T., Nakade S. et al. 2-APB, 2-aminoethoxydiphenylborate, a membrane penetrable modulator of Ins(1,4,5)P3-induced Ca2+ release. Japan. J. Biochem, 1997; 122(3): 498–505.

43. Mauduit P., Jammes H., Rossignol B. M3 muscarinic acetylcholine receptor coupling to PLC in rat exorbital lacrimal acinar cells. American Journal of Physiology – Cell Physiology, 1993; 264(6 Pt 1): C1550–C1560.

44. McMillian M.K., Soltuff S.P., Lechleiter J.D. et al. Extracellular ATP increases free cytosolic calcium in rat parotid acinar cells. Biochemical Journal, 1988; 255(1): 291–300.

45. Medina-Ortiz W.E., Gregg E.V., Brun-Zinkernagel A.M. et al. Identification and functional distribution of intracellular Ca channels in mouse lacrimal gland acinar cells. Open Ophthalmology Journal, 2007; 1: 8–16.

46. Missiaen L., Callwaert G., De Smedt H. et al. 2-Aminoethoxydiphenylborate affects the inositol 1,4,5-trisphosphate receptor, the intracellular Ca2+ pump and the non-specific leak from the non-mitochondrial Ca2+ stores in permeabilised A7r5 cells. Cell Calcium, 2001; 29(2): 111–116.

47. Ozawa T. Ryanodine-sensitive Ca2+ release mechanism in non-excitable cells. International Journal of Molecular Medicine, 2001; 7(1): P. 21–25.

48. Percival A.L., Williams A.J., Kenyon J.L. et al. Chicken skeletal muscle ryanodine receptor isoforms: ion channel properties. Biophysical Journal, 1994; 67 (5): 1834–1850.

49. Putney J.W., Jr. A model for receptor-regulated calcium entry. Cell Calcium, 1986; 7 (1): 1–12.

50. Rizzuto R., De Stefani D., Raffaello A. et al. Mitochondria as sensors and regulators of calcium signalling. Nature Reviews Molecular Cell Biology, 2012; 13(9): 566–578.

51. Sasaki T., Gallacher D.V. Extracellular ATP activates receptor operated cation channels in mouse lacrimal acinar cells to promote calcium influx in the absence of phosphoinositide metabolism. FEBS Letters, 1990; 264(1): 130–134.

52. Shatos M., Masli S., Hodges R., Dartt D. Expression of purinergic receptors and ectonucleosidases in lacrimal glands of normal and thrombospondin-1 deficient mice. Investigative Ophthalmology & Visual Science, 2011; 52: 3706.

53. Slinchenko N.M., Chernysh I.G., Kosterin S.O. Utilization of purified myometrium cell plasma membrane Ca2+-Mg2+-ATPase for comparative estimation of efficacy of energy-dependent Ca2+-transport inhibitors. Ukrainian Biochemical Journal, 2003; 75(2): 33–38. (In Ukrainian).

54. Thastrup O., Dawson A.P., Scharff O. et al. Thapsigargin, a novel molecular probe for studying intracellular calcium release and storage. Agents and Actions, 1989; 27(1): 17–23.

55. Toescu E.C., Lawrie A.M., Petersen O.H. et al. Spatial and temporal distribution of agonist-evoked cytoplasmatic Ca2+ signals in exocrine acinar cells analysed by digital image microscopy. EMBO Journal, 1992; 11(4): 1623–1629.

56. Velykopolska O.Yu., Manko V.V. The role of endoplasmic Ca2+-functional unit in P2Y-receptors signal transduction processes in chironomus plumosus larvae secretory cells of salivary glands. Studia Biologica, 2009; 3(3): 39–50. (In Ukrainian).

57. Voronina S. Tepikin A. Mitochondrial calcium in the life and death of exocrine secretory cells. Cell Calcium, 2012; 52(1): 86–92.

58. Wu J., Kamimura N., Takeo T. et al. 2-Aminoethoxydiphenyl borate modulates kinetics of intracellular Ca2+ signals mediated by inositol 1,4,5-trisphosphate-sensitive Ca2+ stores in single pancreatic acinar cells of mouse. Mol. Pharmacol, 2000; 58(6): 1368–1374.

59. Wu J., Takeo T., Suga S. et al. 2-Aminoethoxydiphenyl borate inhibits agonist-induced Ca2+ signals by blocking inositol trisphosphate formation in acutely dissociated mouse pancreatic acinar cells. Pflugers Archiv, 2004; 448(6): 592–595.

60. Xing J., Petranka J.G., Davis F.M. et al. Role of Orai1 and store-operated calcium entry in mouse lacrimal gland signalling and function. The Journal of Physiology, 2014; 592 (Pt 5): 927–939.

61. Zimmermann B. Control of InsP3-induced Ca2+ oscillations in permeabilized blowfly salivary gland cells: contribution of mitochondria. The Journal of Physiology, 2000; 525(3): 707–719.
РОЛЬ ВНУТРІШНЬОКЛІТИННИХ ОРГАНЕЛ У ПІДТРИМАННІ Ca²⁺-ГОМЕОСТАЗУ В СЕКРЕТОРНИХ КЛІТИНАХ СЛЬЗОЗОВИХ ЗАЛОЗ

А. Б. Котлярова¹², В. В. Манько²

¹Інститут фізіології імені О.О. Богомольця НАН України
вулиця Академіка Богомольця, 4, Київ 01024, Україна
²Львівський національний університет імені Івана Франка
вулиця Грушевського, 4, Львів 79005, Україна
e-mail: annkotliarova@gmail.com

Відомо, що у різних секреторних клітинах вклад тієї чи іншої Ca²⁺-транспортної системи у формування Ca²⁺-сигналу є різним. В останні роки спостерігається суттєве підвищення інтересу дослідників до вивчення функціонування сльозових залоз. Попри те, системних узагальнених результатів дослідження особливостей функціонування Ca²⁺-транспортних систем сльозових залоз у літературі досі немає.

Огляд присвячений системному аналізу особливостей функціонування Ca²⁺-транспортних систем секреторних клітин зовнішньоорбітальної сльозової залози щура.

ІФ₃-чутливі Ca²⁺-канали секреторних клітин зовнішньоорбітальної сльозової залози ефективно інгібуються 2-АФБ (10 мкмоль/л) й активуються ІФ₃, а також за дії на плазматичну мембрану холіноміметиків (карбахолін) і агоністів пуринових рецепторів (АТФ). Сигнальний шлях при активації P2Y-рецепторів у секреторних клітинах сльозових залоз частково опосередковується активацією ІФ₃-чутливих Ca²⁺-каналів.

У секреторних клітинах зовнішньоорбітальної сльозової залози щура функціонують ріанодинчутливі Ca²⁺-канали, які активуються ріанодином (0,05–1 мкмоль/л) і модулюються катіонами Ca²⁺ з вираженим максимумом чутливості до ріанодину при 10⁻⁷ моль/л Ca²⁺. За одночасної активації ріанодинчутливих та ІФ₃-чутливих Ca²⁺-каналів клітини досліджуваних залоз Ca²⁺ вивільняються, очевидно, з одного і того самого депо.

Спustoшення внутрішньоклітинних депо Ca²⁺ за допомогою мобілізації карбахоліним (10 мкмоль/л) чи внаслідок інгібування Ca²⁺-помпи ендоплазматичного ретикулуму тапсигаргіном (1 мкмоль/л) активує депокерований вхід Ca²⁺ у секреторні клітини досліджуваних залоз, який частково інгібуються 2-АФБ.

Ca²⁺-помпа ендоплазматичного ретикулуму секреторних клітин зовнішньоорбітальної сльозової залози ефективно інгібуються еозином Y (5–10 мкмоль/л) і тапсигаргіном (1 мкмоль/л). На відміну від пермеабілізованих клітин, вміст Ca²⁺ у інтактних клітинах практично не змінювався під впливом еозину Y (5–20 мкмоль/л), що свідчить про інгібування Ca²⁺-помпи плазматичної мембрани.

У секреторних клітинах сльозових залоз функціонує Ca²⁺-уніпортер мітохондрій, який інгібуються рутенієм червоним. Ефекти ріанодину і рутенію червоного на вміст Ca²⁺ у клітинах є статистично достовірно неадитивними. Крім того, ріанодин у концентраціях 1–3 мкмоль/л дозозалежно зменшував швидкість дихання досліджуваних клітин, і цей ефект зберігався за преінкубації клітин із ругенієм червоним чи тапсигаргіном. Це свідчить про те, що, крім активації ріанодинчутливих Ca²⁺-каналів
The role of intracellular organelles in Ca²⁺-homeostasis in secretory cells of the lacrimal gland

A. B. Kotlyarova¹, ², V. V. Manyko²

¹Institute of Physiology of Academic City, Kiev 01024, Ukraine
²Lviv National University of Ivan Franko, Lviv 79005, Ukraine
e-mail: annkotliarova@gmail.com

It is known that in different secretory cells, the role of Ca²⁺-transport systems in the formation of Ca²⁺-signal is different. In recent years, there has been a significant increase in the interest of researchers to study the functioning of lacrimal glands. However, systematic comprehensive results of the study of Ca²⁺-transport systems in lacrimal glands are still absent in the literature.

The review is devoted to a systematic analysis of the peculiarities of functioning Ca²⁺-transport systems of secretory cells of the lacrimal gland of the mouse.

Ca²⁺-sensitive secretory cells of lacrimal gland are inhibited by 2-APB and activated by IF₃, as well as upon action of cholinomimetics (carbachol) and purinergic agonists (ATP).

Ca²⁺-mobilizing ATPase activity of permeabilized cells is inhibited by eosin Y (5–20 µM) and taspigrarine (1 µM), which is consistent with the inhibition of Ca²⁺-pump of the plasma membrane. Ca²⁺-ATPase activity of intact cells is not affected by eosin Y (5–20 µM), indicating the inhibition of Ca²⁺-pump of the plasma membrane.

Ca²⁺-pump of the endoplasmic reticulum in extrabiliary lacrimal gland is effectively inhibited by eosin Y (5–10 µM) and taspigrarine (1 µM). In the absence of permeabilized cells, the activity of Ca²⁺-ATPase is not significantly influenced by eosin Y (5–20 µM), indicating the inhibition of Ca²⁺-pump of the plasma membrane.
ємых желез зависит от времени инкубации, от количества субстрата и концентрации Ca^{2+} в среде инкубации.

В секреторных клетках слезных желез функционирует Ca^{2+}-унипортер митохондрий, который ингибируется рутением красным. Эффекты рианодина и рутения красного на содержание Ca^{2+} в клетках статистически достоверно неаддитивные. Кроме того, рианодин в концентрациях 1–3 мкмоль/л дозозависимо уменьшал скорость дыхания исследуемых клеток, и этот эффект сохранялся по преинкубации клеток с рутением красным или тапсигаргином. Это свидетельствует о том, что, кроме активации рианодинчувствительных Ca^{2+}-каналов эндоплазматического ретикулума рианодин ингибирует поступление ионов Ca^{2+} в матрикс митохондрий, которое нечувствительно к рутению красному.

**Ключевые слова:** слезная железа, ИФ_{3}-чувствительные Ca^{2+}-каналы, рианодинчувствительные Ca^{2+}-каналы, депоуправляемый вход Ca^{2+}, Ca^{2+}-насос, Ca^{2+}-унипортер.

Одержано: 10.08.2015