EXPERIMENTAL WORKS

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THE RELATIONSHIP BETWEEN THE IONIZED Ca CONCENTRATION AND MITOCHONDRIAL FUNCTIONS

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The aim of the study was to show the relationships between ionized Ca concentration ($[Ca^{2+}]_m$) in the mitochondria matrix and functional activity of this organelle. $[Ca^{2+}]_m$ was determined using the fluorescent probe Fluo-4, AM. Total level of Ca^{2+} accumulation in mitochondria was monitored using $^{45}Ca^{2+}$ as radioactive tracer. It was shown that incubation of myometrium mitochondria with 3 mM Mg^{2+} resulted in the low level of $[Ca^{2+}]_m$. Subsequent addition of 100 μM Ca^{2+} resulted in 8 times increase of $[Ca^{2+}]_m$ but in low level of total calcium accumulation. Normalized fluorescence of Ca^{2+}-sensitive probe Fluo-4 in response to the Ca^{2+} addition was higher than 2.5. At the same time, $[Ca^{2+}]_m$ was considerably higher in the medium containing 3 mM ATP and 3 mM Mg^{2+}. Subsequent addition of 100 μM Ca^{2+} to the incubation medium resulted in only 2.4 times increase of $[Ca^{2+}]_m$ but considerably higher level of total calcium accumulation was observed. Normalized fluorescence of Fluo-4 in response to the Ca^{2+} addition was lower than 1.3. In liver mitochondria higher rate of oxygen consumption was detected in the presence of an oxidative substrate succinate than of pyruvate or α-ketoglutarate. At the presence of an oxidative substrate succinate normalized fluorescence of Fluo-4 in liver mitochondria in response to the Ca^{2+} addition was lower than 1.3. It was concluded that low level of $[Ca^{2+}]_m$ was correlated with low functional activity of this organelle and, vise versa, high level of $[Ca^{2+}]_m$ was correlated with high functional activity. It was suggested that normalized fluorescence changes in response to the Ca^{2+} addition could be used as a test of the mitochondrial functional activity: lower normalized fluorescence values − higher functional activity.

K e y w o r d s: mitochondria, ionized Ca concentration, total Ca^{2+} accumulation, myometrium, liver.

Mitochondria are known to be “power plants” of cells equally important in cell survival and death [1-3]. Researchers are interested in studying both processes to understand how to save mitochondria and to support life of cell and, vice versa, how to kill mitochondria and, consequently, to delete undesirable cells. Intramitochondrial free calcium ($[Ca^{2+}]_m$) plays an important role in these processes [4-7] and it was suggested that low level of Ca^{2+} in the mitochondrial matrix is needed

Abbreviations: $[Ca^{2+}]_m$, an intramitochondrial free calcium concentration; MCU, the mitochondrial calcium uniporter; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Fluo-4, AM, acetoxymethyl (AM) ester derivative of fluorescent Ca^{2+} indicator; Triton X-100, 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol, non-ionic detergent; TMRM, Tetramethylrhodamine methyl ester perchlorate, sensitive probe for mitochondrial membrane potential; SEM, standard error of the mean.

for the normal functioning of these organelles [5, 8, 9]. For example, Miyata et al. found that the recovery of rat cardiomyocytes from hypoxia depended on the level of $[Ca^{2+}]_m$ at the end of the hypoxic period: cells having $[Ca^{2+}]_m$ greater than about 250-300 nM invariably hypercontracted upon reperfusion [9]. These results were supported by Griffiths et al. who conducted their research on individual cardiomyocytes [5, 8]. So, it was concluded that to decrease the excessive rise of $[Ca^{2+}]_m$ is a main task for maintenance of cell life. The Ca^{2+} concentration in the mitochondria matrix, undoubtedly, plays an important role in functioning of these organelles, but role of "Ca^{2+} overload", which typically happens in the damaged heart during ischemia/reperfusion, has recently been challenged [10, 11]. It was shown that in MCU (the mitochondrial calcium uniporter) null mitochondria – where "Ca^{2+} overload" does not occur during reperfusion – the extent of necrosis was the same as that observed in the hearts from wild type littermates. It was suggested that there is enough Ca^{2+} in the matrix of MCU null mitochondria to allow permeability transition pore opening.

We have shown, that 1) $[Ca^{2+}]_m$ could be changed in the absence of exogenous Ca^{2+}; 2) higher $[Ca^{2+}]_m$ in the absence of exogenous Ca^{2+} was the requirement for higher total Ca^{2+} accumulation (myometrium) and higher rate of oxygen consumption (liver). The suggestion has been made that normalized fluorescence changes in response to the Ca^{2+} addition could be used as the test of the mitochondrial functional activity: lower normalized fluorescence changes – higher functional activity.

Materials and Methods

The treatment of the lab animals was carried out according to "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). A chloroform anesthesia was administered before animals were sacrificed by cervical dislocation and their uteri and livers were promptly removed. Mitochondria from myometrium of non-pregnant rats were isolated using differential centrifugation method [12]. The mitochondria were suspended in a solution with the following composition (mM): sucrose – 250, EGTA – 1, Hepes – 20, and buffered pH 7.4 at 4 °C. Fatty acid free bovine serum albumin (0.1% w/v) was also added. Protein concentration of the mitochondrial fraction was determined by Bradford assay [13]. The concentration of mitochondrial protein in the sample was 25 μg/ml.

Rat liver mitochondria were isolated by the method of differential centrifugation. Liver was removed rapidly and perfused with a solution of the following composition (mM): NaCl − 140, KCl – 4.7, $MgCl_2$ − 1, glucose − 5, Hepes – 10; pH 7.4 to wash out the blood. The obtained preparation was suspended in a solution with the following composition (mM): sucrose – 250, EGTA – 1, Hepes – 10; pH 7.2 at 4 °C. Protein concentration of the mitochondria fraction was determined by Lowry assay [14].

Myometrial cells from non-pregnant rats were isolated according to Mollard et al. [15]. Cell counting was performed using hemocytometer. Cell viability was determined to be higher than 95% using trypan blue method.

Rate of oxygen consumption was determined using polarographic method at 26 °C. 100 μl of mitochondria suspension was added to polarographic chamber that contained the solution of appropriate oxidation substrate. The concentration of protein in the chamber was 5-7 mg/ml. Mitochondrial respiration medium contained (mM): sucrose – 250, K_2HPO_4 – 2, EGTA – 0.1, $CaCl_2$ – 0.1, Hepes – 10; pH 7.2. Pyruvate, α-ketoglutarate and succinate (5 mM) were used as the oxidation substrates. Respiration was stimulated by the addition of ADP (final concentration in the chamber was 200 μM). The rate of respiration was determined in state S_3, S_4 and S_4ATP according to Chance and Williams [16].

Free calcium concentration in the mitochondria ($[Ca^{2+}]_m$) from rat myometrium and liver was determined using the QuantaMaster™ 40 spectrofluorometer (Photon Technology International) and the fluorescent probe Fluo-4, AM (λ_{exc} = 490 nm, λ_{em} = 520 nm).

Myometrial mitochondria were loaded with 2 μM Fluo-4, AM for 30 min at 37 °C in a medium with following composition (mM): sucrose – 250, EGTA – 1, Hepes – 20; pH 7.4. Thereafter, the suspension of mitochondria was diluted (1 : 10) in the same medium containing no fluorescence probe followed by centrifugation. The pellet was resuspended in the same medium containing no fluorescence probe. The ($[Ca^{2+}]_m$) was measured in a medium containing (mM): sucrose – 250, K^+-phospate buffer – 2, sodium succinate – 5, $MgCl_2$ – 3, ±ATP – 3, ±$CaCl_2$ – 0.1, Hepes – 20; pH 7.4.

Liver mitochondria were loaded with 2 μM Fluo-4, AM for 30 min at 37 °C in a medium with following composition (mM): Hepes – 20, sucrose – 250, KH_2PO_4 – 2, ADP – 0.2 (pH 7.4). Thereafter, the suspension of mitochondria was diluted (1 : 10)

by the same medium containing no fluorescence probe followed by centrifugation. The pellet was resuspended in the same medium containing no fluorescence probe. The studies were carried out in a medium containing (mM): Hepes – 20, sucrose – 250, KH_2PO_4 – 2, ADP – 0.2 (pH 7.4), pyruvate – 5 or α-ketoglutarate – 5 or succinate – 5, ± $CaCl_2$ – 0.1.

The calibration of the Fluo-4 fluorescence was performed at the end of the experiments by adding 0.1% Triton X-100 and, in 1 min, 5 mM EGTA (fluorescence intensities F_{max} and F_{min}, respectively). The concentration of ionized Ca in the mitochondria matrix was calculated using the Grynkiewicz equation [17].

Total level of Ca^{2+} accumulation in isolated pig myometrium mitochondria and digitonin-treated rat myometrium cells was monitored using $^{45}Ca^{2+}$ as radioactive tracer. The composition of the standard incubation medium was (mM): KCl – 125, NaCl – 25, ± ATP – 3, $MgCl_2$ – 3, sodium succinate – 3, K^+-phospate buffer – 2, ($^{40}CaCl_2$ + $^{45}CaCl_2$) – 0.01 (0.1 μCi/ml), Hepes – 20; pH 7.4 at 37 °C. The digitonin concentration in the incubation medium was 0.1 mg/ml. This digitonin concentration is known to disrupt the integrity of the plasma membrane, but not to affect the intracellular membrane structures [18]. 100 nM thapsigargin (sarcoplasmic reticulum Ca^{2+} pump inhibitor [19]) was added to the incubation medium to suppress Ca^{2+} accumulation in the sarcoplasmic reticulum of the myometrium cells. Ca^{2+} uptake was terminated by rapid filtration (5-10 sec) of the incubation medium through 0.45 μm Millipore filters. The filters were then washed with isotonic cold stop solution containing 5 mM $CoCl_2$. Radioactivity trapped on the filters was determined on SL-4000 liquid scintillation spectrometer (Intertechnique, France).

Results and Discussion

$[Ca^{2+}]_m$ was determined in isolated rat myometrial and liver mitochondria. Fluo-4 loaded myometrial mitochondria were incubated for 5 min in Mg^{2+}- and Mg^{2+},ATP-containing medium without exogenous Ca^{2+}. It was shown, that $[Ca^{2+}]_m$ in Mg^{2+}-containing medium was 64 ± 6 nM and in Mg^{2+},ATP-containing medium – 185 ± 39 nM (mean ± SEM, n = 8, P < 0.01) (Fig. 1). So, in Ca^{2+} free medium $[Ca^{2+}]_m$ was around 3 times higher in the presence of Mg^{2+} and ATP than with Mg^{2+} only. However, after addition of 100 μM Ca^{2+} $[Ca^{2+}]_m$ increased 8 times in Mg^{2+}-containing (513 ± 64 nM)

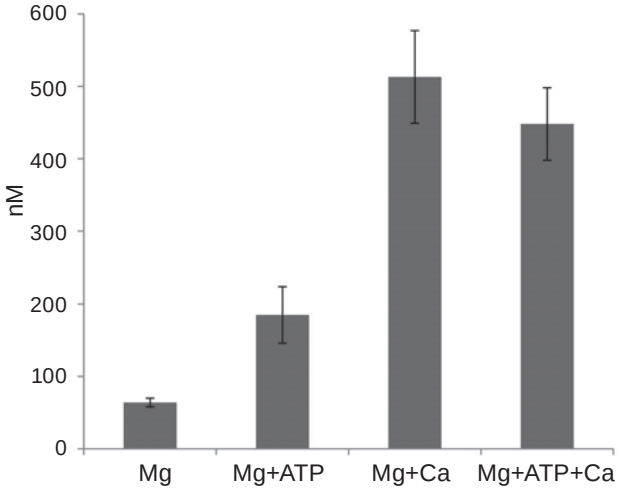

Fig. 1. $[Ca^{2+}]_m$ in myometrium mitochondria (mean ± SEM, n = 8). $[Ca^{2+}]_m$ was determined using the fluorescent probe Fluo-4, AM

and only 2.4 times in Mg^{2+},ATP-containing medium (448 ± 50 nM) (Fig. 1).

The kinetics of $[Ca^{2+}]_m$ changes expressed in Fluo-4 normalized fluorescence units is shown in Fig. 2. These data show that addition of exogenous Ca^{2+} to the myometrial mitochondria incubated in the Mg^{2+},ATP-containing medium caused smaller increase of normalized fluorescence compare to the one in Mg^{2+}-containing medium. It was concluded that the incubation medium composition had a great impact on the normalized fluorescence.

Next we explored the effect of different media on functional activity of mitochondria. It is known that total Ca^{2+} accumulation is the highest in functionally active mitochondria [20] and $^{45}Ca^{2+}$ as radioactive tracer is often used to monitor these values in various cells. Therefore, we determined the total level of Ca^{2+} accumulation in myometrial mitochondria in both incubation media. It was shown that upon addition of 10 μM $^{45}Ca^{2+}$ myometrial mitochondria accumulated 149 ± 18 and 5 ± 2 nmol Ca^{2+}/mg of protein/5 min in Mg^{2+},ATP- and Mg^{2+}-containing medium, respectively (Fig. 3).

These data provide evidence that incubation of mitochondria in 3 mM ATP and 3 mM Mg^{2+}-containing medium resulted in high level of total Ca^{2+} accumulation, i. e., to be functionally active, while in 3 mM Mg^{2+}-containing medium, the level of total Ca^{2+} accumulation is low, meaning low activity of the organelles.

We also used another experimental model – a suspension of myometrial myocytes treated with

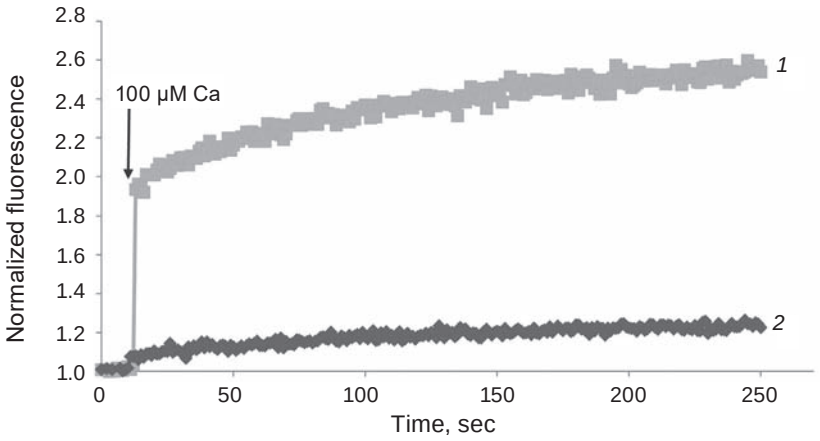

Fig. 2. The kinetic of Ca^{2+} accumulation in the myometrial mitochondria incubated in the Mg^{2+}-containing medium (1) and in the Mg^{2+},ATP-containing medium (2). Results are expressed in Fluo-4 normalized fluorescence units. The results of a typical experiment are presented (n = 8). 100 μM Ca^{2+} additions were made at the times indicated by the arrow

digitonin (0.01%) to study the total level of Ca^{2+} accumulation in mitochondria (using $^{45}Ca^{2+}$ as radioactive tracer). The model provides a more adequate environment, i.e. to study the mitochondria in situ. Ca^{2+} accumulation in mitochondria was tested as such that was not sensitive to thapsigargin (100 nM) and was blocked by ruthenium red (10 μM). The cells permeabilized with digitonin were preincubated for 5 min in Mg^{2+}- and Mg^{2+},ATP-containing media. Then 3 mM ATP was added to the Mg^{2+}-containing medium and the Ca^{2+} transport was started by the addition of 10 μM $^{45}Ca^{2+}$ to both media. It resulted in the Ca accumulation of 459 ± 32 pmol/10^6 cells/5 min in Mg^{2+}-containing medium and 1933 ± 182 pmol/10^6 cells/5 min in Mg^{2+},ATP-containing medium, respectively (Fig. 4). The data suggested that: 1) the level of Ca^{2+} accumulation was higher in the mitochondria that were preincubated in Mg^{2+},ATP-containing medium compared to Mg^{2+}-containing medium; 2) in the case of short time myometrium mitochondria preincubation in the absence of ATP, Ca^{2+} accumulation did not reach the level of one at initial ATP presence in the incubation medium.

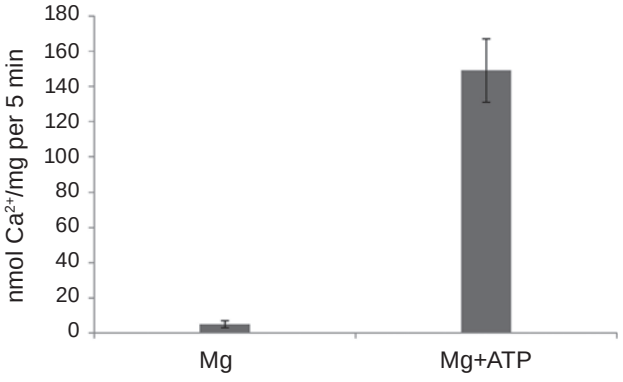

Fig. 3. The total level of Ca^{2+} accumulation in myometrial mitochondria (mean ± SEM, n = 5, P < 0.0001). Experimental model – isolated mitochondria. Total level of Ca^{2+} accumulation in mitochondria was monitored using $^{45}Ca^{2+}$ as radioactive tracer

So, using two experimental models (isolated mitochondria and digitonin-permeabilized myometrial cells) it was shown that the total level of Ca^{2+} accumulation in mitochondria was high in the case of incubation in Mg^{2+},ATP-containing medium and low in Mg^{2+}-containing medium. It was concluded that functional activity of mitochondria was high in Mg^{2+},ATP-containing medium and low in Mg^{2+}-containing medium.

Earlier, using isolated myometrial mitochondria loaded with potential-sensitive probe TMRM, we have shown, that addition of Ca^{2+} to the Mg^{2+}-containing medium induced mitochondrial membrane depolarization [21]. This effect was observed in Mg^{2+} – but not in Mg^{2+},ATP-containing medium. It is known that Ca^{2+} accumulation in the mitochondrial matrix activates Ca^{2+} efflux systems, such as H^+/Ca^{2+} exchanger [20]. It was suggested that activation of the Ca^{2+} efflux through the H^+/Ca^{2+} exchanger led to the elevation of H^+ concentration in the matrix, causing the mitochondrial membrane potential dis-

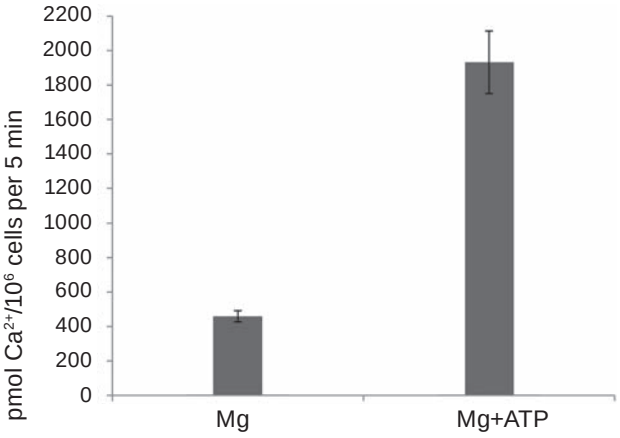

Fig. 4. The total level of Ca^{2+} accumulation in myometrium mitochondria after 5 minute preincubation of cells in Mg^{2+}- and Mg^{2+},ATP-containing medium. Preincubation was followed by 3 mM ATP addition to the Mg^{2+}-containing medium. Ca^{2+} accumulation was started by 10 μM Ca^{2+} addition to both tubes. Time of incubation – 5 min (mean ± SEM, n = 5, P < 0.0001). Experimental model – a suspension of myometrium myocytes treated with digitonin (0.01%). Total level of Ca^{2+} accumulation in mitochondria was monitored using $^{45}Ca^{2+}$ as radioactive tracer

sipation. It is also known that mitochondria can act as ATP consumers [1]. In the case of mitochondrial membrane depolarization F_1F_0 ATP-synthase acts as an ATPase, consuming ATP and pumping protons out across the mitochondrial inner membrane. The mitochondria consume ATP 'in order' to maintain their potential [1]. It was suggested that ATP addition to the incubation medium prevent Ca^{2+}-induced myometrial mitochondria membrane depolarization. Perhaps these results could explain at least one of the reasons of the low level of Ca^{2+} accumulation in mitochondria in Mg^{2+}-containing medium – Ca^{2+}-induced depolarization resulted in inactivation of potential-sensitive Ca^{2+} uniporter, that is the main Ca^{2+}-transporting system in the mitochondria.

Thus, preincubation of myometrial mitochondria in Mg^{2+}-containing medium resulted in low endogenous $[Ca^{2+}]_m$, subsequent addition of 100 μM Ca^{2+} caused a significant increase of free calcium concentration in the mitochondrial matrix but low level of total Ca^{2+} accumulation, so – low functional activity. At the same time, preincubation of myometrial mitochondria in the Mg^{2+},ATP-containing medium resulted in relatively high endogenous $[Ca^{2+}]_m$, subsequent 100 μM Ca^{2+} addition caused relatively low increase of free calcium concentration in the matrix but high level of total Ca^{2+} accumulation meaning high functional activity. Exogenous Ca^{2+} addition to the myometrial mitochondria incubated in the Mg^{2+},ATP-containing medium caused smaller increase of normalized fluorescence compare to the one in Mg^{2+}-containing medium. Thus, we concluded that (1) low endogenous $[Ca^{2+}]_m$ was not correlated with high functional activity; (2) exogenous Ca^{2+} addition resulted in approximately the same level of $[Ca^{2+}]_m$; (3) low level of Fluo-4 normalized fluorescence correlated with high functional activity.

The possibility exist that our conclusions are tissue-specific and not working on others. Therefore, the next series of experiments were conducted on the liver mitochondria. We have tested kinetic of Ca^{2+} accumulation in liver mitochondria that were incubated in medium containing respiratory substrates such as succinate, α-ketoglutarate and pyruvate (5 mM). As shown in Fig. 5 addition of Ca^{2+} caused an increase of Fluo-4 normalized fluorescence with the lowest effect in the presence of succinate and the highest with pyruvate.

Next we studied the effect of the pyruvate, α-ketoglutarate and succinate oxidation on the liver mitochondria respiration rates (Fig. 6).

It was shown that in S_4 state at succinate oxidation rate of oxygen consumption in the liver mitochondria was 17.8 (ng-at. O_2/(mg protein·min)), it is at 36.3% ($P < 0.01$, $n = 4$) higher compared with rate of oxygen consumption in mitochondria at the case of pyruvate oxidation (11.3 ng-at. O_2/(mg protein·min) and 33.0% ($P < 0.01$) above regarding the indicators obtained at α-ketoglutarate oxidation (11.9 ng-at. O_2/(mg protein·min)).

In S_3 state rates of oxygen consumption in the liver mitochondria with succinate, α-ketoglutarate and pyruvate oxidation were 20.4, 14.7 and 16.8 ng-at. O_2/(mg protein·min), respectively. That is, markers of mitochondria respiration in the presence of succinate in the medium were higher on 27.9% ($P < 0.01$) and 17.7% ($P < 0.01$) for the oxidation of pyruvate and α-ketoglutarate, respectively.

After depletion of exogenous ADP equilibrium state S_4^{ATP} was mounted. In this condition the rates of mitochondrial respiration were also higher at the presence of succinate compared to the NAD-dependent substrates – pyruvate and α-ketoglutarate, to 37.5% ($P < 0.01$) and 25.3% ($P < 0.05$) (succinate – 14.9, pyruvate – 9.34, and α-ketoglutarate – 11.1).

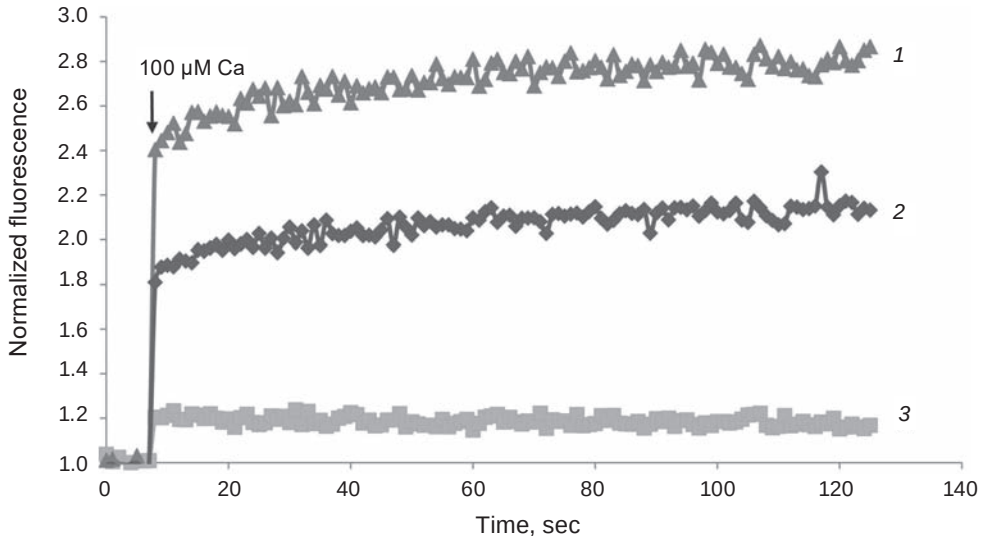

Fig. 5. The kinetic of Ca^{2+} accumulation in liver mitochondria that were incubated in media containing pyruvate (1), α-ketoglutarate (2) and succinate (3) (5 mM). Results are expressed in Fluo-4 normalized fluorescence units. The results of a typical experiment are presented (n = 3). 100 μM Ca^{2+} additions were made at the times indicated by the arrow

We also observed different time of ADP phosphorylation depending on oxidation substrate: succinate – 155 s, pyruvate – 266 s and α-ketoglutarate – 183 s.

Analysis of the data obtained on isolated liver mitochondria suggests that higher rate of oxygen consumption (took place at oxidation of succinate) is accompanied by the lower changes of Fluo-4 normalized fluorescence in response to exogenous Ca^{2+} addition.

It is well documented that a key signaling messenger that is able to transduce life or death signals to mitochondria is intracellular Ca^{2+} [22-24]. It was shown that low level of Ca^{2+} in the mitochondrial matrix provides the normal functioning of these organelles [5, 8, 9]. In this study we have examined the relationship between $[Ca^{2+}]_m$ and mitochondrial function (myometrium and liver). We showed that concentration of ionized Ca in the myometrium and liver mitochondrial matrix depends on incubation medium composition. Specifically the presence or absence of ATP in the incubation medium had a profound effect on rat myometrium $[Ca^{2+}]_m$. For instance, incubation of myometrial mitochondria in Mg^{2+}-containing medium resulted in lower $[Ca^{2+}]_m$ than in Mg^{2+},ATP-containing medium. Ca^{2+} addition

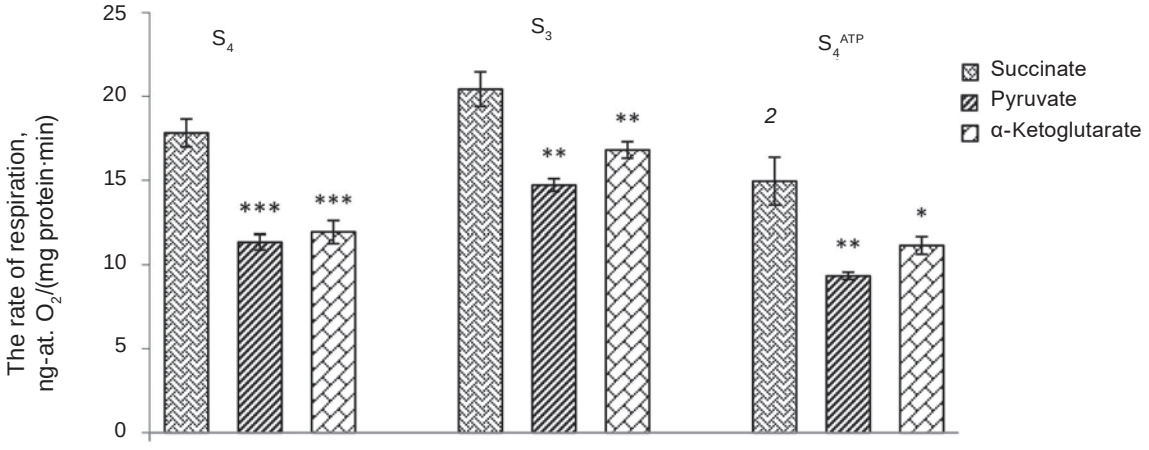

*Fig. 6. The rates of oxygen consumption in the liver mitochondria with succinate, α-ketoglutarate and pyruvate oxidation (mean ± SEM, n = 4, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001)*

to the incubation medium yielded in the same $[Ca^{2+}]_m$ but the different total Ca^{2+} accumulation levels. We also found that oxidation of different substrates in rat liver mitochondria cause changes of $[Ca^{2+}]_m$.

$[Ca^{2+}]_m$ is an important parameter of mitochondria but is not always correlated with the functional activity of this organelle. At the same time it was shown that the lower value of Fluo-4 normalized fluorescence changes in response to the exogenous Ca^{2+} addition was correlated with the higher functional activity of these organelles. Our findings suggest that normalized fluorescence changes in response to the exogenous Ca^{2+} addition can be used as a simple, quantitative test of the mitochondrial functional activity.

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ВЗАЄМОЗВ'ЯЗОК МІЖ КОНЦЕНТРАЦІЄЮ ІОНІЗОВАНОГО Ca І ФУНКЦІОНУВАННЯМ МІТОХОНДРІЙ

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Мета дослідження – показати взаємозв'язок між концентрацією іонізованого Ca ($[Ca^{2+}]_m$) в матриксі мітохондрій та функціональною активністю цих органел. $[Ca^{2+}]_m$ визначали за допомогою флуоресцентного барвника Fluo-4, AM. Загальну акумуляцію Ca^{2+} в мітохондріях визначали за допомогою ізотопного методу з використанням $^{45}Ca^{2+}$. Встановлено, що інкубація мітохондрій міометрія в середовищі, до складу якого входить 3 мМ Mg^{2+}, призводить до низького рівня $[Ca^{2+}]_m$. Подальше додавання 100 мкМ Ca^{2+} супроводжувалось збільшенням концентрації іонізованого Ca в 8 разів, проте, за таких умов, реєстрували низький рівень загальної акумуляції цього катіона. Нормована флуоресценція Ca^{2+}-чутливого зонда Fluo-4 у відповідь на додавання Ca^{2+} була понад 2,5 умовних одиниць. У той же час, $[Ca^{2+}]_m$ була значно вище за інкубації мітохондрій в присутності 3 мМ АТР та 3 мМ Mg^{2+}. Подальше додавання 100 мкМ Ca^{2+} супроводжувалось збільшенням концентрації іонізованого Ca лише у 2,4 раза, проте реєструвався високий рівень загальної акумуляції цього катіона. У присутності 3 мМ АТР та 3 мМ Mg^{2+} нормована флуоресценція Ca^{2+}-чутливого зонда Fluo-4 у відповідь на додавання Ca^{2+} була меншою за 1,3 умовних одиниць. У мітохондріях печінки найвищу швидкість поглинання кисню реєстрували в присутності сукцинату, ніж пірувату або α-кетоглутарату. Зауважимо, що у присутності сукцинату нормована флуоресценція Ca^{2+}-чутливого зонда Fluo-4 в мітохондріях печінки у відповідь на додавання Ca^{2+} була меншою за 1,3 умовних одиниць. Отже, дійшли висновку про те, що низький рівень іонізованого Ca в матриксі мітохондрій корелює з низькою функціональною активністю і, навпаки, високий рівень $[Ca^{2+}]_m$ корелює з високою функціональною активністю. Зроблено припущення, що зміни нормованої флуоресценції Ca^{2+}-чутливого зонда у відповідь на додавання Ca^{2+} можна використовувати як тест на функціональну мітохондрійну активність: менше значення нормованої флуоресценції – більше функціональна активність.

Ключові слова: мітохондрія, концентрація іонізованого Ca, загальна акумуляція Ca^{2+}, міометрій, печінка.

ВЗАИМОСВЯЗЬ МЕЖДУ КОНЦЕНТРАЦИЕЙ ИОНИЗИРОВАННОГО Ca И ФУНКЦИОНИРОВАНИЕМ МИТОХОНДРИЙ

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Цель исследования – показать связь между концентрацией ионизированного Ca ($[Ca^{2+}]_m$) в матриксе митохондрий и функциональной активностью этих органелл. $[Ca^{2+}]_m$ определяли при помощи флуоресцентного зонда Fluo-4, AM. Общую аккумуляцию Ca^{2+} в митохондриях определяли с помощью изотопного метода с использованием $^{45}Ca^{2+}$. Показано, что инкубация митохондрий миометрия в среде, содержащей 3 мМ Mg^{2+}, приводила к низкому уровню $[Ca^{2+}]_m$. Последующее внесение 100 мкМ Ca^{2+} сопровождалось увеличением концентрации ионизированного Ca в 8 раз, однако, в этих условиях, регистрировался низкий уровень общей аккумуляции этого катиона. Нормированная флуоресценция Ca^{2+}-чувствительного зонда Fluo-4 в ответ на внесение 100 мкМ Ca^{2+} была выше 2,5 условных единиц. Если же митохондрии инкубировали в среде, содержащей 3 мМ ATP и 3 мМ Mg^{2+}, концентрация ионизированного Ca была значительно выше (по сравнению со средой, содержащей 3 мМ Mg^{2+}). Последующее внесение 100 мкМ Ca^{2+} сопровождалось увеличением концентрации ионизированного Ca только в 2,4 раза, однако именно в этих условиях регистрировался высокий уровень общей аккумуляции этого катиона. В присутствии 3 мМ ATP и 3 мМ Mg^{2+} нормированная флуоресценция Ca^{2+}-чувствительного зонда Fluo-4 в ответ на внесение 100 мкМ Ca^{2+} не превышала 1,3 условных единиц. В митохондриях печени наибольшую скорость поглощения кислорода регистрировали при наличии в среде инкубации сукцината. Отметим, что в присутствии сукцината нормированная флуоресценция Ca^{2+}-чувствительного зонда Fluo-4 в ответ на внесение Ca^{2+} не превышала 1,3 условных единиц. Сделан вывод о том, что низкий уровень ионизированного Ca в матриксе митохондрий коррелирует с низкой функциональной активностью и, наоборот, высокий $[Ca^{2+}]_m$ коррелирует с высокой функциональной активностью. Высказывается предположение, что изменение нормированной флуоресценции Ca^{2+}-чувствительного зонда в ответ на введение Ca^{2+} можно использовать в качестве теста на функциональную активность митохондрий: меньше значение нормированной флуоресценции – больше функциональная активность и наоборот.

Ключевые слова: митохондрия, концентрация ионизированного Ca, общая аккумуляция Ca^{2+}, миометрий, печень.

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