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THE ROLE OF INTRACELLULAR ORGANELLES IN Ca^{2+}-HOMEOSTASIS IN SECRETORY CELLS OF THE LACRIMAL GLAND

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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. This review is devoted to analysis of functioning of Ca^{2+}-transport system of secretory cells in the rat exorbital lacrimal gland. IP_3Rs in exorbital lacrimal gland cells were effectively inhibited by 2-APB (10 μM) and activated by IP_3 (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 IP_3R activation. RyRs of lacrimal gland secretory cells were activated by Ca^{2+} and low concentrations of ryanodine (0,05–1 μM). Simultaneous activation of RyRs and IP_3Rs in these cells caused Ca^{2+} release from the same store. Ca^{2+} mobilization from the intracellular stores induced by carbacholine (10 μM) or thapsigargin (1 μM) caused store-operated Ca^{2+} 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 thapsigrgin (1 μM). 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 inhibition of PMCA. 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. Mitochondrial Ca^{2+}-uniporter of lacrimal gland secretory cells was inhibited by ruthenium red (10 μM). Effects of ryanodine and ruthenium red on the Ca^{2+} 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 Ca^{2+} transport to the mitochondrial matrix, which was insensitive to the ruthenium red.

Keywords: lacrimal glands, IP_3Rs, RyRs, SOCC, SERCA, Ca^{2+}-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 or ders 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 μM) 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_3R 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, stimulatesfluid and protein secretion by the lacrimal glands [14, 43].

Under the influence of carbacholine (1 μM) – 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_3Rs inhibitor – 2-APB [42, 46, 56, 58, 59] in concentration of 10 μM completely prevented carbacholine-induced reduction of Ca^{2+} content in cells [36].

In the lacrimal gland secretory cells IP_3Rs retain their functional activity in the dygitonin-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 μM) 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_3Rs presence in the secretory cells of rat exorbital lacrimal gland.

IP_3Rs 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].

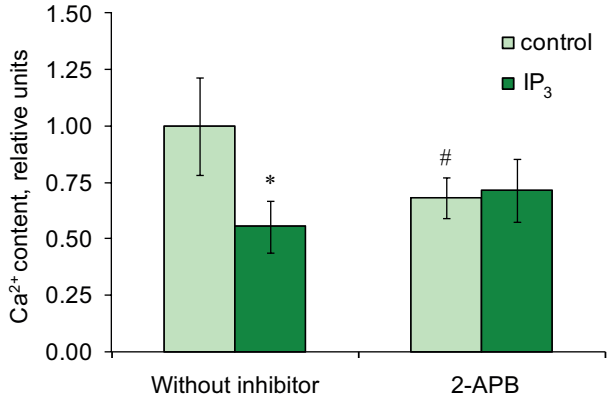

Fig. 1. The effect of IP_3 on Ca^{2+} content in the permeabilized secretory cells of rat exorbital lacrimal gland: tissue Ca^{2+} content in the absence of inhibitors/agonists was normalized to one; incubation time – 15 min; $[K^+]$ = 140 mM, $[Ca^{2+}] = 10^{-7}$ M; $[IP_3]$ = 2 μM; [2-APB] = 10 μM; * – the difference compared with the control reliable with $P < 0.05$; # – difference compared with the sample without inhibitor/agonist significant with $P < 0.05$; $n = 6$ [36]

Рис. 1. Зміни вмісту Ca^{2+} у пермеабілізованих клітинах зовнішньоорбітальної сльозової залози за дії $IФ_3$: вміст Ca^{2+} у тканині нормалізували, прийнявши за одиницю його значення за відсутності агоністів/інгібіторів; час інкубації 15 хв; * – різниця порівняно з контролем достовірна з $P < 0,05$, # – різниця порівняно з пробою за відсутності інгібітора/агоніста достовірна з $P < 0,05$; $n = 6$ [36]

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_3R 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_3R 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 μM, 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_3Rs 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 μM) 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].

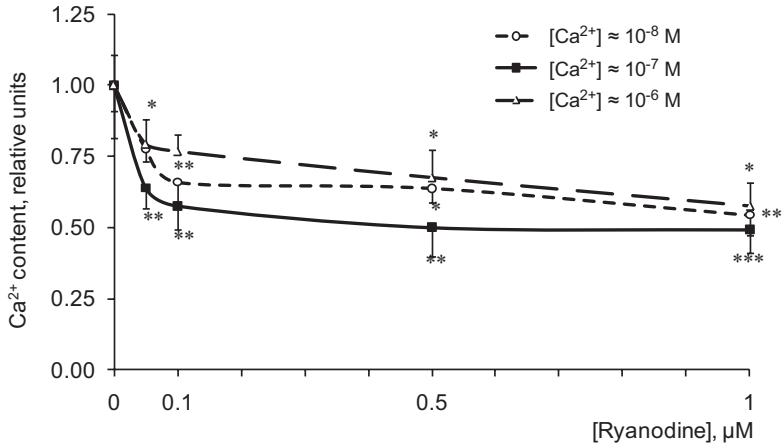

Fig. 2. The effect of ryanodine on Ca^{2+} content in the permeabilized secretory cells of rat exorbital lacrimal gland: tissue Ca^{2+} content in the absence of ryanodine was normalized to one; incubation time – 15 min; $[K^+]$ = 140 mM; * – difference compared with control reliable with P < 0.05, ** – with P < 0,01; *** – with P < 0,001; n = 4–5 (according to the publication [32])

Рис. 2. Вплив ріанодину різної концентрації на вміст Ca^{2+} у пермеабілізованих клітинах зовнішньоорбітальної сльозової залози щура: вміст Ca^{2+} у тканині нормалізували, прийнявши за одиницю його значення у пробі, котра не містила ріанодину; час інкубації – 15 хв; $[K^+]$ = 140 ммоль/л; * – різниця порівняно з контролем достовірна з P < 0,05, ** – з P < 0,01; *** – з P < 0,001; n = 4–5 (за публікацією [32])

Simultaneous activation of RyRs and IP_3Rs 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].

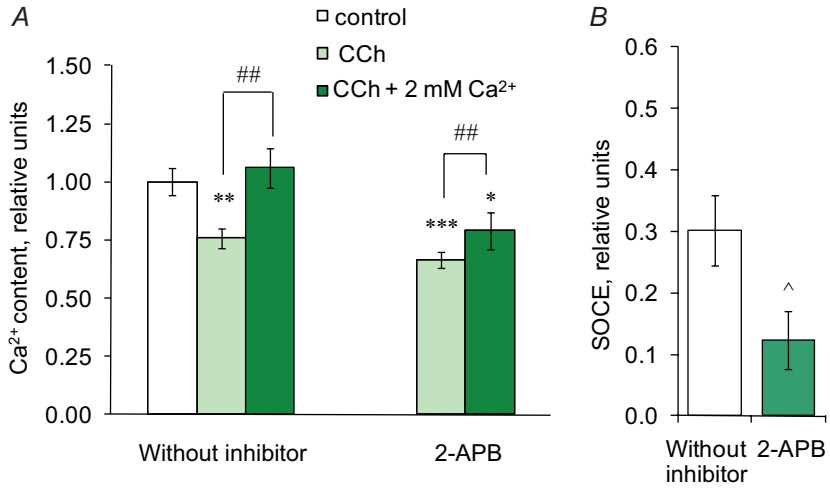

Fig. 3. SOCE into cells of the exorbital lacrimal gland in control and in the presence of 2-APB in medium: *A* – Ca^{2+} content in the tissue measured under appropriate conditions, *B* – calculated SOCE (increase in the Ca^{2+} content in cells after restoring its concentration in the medium), induced by carbacholine (10 μM); stars – a degree of reliability of the difference with respect to control or above the lines, to this indicator in other experiment; * – the difference significant with $P < 0.05$, ** – with $P < 0.01$, *** – with $P < 0.001$; ^ – significant changes in SOCE with $P < 0.05$; $n = 7$ [36]

Рис. 3. Депокерований вхід Ca^{2+} у клітини зовнішньоорбітальної сльозової залози щура у контролі та за наявності 2-АФБ у середовищі:
A – вміст Ca^{2+} у тканині, визначений за відповідних умов, *B* – депокерований вхід Ca^{2+} розраховували як приріст вмісту Ca^{2+} у клітинах після відновлення його концентрації у середовищі, індукували карбахоліном (10 мкмоль/л); зірочками позначено ступінь достовірності різниці стосовно контролю або, над лініями, стосовно цього показника в іншому досліді; * – різниця достовірна з $P < 0,05$, ** – з $P < 0,01$, *** – з $P < 0,001$; ^ – достовірна зміна депокерованого входу Ca^{2+} з $P < 0,05$; $n = 7$ [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].

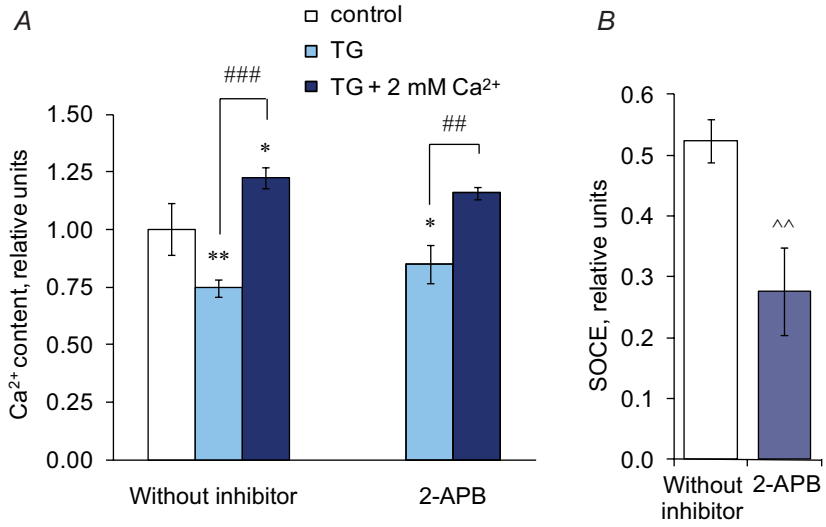

Fig. 4. SOCE into cells of the exorbital lacrimal gland in control and in the presence of 2-APB in medium: *A* – Ca^{2+} content in the tissue measured under appropriate conditions, *B* – calculated SOCE (increase in Ca^{2+} content in cells after restoring its concentration in the medium), induced by thapsigargin (10 μM); stars – a degree of reliability of the difference with respect to control or, above the lines, to this indicator in other experiment; * – difference significant with $P < 0.05$, ** – with $P < 0.01$, *** – with $P < 0.001$; ^^ – significant changes in SOCE with $P < 0.01$; $n = 7$ [36]

Рис. 4. Депокерований вхід Ca^{2+} у клітини зовнішньоорбітальної сльозової залози щура у контролі та за наявності інгібітора $IФ_3$-чутливих Ca^{2+}-каналів 2-АФБ у середовищі: *A* – вміст Ca^{2+} у тканині, визначений за відповідних умов, *B* – депокерований вхід Ca^{2+} розраховували як приріст вмісту Ca^{2+} у клітинах після відновлення його концентрації у середовищі, індукували тапсигаргіном; зірочками позначено ступінь достовірності різниці стосовно контролю або, над лініями, стосовно цього показника в іншому досліді; * – різниця достовірна з $P < 0,05$, ** – з $P < 0,01$, *** – з $P < 0.001$; ^^ – достовірна зміна депокерованого входу Ca^{2+} з $P < 0,01$; $n = 7$ [36]

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 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_3R 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].

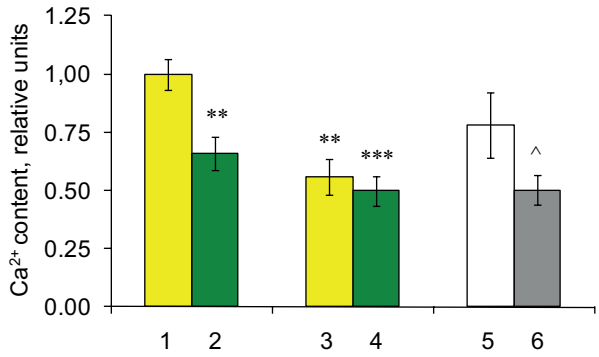

Fig. 5. The effect of ryanodine on Ca^{2+} content in permeabilized cells of the lacrimal glands in the presence of ruthenium red: $[K^+]$ = 140 mM, $[Ca^{2+}] = 10^{-7}$ M; 1 – control (K), 2 – Ca^{2+} content in presence of ryanodine (A), 3 – in presence of ruthenium red (B), 4 – for the simultaneous 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]

Рис. 5. Вплив ріанодину на вміст Ca^{2+} у пермеабілізованих клітинах сльозових залоз за наявності рутенію червоного: $[K^+]$ = 140 ммоль/л, $[Ca^{2+}] = 10^{-7}$ моль/л; *1* – контроль (*K*), *2* – вміст Ca^{2+} за дії ріанодину (*A*), *3* – за дії рутенію червоного (*B*), 4 – за одночасної дії обох речовин ({*A* + *B*}), 5 – алгебраїчна сума змін вмісту Ca^{2+} за окремої дії ріанодину та рутенію червоного ([*K* – *A*] + [*K* – *B*]), *6* – зміна вмісту Ca^{2+} за одночасної дії обох речовин ([*K* – {*A* + *B*}]); вміст Ca^{2+} у тканині нормалізували, прийнявши за одиницю його значення у пробі, котра не містила інгібіторів/агоністів; ** – різниця достовірна стосовно контролю з $P < 0,01$; ^ – достовірна різниця між сумою змін за окремої дії двох речовин та зміною за їхньої одночасної дії ($P_a < 0,05$); $n = 6$ [37]

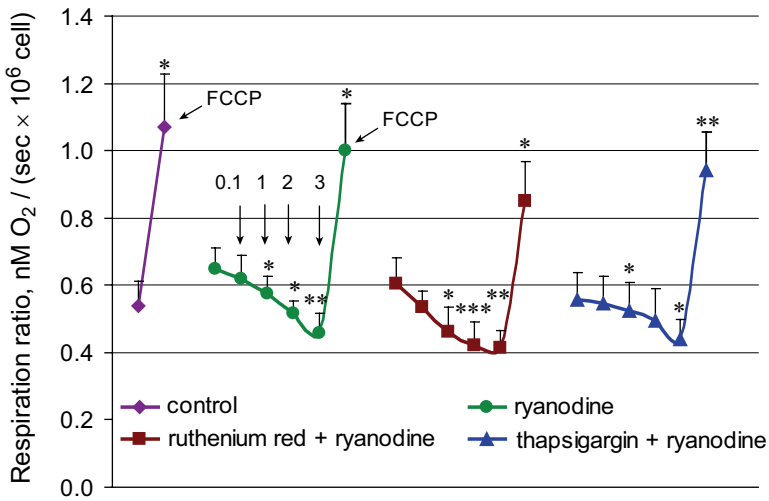

Fig. 6. The level of cell respiration in presence of ryanodine and FCCP in medium under conditions of Ca^{2+}-transport systems inhibition: Arrow shows the addition of FCCP (1 μM) and ryanodine to a final concentration of 0.1, 1.2 or 3 μM; $n = 5$ [37]

Рис. 6. Інтенсивність клітинного дихання за наявності у середовищі ріанодину та FCCP за інгібування Ca^{2+}-транспортувальних 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).

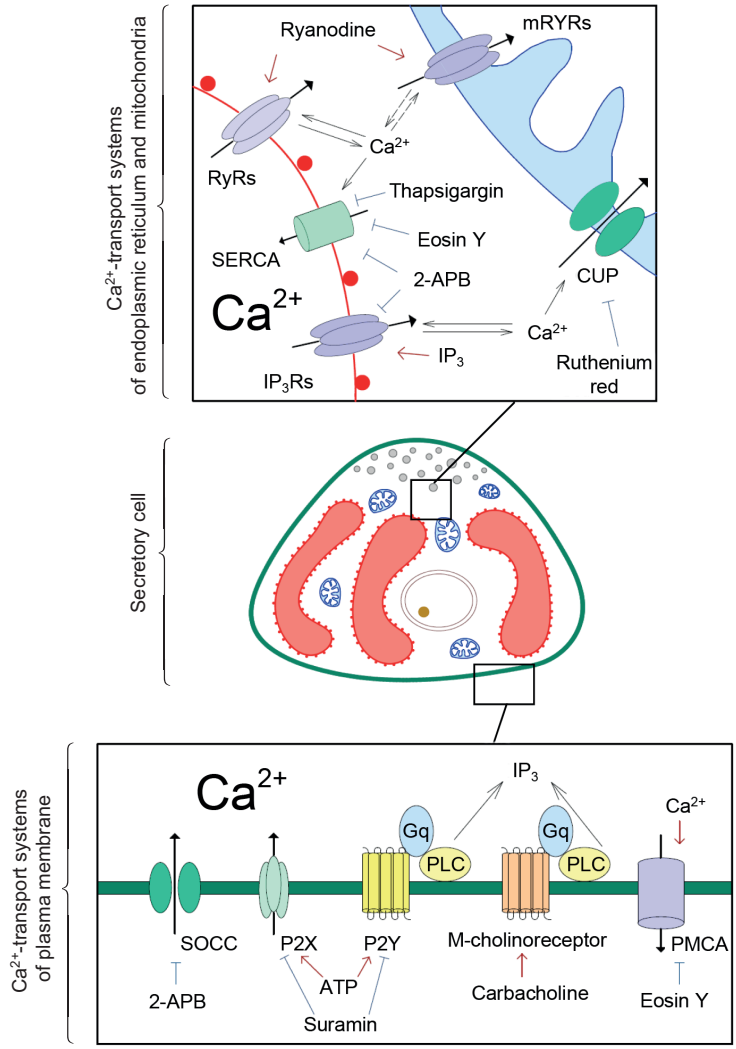

Fig. 7. Scheme of coordinated functioning of different Ca^{2+}-transport systems in extraorbital lacrimal gland cells of the rat:
→ – activation, ┤– inhibition; CUP – Ca^{2+}-uniporter, IP_3 – inositol 1,4,5-trisphosphate, IP_3Rs – IP_3-receptors, mRyRs – mitochondrial ryanodine receptors, PLC – phospholipase 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 взаємоузгодженого функціонування Ca^{2+}-транспортувальних систем секреторних клітин зовнішньоорбітальної сльозової залози щурів:
→ – активація, ┤– інгібування; CUP – Ca^{2+}-уніпортер, IP_3 – інозитол-1,4,5-трифосфат, IP_3Rs – $IФ_3$-чутливі Ca^{2+}-канали, mRyRs – мітохондріальні ріанодинчутливі Ca^{2+}-канали, PLC – фосфоліпаза С, 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-purinoreceptors and acetylcholine and its analogues – agonists of the identified M_3-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 IP_3R activation [36]. IP_3Rs 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, 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 μM, respectively) on the Ca^{2+} content were non-additive [36].

RyRs of endoplasmic reticulum in secretory cells of the lacrimal glands were activated by Ca^{2+} and low concentrations of ryanodine (0,05–1 μM) [32].

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

An increase in cytosolic Ca^{2+} concentration after the IP_3R and RyR activation is accompanied by activation of SERCA, PMCA, Ca^{2+}-uniporter and mRyRs.

SERCA of exorbital lacrimal gland cells was efficiently inhibited by eosin Y (10–20 μM) and by thapsigargin (1 μM) [34]. Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} intake in the mitochondrial matrix and subsequent activation of the mitochondrial oxidation. Mitochondrial Ca^{2+}-uniporter inhibition, which prevents the Ca^{2+} flow in the matrix caused the levelling of carbacholine-stimulating effect, and IP_3Rs 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 Ca^{2+}-transport system functioning in the lacrimal gland secretory cells addesses of their functioning changes during aging and regulation by hormones.

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РОЛЬ ВНУТРІШНЬОКЛІТИННИХ ОРГАНЕЛ У ПІДТРИМАННІ Ca^{2+}-ГОМЕОСТАЗУ В СЕКРЕТОРНИХ КЛІТИНАХ СЛЬОЗОВИХ ЗАЛОЗ

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Відомо, що у різних секреторних клітинах вклад тієї чи іншої Ca^{2+}-транспортувальної системи у формування Ca^{2+}-сигналу є різним. В останні роки спостерігається суттєве підвищення інтересу дослідників до вивчення функціонування сльозових залоз. Попри те, системних узагальнених результатів дослідження особливостей функціонування Ca^{2+}-транспортувальних систем сльозових залоз у літературі досі немає.

Огляд присвячений системному аналізу особливостей функціонування Ca^{2+}-транспортувальних систем секреторних клітин зовнішньоорбітальної сльозової залози щура.

$ІФ_3$-чутливі Ca^{2+}-канали секреторних клітин зовнішньоорбітальної сльозової залози ефективно інгібуються 2-АФБ (10 мкмоль/л) й активуються $ІФ_3$, 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 $ІФ_3$-чутливих Ca^{2+}-каналів.

У секреторних клітинах зовнішньоорбітальної сльозової залози щура функціонують ріанодинчутливі Ca^{2+}-канали, які активуються ріанодином (0,05–1 мкмоль/л) і модулюються катіонами Ca^{2+} з вираженим максимумом чутливості до ріанодину при 10^{-7} моль/л Ca^{2+}. За одночасної активації ріанодинчутливих та $ІФ_3$-чутливих Ca^{2+}-каналів клітин досліджуваних залоз Ca^{2+} вивільняється, очевидно, з одного і того самого депо.

Спустошення внутрішньоклітинних депо Ca^{2+} за допомогою мобілізації карбахоліном (10 мкмоль/л) чи внаслідок інгібування Ca^{2+}-помпи ендоплазматичного ретикулуму тапсигаргіном (1 мкмоль/л) активує депокерований вхід Ca^{2+} у секреторні клітини досліджуваних залоз, який частково інгібується 2-АФБ.

Ca^{2+}-помпа ендоплазматичного ретикулуму секреторних клітин зовнішньоорбітальної сльозової залози ефективно інгібується еозином Y (5–10 мкмоль/л) і тапсигаргіном (1 мкмоль/л). На відміну від пермеабілізованих клітин, вміст Ca^{2+} у інтактних клітинах практично не змінювався під впливом еозину Y (5–20 мкмоль/л), що свідчить про інгібування Ca^{2+}-помпи плазматичної мембрани. Ca^{2+}-АТФазна активність пермеабілізованих клітин досліджуваних залоз залежить від часу інкубації, від кількості субстрату і концентрації Ca^{2+} у середовищі інкубації.

У секреторних клітинах сльозових залоз функціонує Ca^{2+}-уніпортер мітохондрій, який інгібується рутенієм червоним. Ефекти ріанодину і рутенію червоного на вміст Ca^{2+} 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 рутенієм червоним чи тапсигаргіном. Це свідчить про те, що, крім активації ріанодинчутливих Ca^{2+}-каналів

ендоплазматичного ретикулуму, ріанодин інгібує надходження іонів Ca^{2+} у матрикс мітохондрій, яке нечутливе до рутенію червоного.

Ключові слова: сльозова залоза, $ІФ_3$-чутливі Ca^{2+}-канали, ріанодинчутливі Ca^{2+}-канали, депокерований вхід Ca^{2+}, Ca^{2+}-помпа, Ca^{2+}-уніпортер.

РОЛЬ ВНУТРИКЛЕТОЧНЫХ ОРГАНЕЛЛ В ПОДДЕРЖАНИИ Ca^{2+}-ГОМЕОСТАЗА В СЕКРЕТОРНЫХ КЛЕТКАХ СЛЕЗНОЙ ЖЕЛЕЗЫ

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Известно, что в разных секреторных клетках вклад той или иной Ca^{2+}-транспортировочной системы в формирование Ca^{2+}-сигнала различен. В последние годы наблюдается существенное повышение интереса исследователей к изучению функционирования слезных желез. Тем не менее, системных обобщенных результатов исследования особенностей функционирования Ca^{2+}-транспортировочных систем слезных желез в литературе до сих пор нет.

Обзор посвящен системному анализу особенностей функционирования Ca^{2+}- транспортировочных систем секреторных клеток внеорбитальной слезной железы крысы.

$ИФ_3$-чувствительные Ca^{2+}-каналы секреторных клеток слезной железы ингибируются 2-АФБ и активируются $ИФ_3$, а также при действии на плазматическую мембрану холиномиметикив (карбахолин) и агонистов пуриновых рецепторов (АТФ). Сигнальный путь при активации P2Y-рецепторов в секреторных клетках слезных желез опосредуется активацией $ИФ_3$-чувствительных Ca^{2+}-каналов.

В секреторных клетках внеорбитальной слезной железы крысы функционируют рианодинчувствительные Ca^{2+}-каналы, которые активируются рианодином (0,05–1 мкмоль/л) и модулируются катионами Ca^{2+} с выраженным максимумом чувствительности к рианодину при 10^{-7} моль/л Ca^{2+}. При одновременной активации рианодинчувствительных и $ИФ_3$-чувствительных Ca^{2+}-каналов клеток исследуемых желез Ca^{2+} высвобождается, очевидно, из одного и того же депо.

Опустошение внутриклеточных депо Ca^{2+} с помощью мобилизации карбахолином (10 мкмоль/л) или за счет ингибирования Ca^{2+}-насоса эндоплазматического ретикулума тапсигаргином (1 мкмоль/л) активирует депоуправляемый вход Ca^{2+} в секреторные клетки исследуемых желез, который частично ингибируется 2-АФБ.

Ca^{2+}-насос эндоплазматического ретикулума секреторных клеток внеорбитальнои слезной железы эффективно ингибируется эозином Y (5–10 мкмоль/л) и тапсигаргином (1 мкмоль/л). В отличие от пермеабилизованных клеток, содержание Ca^{2+} в интактных клетках практически не менялся под воздействием эозина Y (5–20 мкмоль/л), что свидетельствует об ингибировании Ca^{2+}-насоса плазматической мембраны. Ca^{2+}-АТФазная активность пермеабилизованных клеток исследу-

емых 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.

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