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NAADP-SENSITIVE Ca^{2+} STORES IN PERMEABILIZED RAT HEPATOCYTES

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Nicotinic acid adenine dinucleotide phosphate (NAADP) is a nucleotide that is potent to release calcium from intracellular stores in different cell types. NAADP was shown to target specific type of intracellular store namely endolysosomal system or acidic store. Despite intense studies, its effect on endoplasmatic reticulum (ER) still remains to be elucidated. The main aim of our work was to investigate NAADP-sensitive store in permeabilized rat hepatocytes monitoring the level of Ca^{2+} inside intracellular organelles using chlorotetracycline (CTC). We have shown that NAADP triggered changes of stored Ca^{2+} in rat hepatocytes are dependent on concentration of EGTA-Ca^{2+}-buffer in cell incubation medium, i.e. the higher is the EGTA concentration in incubation medium the smaller or absent is the effect of NAADP. Besides, the effect of NAADP was more pronounced upon cells pretreatment with the inhibitory concentration of ryanodine (100 μM). This might suggest that the effect of NAADP is dependent on ER luminal calcium. We have also found that NAADP-evoked Ca^{2+} release in permeabilized hepatocytes is sensitive to nigericin, bafilomycin A and thapsigargin. Additionally, NAADP triggered changes in stored Ca^{2+} were completely abolished by NED-19 as antagonist of NAADP.

K e y w o r d s: hepatocytes, calcium, acidic calcium stores, NAADP, ryanodine-sensitive Ca^{2+}-channels, two-pore channels.

Nicotinic acid adenine dinucleotide phosphate (NAADP) is a naturally occurring nucleotide that has been shown to be involved in calcium release from intracellular stores in a wide variety of cell types [1]. The first demonstration that NAADP levels increase in response to an extracellular stimulus arose from studying sea urchin fertilization (NAADP changed in both the eggs and sperm upon contact) [2]. Subsequently it was shown on different mammalian preparations that NAADP is a potent Ca^{2+}-releasing second messenger [3–5].

Although, the question about the nature of NAADP-receptor remains open, since NAADP might regulate different ion channels, including ryanodine receptors, two-pore channels, and TRP-ML1 (transient receptor potential channel, subtype mucolipin1) [6]. Accordingly, different models of NAADP effect inside the cell were proposed. Previous results of Mandi et al. [7] reported about NAADP-sensitive store in liver microsome fraction. Additionally, Zhang's group has shown that NAADP-sensitive Ca^{2+} release channel is present in the lysosome of native liver cells [8]. Hence, due to the destroyed apposition of ER and acidic vesicles upon liver homogenization, it appears to be difficult to conclude about the role of NAADP for Ca^{2+} signalling in rat hepatocytes. The aim of this paper was to examine possible mechanisms of NAADP mediated Ca^{2+} signalling pathways in permeabilized rat hepatocytes.

Materials and Methods

Cell isolation. For the preparation of isolated hepatocytes, wild type male rats (180-200 g) were anaesthetized with an inhalation of chloroform and subsequently decapitated. The liver was isolated and digested with the "two step" collagenase perfusion method as recommended [9]. It includes a non-recirculating perfusion of the liver with Ca^{2+}- and Mg^{2+}-free, EGTA-containing (0.1 mM) Hank's Balanced Salt Solution (HBSS), for 10 min, followed by a short wash with EGTA-free HEPES-containing (10 mM) buffer at 37 ºC for 2 min. Blanched liver

was then perfused with 0.01% collagenase in Ca^{2+}-containing (1 mM) HBSS for 10-15 min. After collagenase treatment, the liver was excised, minced in Ca^{2+}-containing HBSS, and the cell suspension was then filtered through serial nylon mesh filters and centrifuged at 50 g for 2 min. The supernatant was discarded and cells were resuspended in Ca^{2+}-containing HBSS supplemented with fetal bovine serum (10%). Hepatocytes viability was tested by Trypan Blue exclusion, and preparation for each experiment contained approximately 90% of viable cells.

The Hank's Balanced Salt Solution (HBSS) contained (mM): 137 NaCl, 5.4 KCl, 0.2 Na_2HPO_4, 0.4 KH_2PO_4, 0.4 $MgSO_4$, 1.3 $CaCl_2$, 4.1 Na_2CO_3 and 5.6 glucose. For Ca^{2+}- and Mg^{2+}-free HBSS $CaCl_2$ and $MgSO_4$ were eliminated from the medium.

Animal experiments were carried out in accordance to the European Community Council Directive of 24 November 1986 (86/609/EEC).

Chlorotetracycline fluorescence as a quantitative measure of stored calcium in hepatocytes. Prior to Ca^{2+}-imaging, isolated hepatocytes were permeabilized in suspension with saponine (0.1 mg/ml,) in intracellular solution for 10 min. Cells were then washed with intracellular solution.

Before loading with chlorotetracycline (CTC) permeabilized hepatocytes suspensions were incubated in intracellular solutions added appropriate reagents for 10 min. Then permeabilized cells suspensions were washed with intracellular solution.

Intracellular solution contained (mM): 20 NaCl, 120 KCl, 1.13 $MgCl_2$, 1.3 $CaCl_2$, 10.0 HEPES, 5 μg/ml oligomycine, 1 μg/ml rotenone and 2.0 ATP (pH 7.0). All experimental procedures were carried out at 37 ºC. In order to modulate free calcium concentration in the incubation medium of permeabilized rat hepatocytes we used EGTA-Ca^{2+} buffer which contained following Ca^{2+} concentrations (maxchelator.stanford.edu/CaEGTA-TS.htm): $[Ca^{2+}]_{cyt.}$ = 2.47 nM (EGTA – 100 μM, $CaCl_2$ – 1 μM); $[Ca^{2+}]_{cyt.}$ = 243 nM (EGTA – 100 μM, $CaCl_2$ – 50 μM); $[Ca^{2+}]_{cyt.}$ = 240 nM (ЕГТА – 50 μM, $CaCl_2$ – 25 μM).

To estimate the changes of internal Ca^{2+} concentrations hepatocytes were loaded with 100 μM CTC in the dark at room temperature for 20 min. This Ca^{2+}-sensitive fluorescent probe has been applied widely in biological systems [10, 11] to monitor the free internal Ca^{2+} concentration in the organelle lumen by the following mechanism: CTC permeates in a neutral uncomplexed form and the internal and external CTC concentrations are equal at equilibrium. Within the lumen, Ca^{2+} complexes with CTC can give a small increase in fluorescence. Further, this complex binds to the inner surface of the membrane yielding a large increase in fluorescence. Thus, it is considered that CTC-Ca^{2+} fluorescence corresponds to the level of Ca^{2+} inside intracellular organelles, as mitochondria were deenergized.

For Ca^{2+}-measurements, cells were plated on the glass slides and imaged single cells using a LUMAM-I-1 luminescent microscopy system (40× objective, NA 0.7). CTC was excited by 380 nm using light-emitting diode and emission fluorescence was collected between 505-520 nm. For each subset of experiments, a group of 30 cells in the filed of view was examined. Ca^{2+} relative changes in CTC fluorescence were normalized to the control value considered as 100%.

Chemicals. Collagenase, ATP, EGTA, Ruthenium Red, NAADP, Ryanodine, Thapsigargin, Nigericine, IP_3 were from Sigma (UK), Saponine (Calbiochem), HEPES (Calbiochem).

Results and Discussion

NAADP releases Ca^{2+} from Ca^{2+} stores in rat permeabilized hepatocytes. We showed that NAADP (7 μM) caused a significant decrease in chlorotetracycline (CTC) fluorescence intensity in permeabilized rat hepatocytes, which testify to Ca^{2+} release from intracellular Ca^{2+} stores (Fig. 1, incubation medium # 4) (34.82 ± 9.93%; $P < 0.01$, $n = 12$). These results were obtained without adding calcium and EGTA to the intracellular medium. Interestingly, NAADP did not cause any changes in calcium content after EGTA-Ca^{2+}-buffer was added to intracellular solution (100 μM EGTA, 50 μM Ca^{2+}) maintaining free Ca^{2+} concentration at 243 nM (Fig. 1, incubation medium # 2). NAADP decreased Ca^{2+} level (by 33.21 ± 4.16%; $P < 0.01$, $n = 36$) when concentration of chelating agent was reduced further (EGTA – 50 μM, Ca^{2+} – 25 μM) without changing free calcium concentration (Fig. 1, incubation medium # 3). We also found that NAADP did not change calcium content in permeabilized rat hepatocytes when concentration of free calcium was buffered to much lower level (2.4 nM) upon addition of 100 μM EGTA (Fig. 1, incubation medium # 1).

Thus, we found that NAADP-triggered changes of Ca^{2+} store in rat hepatocytes are dependent on the concentration of EGTA-Ca^{2+}-buffer in cell incubation medium, i.e. the higher is EGTA concentration

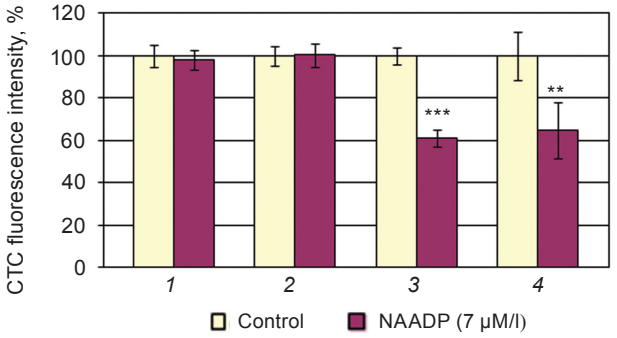

*Fig. 1. Dependence of the NAADP effect on CTC fluorescence intensity in rat permeabilized hepatocytes upon the concentration of the EGTA-Ca^{2+}-buffer in incubation medium: 1 – $[Ca^{2+}]_{cyt.}$ = 2.47 nM (EGTA – 100 μM, $CaCl_2$ – 1 μM); 2 – $[Ca^{2+}]_{cyt.}$ = 243 nM (EGTA – 100 μM, $CaCl_2$ – 50 μM); 3 – $[Ca^{2+}]_{cyt.}$ = 240 nM (EGTA – 50 μM, $CaCl_2$ – 25 μM); 4 – no EGTA-Ca^{2+} buffer. *** Statistically significant difference compared to control $P < 0.001$, $n = 36$; ** statistically significant difference compared to control $P < 0.01$, $n = 12$*

in the incubation medium the smaller or absent is the effect of NAADP. This might suggest that Ca^{2+}-induced Ca^{2+} release (CIRC) plays an important role in NAADP-elicited Ca^{2+} release in hepatocytes. Accordingly, it is likely that concentration of EGTA is critical for NAADP-evoked change of Ca^{2+} content in rat hepatocytes. Hence, studies on liver microsomes did not find free cytosolic Ca^{2+} concentration to be crucial [7].

The effect of thapsigargin on the NAADP-evoked Ca^{2+} release in rat permeabilized hepatocytes. To test which intracellular Ca^{2+} store is involved in this process and whether the effect of NAADP is thapsigargin dependent in rat hepatocytes, we next tested simultaneous application of NAADP (7 μM) and thapsigargin (1 μM) to the incubation medium lacking EGTA-Ca^{2+}-buffer. This resulted in a decrease of the CTC fluorescence to a slightly greater extent (by 43.91 ± 8.764%; $P < 0.001$, $n = 12$) compared to separate effects of NAADP and thapsigargin in the same medium (Fig. 2). We found no effect of NAADP on calcium content in hepatocytes after ER Ca^{2+} store depletion with thapsigargin. In fact, thapsigargin completely prevented NAADP effect in all incubation media that were used. Our finding suggests that NAADP-mediated Ca^{2+} release is thapsigargin-sensitive in rat hepatocytes.

These results are consistent with the findings on NAADP-mediated Ca^{2+} release in liver microsomes and its dependence on thapsigargin [7], but controversial with the data obtained by Zhang et al. on liver lysosomes [8]. Additionally, NAADP-elicited Ca^{2+} release has been shown to be thapsigargin-insensitive in sea urchin eggs [12] and some mammalian cell types such as pancreatic acinar cells [4], pulmonary arterial myocytes [13, 14], rat cortical neurons [15]. On the one hand, the microsomal fraction of liver hepatocytes is a more heterogeneous system compared to the lysosomal ones, as it exhibits Ca^{2+} stores derived both from lysosomes and the ER containing, mainly $InsP_3Rs$, RyRs, NAADP receptors, and Ca^{2+}-ATPase. On the other hand, permeabilized hepatocytes represent a tool of different Ca^{2+}-stores, i.e. ER, mitochondria, Golgi apparatus, nucleus and lysosomes, providing a complex analysis of Ca^{2+} signaling events in these cells.

Interestingly, treatment of permeabilized hepatocytes with either NAADP or thapsigargin followed by the application of either thapsigargin or NAADP, respectively, did not affect fluorescence intensity (Fig. 2). Presumably obtained data confirm bidirectional organelle communication which has been recently shown for sea urchin eggs (NAADP-sensitive acidic store and ER) [16]. This could be due to the fact that Ca^{2+} released from NAADP-sensitive store (acidic store) may contribute to its release from ER via CICR mechanism. In this case, EGTA affects

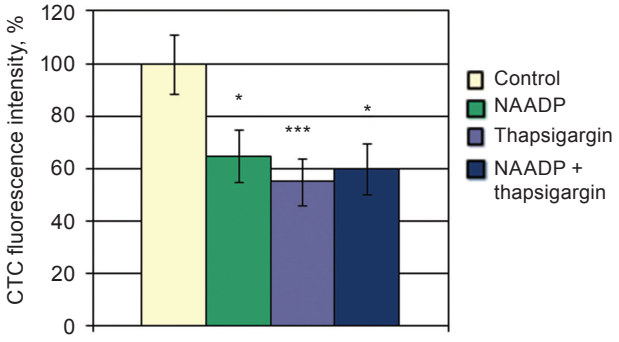

*Fig. 2. The effects of NAADP and thapsigargin on CTC fluorescence intensity in permeabilized rat hepatocytes in the medium lacking EGTA-Ca^{2+}-buffer: *** statistically significant difference compare to control $P < 0.001$, $n = 12$; * statistically significant difference compare to control $P < 0.05$, $n = 12$*

Ca^{2+} diffusion between the acidic store and ER as a possible target, thus, ablating the NAADP response.

Nevertheless, NAADP can initiate regenerative global Ca^{2+} signals (waves and oscillations) through amplification of 'triggered' Ca^{2+} signal by recruitment of IP_3Rs and RyRs that exhibit the characteristic property of CICR [17]. This might occur by two models: most obviously by the trigger Ca^{2+} stimulating ER channels via the CICR, but also by trigger Ca^{2+} being sequestered into and 'priming' the ER (which sensitizes ER channels from the luminal face) [17].

We conclude that the effect of NAADP on Ca^{2+} release is sensitive to the level of the luminal ER calcium. That explains the fact that emptying of the ER Ca^{2+} store with thapsigargin prevented NAADP triggered changes in Ca^{2+} content in permeabilized rat hepatocytes.

The effects of nigericin and bafilomycin A1 on the NAADP-evoked Ca^{2+} release in rat permeabilized hepatocytes. It is known that NAADP-senstive Ca^{2+} release identified in mammals is localized to the acidic lysosomal compartments [18]. We next examined if NAADP-evoked Ca^{2+} release in rat hepatocytes is mediated via acidic pools. For this purpose, nigericin as a well known protonophore, which breaks down the transmembrane H^+ gradient in acidic organelles [19] such as lysosomes, endosomes etc., was applied. As is shown in Fig. 3, treatment of hepatocytes with nigericin (7 μM) induced a decrease in CTC fluorescence intensity compared to the control (without nigericin) (by 36.17 ± 9.79%, $P < 0.05$, $n = 6$) in the medium without addition of

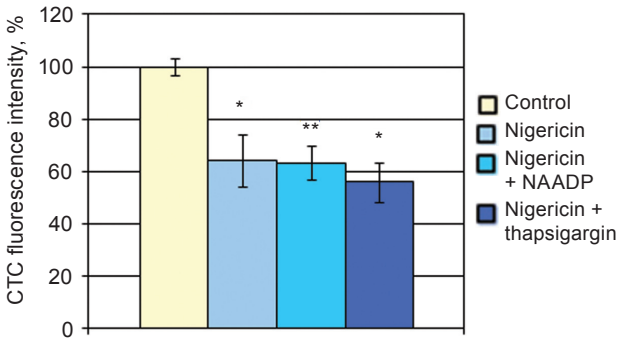

*Fig. 3. The effects of nigericine and thapsigargin on CTC fluorescence intensity in permeabilized rat hepatocytes in the medium lacking EGTA-Ca^{2+}-buffer: ** statistically significant difference compare to control $P < 0.01$, $n = 6$; * statistically significant difference compare to control $P < 0.05$, $n = 6$*

EGTA and Ca^{2+}. Whereas simultaneous application of nigericin (7 μM) and NAADP (7 μM) elicited slightly more enhanced Ca^{2+} efflux from intracellular stores, compared to their separate effects (Fig. 3) (36.21 ± 7.62%; $P < 0.01$, $n = 6$). In contrast, a prior treatment with nigericin prevented NAADP stimulated Ca^{2+} release (Fig. 3). Similarly, nigericin had no effect on the level of the CTC fluorescence after cells were pre-incubated with NAADP (Fig. 3).

Thus, NAADP-evoked Ca^{2+} release in permeabilized hepatocytes is sensitive to nigericin. This can be explained by the expression of NAADP-channels in acidic stores, as it was shown earlier in rat hepatocytes [8]. It is possible that local NAADP-induced Ca^{2+} release from acidic stores might be amplified by CICR through the recruitment of IP_3Rs and RyRs on the ER located proximal to NAADP-activated receptors [14] in intact cells. But such events are unlikely if cells are permeabilized. It is more likely that local NAADP- released Ca^{2+} is sequestered into ER and sensitizes ER channels from the luminal face [17].

Additionally, we observed more substantial decrease (43.69 ± 6.41%; $P < 0.01$, $n = 6$) in CTC fluorescence intensity after incubation of hepatocytes both with nigericin (7 μM) and thapsigargin (1 μM) (Fig. 3). Interestingly, pretreatment of hepatocytes with thapsigargin followed by the application of nigericin led to a decrease in CTC fluorescence (29.34 ± 5.35%; $P < 0.01$, $n = 6$). In contrast, a prior application of nigericin prevented thapsigargin-induced changes of CTC fluorescence. Our findings postulate that nigericin directly or indirectly affects thapsigargin-sensitive (ER) and acidic (lysosomal) Ca^{2+} store which is insensitive to thapsigargin. Perhaps, nigericin affects thapsigargin-sensitive store along with the acidic, e.g. junctions between the acidic store and ER. This suggests the specific membrane contact sites between acidic organelles and the endoplasmic reticulum. Perhaps, possible morphological and functional relationships between ER and acidic cellular compartments make take a place in this case.

The acidic stores do not possess thapsigargin-sensitive Ca^{2+}-pumps, but have Ca^{2+} uptake mechanism that depends on a bafilomycin-sensitive vacuolar H^+-pump [20]. One way of interfering store acidification is to pretreat cells with bafilomycin A1. When permeabilized hepatocytes were pre-treated with bafilomycin A1 (1 μM), NAADP (7 μM) did not cause any significant changes of Ca^{2+} store level in the medium with high and low EGTA concentrations

maintaining the same free calcium concentration (Fig. 4). These results indicate that NAADP releases Ca^{2+} from acidic stores in rat hepatocytes. Nevertheless, bafilomycin A1 did not affect NAADP-induced Ca^{2+} release from liver microsomes [7], as well as from lysosomes [8].

We have found that bafilomycin prevents NAADP-evoked changes of CTC fluorescence in rat hepatocytes. This indicates that NAADP primarily depletes Ca^{2+} from organelles of a lysosomal type. We assume that NAADP induces local microdomains of high Ca^{2+} at junctions between the ER and acidic organelles, which has recently been shown for sea urchin eggs [16].

The effects of ryanodine and ruthenium red on the NAADP-mediated Ca^{2+} release in permeabilized hepatocytes. Studies on isolated pancreatic acinar nuclei have demonstrated that Ca^{2+} liberation elicited by NAADP can be blocked by ryanodine or ruthenium red [3]. In order to test whether NAADP causes Ca^{2+} release from ER by opening RyRs, we used 100 μM ryanodine as an inhibitor of ryanodine receptors [21]. We found that in the presence of ryanodine, NAADP decreased Ca^{2+} content (40.85 ± 4.47%; $P < 0.001$, $n = 37$) in hepatocytes incubated in the medium with 100 μM EGTA upon maintaining free calcium concentration at 240 nM level (Fig.5). Thus, ryanodine does not prevent the effect of NAADP. We also established that upon application of 100 μM ryanodine to the medium with lower EGTA concentration (50μM) (maintaining the same free calcium concentration) NAADP was more potent to decrease Ca^{2+} store (135.33 ± 3.61%; $P < 0.001$, $n = 36$) compared to the effect of ryanodine. Moreover, in the presence of ryanodine NAADP decreased calcium content (91.68 ± 3.95%; $P < 0.001$, $n = 36$) in hepatocytes incubated in the medium with the lowest free calcium concentration (2.4 nM), whereas the effect of NAADP was not observed in cells with no ryanodine pretreatment.

Thus, the effect of NAADP is pronounced upon cells pre-treatment with the inhibitory concentration of ryanodine. In this case RyRs might be involved in indirect modulation of the NAADP effect via increasing the level of ER luminal calcium.

The effect of NED-19 (18 nM) on the NAADP-mediated Ca^{2+} release in permeabilized hepatocytes. Recent studies demonstrate that two-pore channels (TPC) are located to endosomes and lysosomes and form Ca^{2+} release channels that respond to the activation by NAADP mobilizing Ca^{2+} from acidic stores [5, 24]. Ned-19 itself inhibits both NAADP-mediated Ca^{2+} release and NAADP binding [25]. We found that NED-19 (18 nM) completely blocked NAADP-induced Ca^{2+} release from permeabilized hepatocytes (Fig. 6). Therefore, NAADP releases Ca^{2+} through TPCs in rat hepatocytes. New evidence indicates that TPCs are regulated by NAADP, but are not the NAADP receptors. Walseth and collaborators have postulated that the small fraction of 45 and 40 kDa proteins could be receptors for NAADP [26]. It was proposed that NAADP binding proteins could bind to different ion channels [6]. Perhaps, a target to NAADP is localized at specific membrane contact sites between acidic organelles and the endoplasmic reticulum. New results provide strong

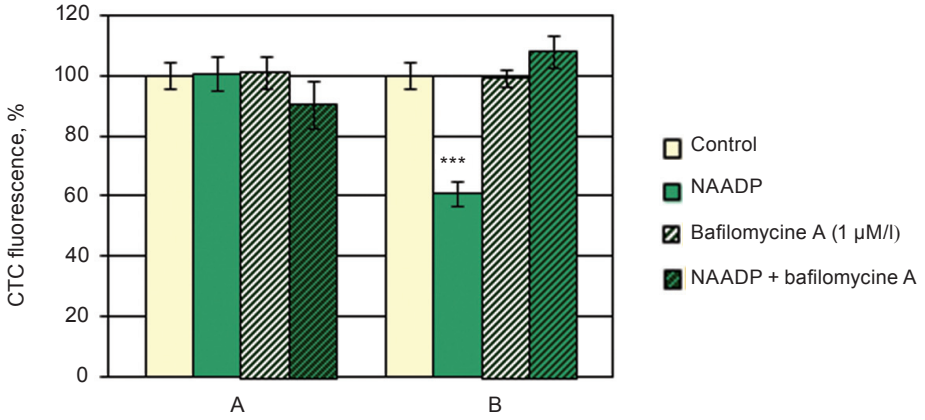

*Fig. 4. Influence of bafilomycin A1 (1 μM) on CTC fluorescence intensity in permeabilized rat hepatocytes in the medium containing high (A) and low (B) EGTA concentration upon physiological level of free calcium: A – $[Ca^{2+}]_{cyt.}$ = 243 nM (EGTA – 100 μM, $CaCl_2$ – 50 μM); B – $[Ca^{2+}]_{cyt.}$ = 240 nM (EGTA – 50 μM, $CaCl_2$ – 25 μM); *** statistically significant difference compare to control $P < 0.001$; $n = 36$*

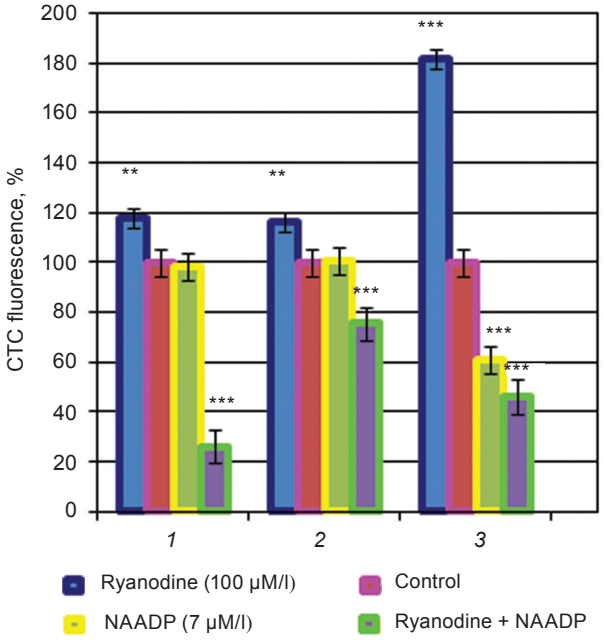

*Fig. 5. Influence of ryanodine and NAADP on CTC fluorescence intensity in permeabilized rat hepatocytes in the medium with different EGTA-Ca^{2+}-buffer: 1 – $[Ca^{2+}]_{cyt.}$ = 2.47 nM (EGTA – 100 μM, $CaCl_2$ – 1 μM); 2 – $[Ca^{2+}]_{cyt.}$ = 243 nM (EGTA – 100 μM, $CaCl_2$ – 50 μM); 3 – $[Ca^{2+}]_{cyt.}$ = 240 nM (EGTA – 50 μM, $CaCl_2$ – 25 μM); *** statistically significant difference compare to control P < 0.001, n = 36; ** statistically significant difference compare to control P < 0.01, n = 36*

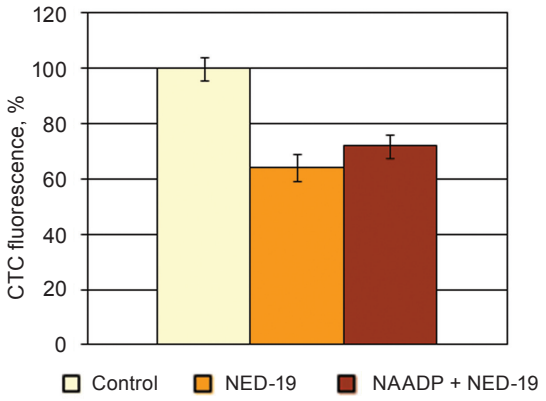

Fig.6. The effect of NED-19 (18 nM) on CTC fluorescence intensity in permeabilized rat hepatocytes in the medium with physiological level of free calcium and low EGTA concentration (EGTA – 50 μM, $CaCl_2$ – 25 μM; $[Ca^{2+}]_{cyt.}$ = 240 nM)

evidence for association between TPC2 and STIM1, as well as Orai1, in human cells only upon depleting of intracellular Ca^{2+} stores [27]. It is possible that this mechanism is responsible for NAADP-evoked calcium release which is related to the level of store depletion.

Thus, we have found that NAADP-evoked changes of store Ca^{2+} in rat hepatocytes are dependent on concentration of EGTA-Ca^{2+}-buffer in cell incubation medium. Additionally, NAADP-sen-

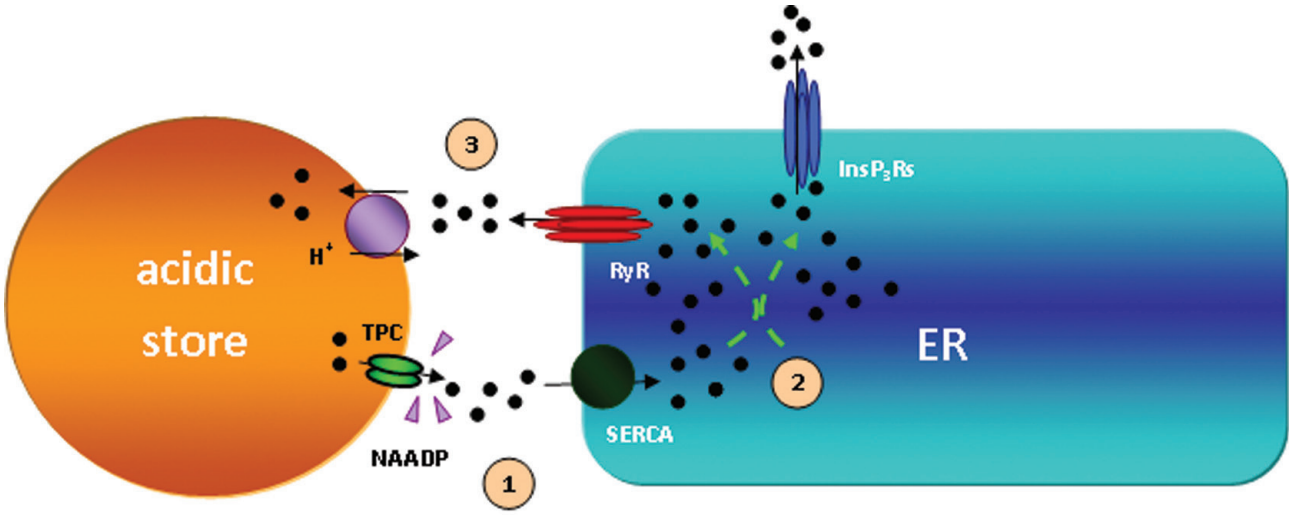

Fig. 7. Schematic representation of proposed Ca^{2+} signaling pathways mediated by NAADP in rat hepatocytes: 1– Ca^{2+} released from acidic store through two-pore channels by NAADP is subsequently sequestered into ER; 2 – Ca2+ overload in the ER triggers Ca^{2+} release via RyRs and/or IP_3Rs; 3 – Ca^{2+} released from the ER is taken back into the acidic store

sitive Ca^{2+} store in rat hepatocytes appears to be sensitive to thapsigargin, nigericin and bafilomicin A. We also observed that the effect of NAADP is dependent on luminal ER calcium since the inhibitory concentration of ryanodine (100 μM) enhanced the effect of NAADP. This was not seen in the presence of ruthenium red (10 μM). Moreover, it is likely that NAADP triggers Ca^{2+} release via TPCs as NED-19 completely abolished changes in store Ca^{2+} level. As a result, NAADP depletes Ca^{2+} from lysosomal type organelles through TPCs. This, in turn, evokes release of Ca^{2+} from ER store that exhibits possible morphological and functional junctions with acidic cellular compartments (Fig. 7). Our results are consistent with a proposed two-pool mechanism where Ca^{2+} released from one compartment may be taken up by the second compartment, which would overload and spontaneous release Ca^{2+} [27].

NAADP-ЧУТЛИВІ Ca^{2+}-ДЕПО У ПЕРМЕАБІЛІЗОВАНИХ ГЕПАТОЦИТАХ ЩУРІВ

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Нікотинацидаденіндинуклеотидфосфат (NAADP) – нуклеотид, здатний вивільнювати Ca^{2+} із внутрішньоклітинних депо різних типів клітин. Показано, що він може впливати на окремий вид кальцієвих депо з кислим вмістом, а саме на ендолізосомальну систему клітин. Проте дія NAADP на ендоплазматичний ретикулум (ЕПР) залишається предметом дискусій. Метою роботи було вивчити вплив NAADP на вміст Ca^{2+} у внутрішньоклітинних депо пермеабілізованих гепатоцитів щурів. Зміни депонованого кальцію реєстрували за інтенсивністю флуоресценції Ca^{2+}-хлортетрациклінового комплексу (ХТЦ). Встановлено, що зменшення депонованого кальцію у клітинах, спричинене NAADP, залежало від наявності ЕГТА-Ca^{2+}-буфера. Статистично вірогідне зменшення вмісту Ca^{2+} спостерігалось у середовищі з низькою концентрацією ЕГТА або без додавання ЕГТА. Показано, що ріанодин (100 мкМ) підсилював, зумовлене NAADP зменшення депонованого Ca^{2+}. Ми припускаємо, що вплив NAADP залежить від люмінального Ca^{2+} в ЕПР. Виявлено, що дія NAADP блокується тапсигаргином, бафіломіцином А1 (1 мкМ) та нігеріцином (7 мкМ), а NED-19 (антагоніст NAADP) пригнічує зміни депонованого Ca^{2+}, що спричинені NAADP.

Ключові слова: гепатоцити, кальцієві депо з кислим вмістом, NAADP, двопорові канали, ріанодинчутливі кальцієві канали.

NAADP-ЧУВСТВИТЕЛЬНЫЕ Ca^{2+}-ДЕПО В ПЕРМЕАБИЛИЗИРОВАННЫХ ГЕПАТОЦИТАХ КРЫС

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Никотинацидадениндинуклеотидфосфат (NAADP) – нуклеотид, освобождающий Ca^{2+} с внутриклеточных депо разных типов клеток. Показано, что он может влиять на отдельный вид кальциевых депо с кислой средой, а именно на эндолизосомальную систему клеток. Однако влияние NAADP на эндоплазматический ретикулум (ЭПР) остается предметом дискуссий. Целью работы было изучить влияние NAADP на содержание Ca^{2+} во внутриклеточных депо пермеабилизированных гепатоцитов крыс. Изменения депонированного кальция регистрировали по интенсивности флуоресценции Ca^{2+}-хлортетрациклинового комплекса (ХТЦ). Установлено, что изменение депонированного Ca^{2+} в исследуемых клетках, вызванное NAADP, зависело от наличия ЭГТА-Ca^{2+}-буфера. Статистически достоверное снижение содержания Ca^{2+} наблюдалось в среде с низкой концентрацией ЭГТА или без добавления ЭГТА. Показано, что рианодин (100 мкМ) усиливал вызванное NAADP снижение Ca^{2+}. Мы предполагали, что влияние NAADP зависит от уровня люминального Ca^{2+} в ЭПР. Показано, что NAADP-индуцированные изменения содержания Ca^{2+} во внутриклеточных депо гепатоцитов крыс зависят от тапсигаргина, бафиломицина А1 (1 мкМ), ни-

герицина (7 мкМ), а NED-19 (антагонист NAADP) угнетает NAADP-вызванные изменения депонированного Ca^{2+}.

Ключевые слова: гепатоциты, кальциевые депо с кислой средой, NAADP, двупоровые каналы, рианодинчувствительные кальциевые каналы.

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