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KINETIC PARAMETERS OF RESPIRATION IN RAT PERMEABILIZED HEPATOCYTES UPON Ca²⁺ IN VARIOUS CONCENTRATIONS IN MEDIUM AND PROLONGED INFLUENCE OF TAURINE

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The dependence of permeabilized rat hepatocytes respiration rate on oxidation substrates concentration at presence 0.1 and 1 μM Ca²⁺ in the medium and amino sulfonic acid – taurine – effect on this subordination was studied. Experimental animals were injected with taurine (40 mg/kg of weight) for 28 days and control rats were injected with equal volume of water. Liver cells were permeabilized with digitonin (20 μg per 1 million cells). Respiration rate was determined by polarographic method using Clark's electrode, upon either succinate or pyruvate with malate oxidation. Hill equation parameters were calculated applying {v; v/[S]^h} coordinates. The kinetic dependence of respiration upon succinate and pyruvate oxidation is well described by Hill equation in the mediums with studied concentration of Ca²⁺ in control and under taurine action. Hill coefficient h and semi activation constant $K_{0.5}$ for succinate, at rotenone presence, are not changed in control animals after increase of Ca²⁺ concentration from 0.1 to 1 μM. Maximal velocity V_{max} was slightly increased under such conditions. Kinetic parameters of respiration upon succinate oxidation and both Ca²⁺ concentration, are not changed significantly under prolonged taurine injection. There is more essential difference between kinetic parameters of pyruvate-stimulated respiration at various Ca²⁺ concentrations. Thus, in control, V_{max} is 1.5 times lower, and $K_{0.5}$ – 10.7 times major at 0.1 μM Ca²⁺ in comparison with 1 μM Ca²⁺. Hill coefficient becomes less than 1 at both Ca²⁺concentration. As a result of taurine injection, V_{max} is 1.6 times less at low Ca²⁺ and $K_{0.5}$ is higher, but only 6 times. Hill coefficient of this process increases at 0.1 μM Ca²⁺ and is 1.12, upon taurine impact. Substrate inhibition, inherent to the dependence of respiration rate on pyruvate concentration within the medium with 1 μM Ca²⁺, starts developing, under taurine effect, at higher concentration of this substrate in comparison with control (3 mM comparatively to 0.35 mM). Thus, Ca²⁺ concentration rise from 0.1 to 1 μM stimulates the rat hepatocytes mitochondria respiration upon exogenous pyruvate oxidation, but not succinate. Taurine under the prolonged *in vivo* action does not influence succinate-stimulated rotenone-insensitive respiration and intensifies processes of pyruvate-stimulated oxygen consumption in hepatocytes.

Keywords: hepatocytes, mitochondria, respiration, calcium, taurine.

INTRODUCTION

Hepatocytes play a vital role in providing of the entire organism functioning, because they are involved in the processes of endogenous and exogenous compounds detoxication, blood proteins synthesis and secretion, choleresis, glucose and temperature homeostasis maintenance etc.

Calcium cations have the unique properties and universal ability of diverse signals transduction on the cellular level. Increase in Ca^{2+} concentration in the cytosol activates various cell processes in different tissues or organs (including liver). It may cause the ATP lack in the cytosol. Living cells need the balance between producing and using of ATP for homeostasis maintenance. It requires the effective coordination of the cytosolic ATP-dependent processes velocity with the mitochondrial oxidative phosphorylation intensity [20]. It is found out that exactly Ca^{2+} oscillations provide the conformity between the intramitochondrial ATP synthesis and the cell energy demand in hepatocytes [6]. Properly accumulation of Ca^{2+} by mitochondria serves as a signal to intensify ATP producing if necessary, and transduction of this signal is based on activation of the mitochondrial Ca^{2+}-sensitive enzymes: α-ketoglutarate dehydrogenase, isocitrate dehydrogenase and pyruvate dehydrogenase [15].

It is postulated that a change in use of substrates of oxidation by cell modulates the mitochondrial Ca^{2+} accumulation processes. This assumption is based greatly on the experimental data according to which the Ca^{2+}-accumulation process in the mitochondria is maintained during succinate oxidation mainly [22]. Conversely, the succinate oxidation limitation and switching to the predominant NAD-dependent substrates using prevents the excessive Ca^{2+}-accumulation by mitochondria and overloading of their Ca^{2+}-removal systems. It has to provide the stability of the mitochondrial energy-producing function, the tissues ATP and GTP pool restoring, the organs functioning maintenance under conditions of various physiological states. However, the molecular mechanisms and physiological direction of this process are unclear.

Taurine – one of the most widespread amino sulfonic acids in mammals – is also involved in the maintenance of Ca^{2+}-homeostasis and mitochondria functioning [9]. Lobo and co-authors [11], using electron microscopy with immunohistochemical marker, revealed that the highest taurine concentration in the cell is within mitochondria. It was shown that taurine caused the increase in the rate of Ca^{2+} uptake by the hepatocytes mitochondria already at the minimal concentration of 1 mM [17]. However, the peculiarities of various substrates oxidation in mitochondria upon taurine action and at the different Ca^{2+} concentrations are not researched. That is why a part of our work was devoted to this problem.

Thus, maintenance of cell energy supply at the physiological level is a complicated regulated process dependent on qualitative and quantitative system parameters. The majority of papers, concerning the correlation between intracellular signaling (including Ca^{2+}-signaling) and metabolic (mitochondria respiration) pathways, are focused on the qualitative system characteristics. The works dedicated to the quantitative characteristics, in particular to the dependence of kinetic parameters of mitochondria respiration (during different substrates oxidation) on Ca^{2+} concentration are far in minority. This fact has determined the aim of our study.

MATERIALS AND METHODS

Experiments were carried out using male Wistar rats of 180–220 g weighit. All manipulations with animals were conducted according to "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" and the Law of Ukraine "On protection of animals from cruelty". Rats were kept under the stationary vivarium conditions at constant temperature and basic allowance. Experimental animals were injected with taurine solution (40 mg/kg of weight) through a tube intragastrically for 28 days and control rats were injected with water. Animals were narcotized with diethyl ether after that period, then they were decapitated, abdominal dissection was made and a liver was eliminated rapidly. Decapitation procedure was executed in a laboratory from the other rats separately.

Hepatocytes were isolated by two-staged Seglen method [23]. Liver was withdrawn immediately after decapitation and perfused with non-calcium extracellular solution to wash out the blood. The next stage was recirculating perfusion of the liver with collagenase solution for 10–15 minutes. The organ was consistently rinsed with the basic extracellular solution to wash out collagenase after the collagen matrix destroying. All procedures listed above were carried out at 37 °C. The solutions flux velocity was stable. Collagenase was dissolved ex tempore because of its autoproteolysis ability.

Liver was disposed within basic extracellular solution after perfusion and hepatocytes were isolated by gentle pipetting. Suspension was filtrated through the nylon filter to exclude clots of the cells and three times centrifuged at 50 g to remove the metabolites, the intercellular matrix residues and the damaged cells. Hepatocytes were dyed with 0.1 % tripan blue to examine their plasma membrane integrity. The portion of the cells with intact plasmalemma was 84–94 %. Cells were counted using hemocytometer.

A polarographic method was applied to estimate the intact and permeabilized hepatocytes respiration. The diffuse current value was registered using a unit, constructed on a base of Clark's electrode, oxygen monitor YSI 5300, multimeter UT60E, magnetic stirrer for suspension and glass enclosed chamber with volume of 1.6 ml, connected to a thermostat.

Hepatocytes were permeabilized with digitonin for detailed researching of the respiration and oxidative phosphorylation. This procedure was used because transport rate of exogenous substrates through intact plasma membrane is insufficient for their effective involving in the oxidative processes in real time. Isolated cells were centrifuged and extracellular medium was replaced with the one of solutions for permeabilized hepatocytes incubation, containing various Ca^{2+} concentrations. Then digitonin was added (20 µg/ million cells within 1 ml of suspension) and the cells were incubated for 10 min at 37 °C.

Oxygen consumption rate by permeabilized liver cells was registered at 37 °C using succinate (0.1–5 mM) or pyruvate (0.01–5 mM) as substrate of oxidation. Substrates were added into the chamber in increasing concentrations. Respiration was stimulated by ADP (750 µM) before application of substrates. Rotenone (10 µM) was applied to selectively block respiratory chain complex I.

Such reagents were used in experiments: $CaCl_2$, HEPES, BSA, succinate, malate, sodium pyruvate, type IV collagenase – Sigma-Aldrich (USA); NaCl, KCl, KH_2PO_4, $MgCl_2$, glucose, Tris – Alpharus (Ukraine); digitonin – Fisher scientific (USA); EGTA – Acros Organics (Belgium).

Basic extracellular solution was of such composition, mM: NaCl – 140.0, KCl – 4.7, $CaCl_2$ – 1.3, $MgCl_2$ – 1.0, HEPES – 10.0, glucose – 5.0; pH 7.4. Composition of the solu-

tion was modified in some cases: $CaCl_2$ was not added, to create nominally non-calcium medium; collagenase (120 u/ml) and $CaCl_2$ (3 mM) were admixed.

Intracellular solution was of such composition, mM: KCl – 90.0, NaCl – 15.0, $MgCl_2$ – 1.0, KH_2PO_4 – 2, EGTA – 0.5, HEPES –10.0; pH 7.2. Composition of the solution basically meets the ion composition of the hepatocytes intracellular medium [7]. Media with 0.1 and 1 μM Ca^{2+} concentration were made on the basis of the previous one, using Ca^{2+}-EGTA buffers. Those Ca^{2+} concentrations are chosen, since our previous studies (unpublished data) show, that the most significant differences in mitochondria functional ability to response the substrates, ADP and DNP influence appear upon the transition from 0.1 to 1 μM Ca^{2+} within the medium. These means are close to cytosolic Ca^{2+} content under conditions of resting (0.1 μM Ca^{2+}) and activated (1 μM Ca^{2+}) states of the liver cells [1], in particular, physiological agonist (vasopressin) causes the rise of Ca^{2+} concentration from 0.2 (approximately) to 1 μM [21]. Unbounded Ca^{2+} concentration was calculated by the Ca/Mg/ATP/EGTA Calculator v1 programme (http://maxchelator.stanford.edu).

Statistical analyses were performed with computer application, via *Microsoft Office Excel* package. Coefficient of the difference adequacy between two statistical groups was determined using the Student's test [4]. Kinetic parameters of Hill equation were calculated within coordinates {v; v/[S]^h} (modified Eadie-Hofstee coordinates) using a method of index *h* iteration. The best accordance of the achieved dependence to the experimental data was assessed by linear regression [14]. The approximation authenticity was determined by applying F-statistics. The approximation coefficient was considered to be authentic if $P \leq 0.05$.

RESULTS AND DISCUSSION

Dependence of succinate-stimulated respiration rate on calcium concentration in the medium. Kinetic analysis of the succinate oxidation in hepatocytes mitochondria was carried out upon low temperatures [2], hypoxia [12], immobilization stress [22], and upon the various physiological states [3]. Brown has revealed that the substrate oxidation kinetics is modified during hibernation and it provides the metabolism regulation by mitochondria [3]. Priority use of succinate compared to the other substrates was noted in most works [2, 3, 18, 19]. Its oxidation rate is much higher in comparison with other substrates of tricarboxylic acid cycle and the power of transmembrane potential generation is so large that supplies the maximal ADP phosphorylation intensity. Succinate is usually applied at saturating concentrations in the researches, but making clear of its oxidation kinetics with different substrate concentrations gives an opportunity to reveal the interesting regularities. In particular, in experiments with hibernating animals, higher respiration rate indexes were received upon lower succinate concentration than in condition of saturating succinate content [3]. It is considered to maintenance of provide energy processes intensity at sufficient level during hibernation, preserve cell and mitochondria against damage and give a chance to turn into active state quickly.

Although change in cytosolic Ca^{2+} concentration is an important instrument of mitochondria respiration processes regulation [8, 24], that does not occurred succinate oxidation. Such conclusion is a result of fact that succinate dehydrogenase is not Ca^{2+}-dependent enzyme [18]. That is why, the first task in the approbation of a new protocol – study *in situ* of mitochondria respiration kinetics, was to check a dependence of the parameters of that kinetics upon succinate oxidation and Ca^{2+} concentration.

As a result of studying the permeabilized hepatocytes oxygen consumption in media containing various Ca^{2+} concentrations, we have revealed that: kinetics of the respiration processes upon exogenous succinate oxidation in those cells may be well described by Hill equation. Moreover, such kinetic parameters as Hill coefficient h and maximal velocity V_{max}, are not modified with Ca^{2+} concentration in the medium from 0.1 to 1 μM increasing. While $K_{0.5}$ for succinate is 2.1 times less at Ca^{2+} concentration 1 μM (1.05 ± 0.06 mM) compared to 0.1 μM Ca^{2+} (2.25 ± 0.16 mM, n = 4; Fig. 1). Such increase of affinity to substrate shows the existence of some dependence between oxidative processes velocity in hepatocytes mitochondria upon succinate oxidation and cytosolic Ca^{2+} concentration. However there is a probability of activation by Ca^{2+} cations (either directly or indirectly) of the other TCA cycle enzymes: α-ketoglutarate dehydrogenase, isocitrate dehydrogenase and pyruvate dehydrogenase [15], under such experimental conditions. Elimination of effect of these enzymes is necessary for study of kinetic dependence. It can be achieved, for example, by inhibiting the respiratory chain components which are not involved in succinate oxidation. This is the reason to add rotenone – an inhibitor of the respiratory chain complex I [5] – during the later investigation of succinate oxidation kinetic parameters. The endogenous respiration rate of the control cells is not changed by rotenone within medium, containing 0.1 μM of Ca^{2+} (0.09 ± 0.03 – before and 0.07 ± 0.02 nmol O_2 / (s × million cells) after rotenone applying). From other hand, rotenone lowers respiration rate in medium with higher Ca^{2+} concentration 1 μM to 0.09 ± 0.02 nmol O_2 / (s × million cells), i.e. by 33 ± 9 % ($n = 5$, $P ≤ 0.05$). Thus, Ca^{2+} concentration increase in non-rotenone experiments causes a repartition of the substrates oxidation, in fact, the limitation of succinate use in the isolated hepatocytes. Probably, it could be a mechanism of the mitochondria protection against Ca^{2+} overload and it is showed in Saakyan's study [22].

Succinate increases the hepatocytes oxygen consumption velocity in dose dependent manner, under conditions of inhibition of the respiratory chain complex I. Intensification of oxygen consumption by liver cells at 0.1–3 mM succinate is ascertained. This process is described by the Hill equation quite strictly. $K_{0.5}$ and h are not dependent on Ca^{2+} concentration in the medium, and form 0.47–0.49 mM and 0.91, respectively. V_{max} is characterised by dependence on Ca^{2+} concentration: it is 0.98 and 1.15 nmol O_2 / (s × million cells) at 0.1 and 1 μM, respectively. In both cases, some respiration rate decreasing is observed at 5 mM succinate. It is a result of substrate inhibition, obviously (see Fig. 1).

Received kinetic parameters of hepatocytes mitochondria respiration during succinate oxidation are somewhat different from ones in the pancreas cells. In particular, Hill coefficient h of permeabilized pancreas cells succinate-stimulated respiration is significantly higher, than 1 (1.43 and 1.40 at 0.1 and 1 μM Ca^{2+} [13]), but it is also practically not dependent on Ca^{2+} content in the medium. Such a difference is caused, probably, by the development of the substrate inhibition of succinate-stimulated respiration in hepatocytes (that is why the virtual Hill coefficient becomes quite lower, than 1), but not in pancreas cells. The existence of such "negative cooperation" during succinate oxidation, as well as the substrate inhibition, indicates the presence of a complex system of the feedbacks directed at oxidative processes that decrease upon the high rate of the mitochondria membrane potential.

$K_{0.5}$ amount of hepatocytes succinate-stimulated respiration though has revealed two times less than in pancreas cells [13], but is also quite high and does not depend on

Ca^{2+} concentration in the medium. Obviously, it is caused by low affinity of either succinate dehydrogenase or mitochondrial transporters to the exogenous succinate in hepatocytes (as well as in pancreas cells [13]). Ca^{2+} cations in researched concentrations do not have significant influence on the metabolic flux through succinate oxidation system consisting of the units for succinate transporting, succinate dehydrogenase, and respiratory chain complexes III and IV.
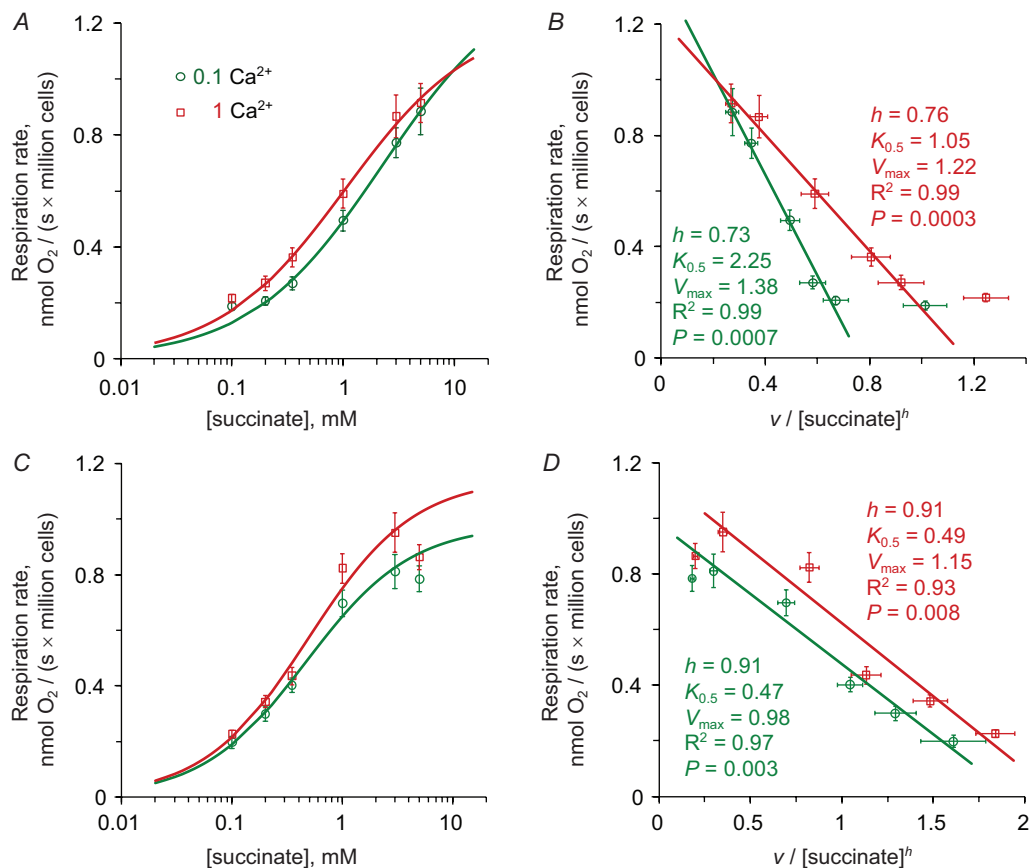

Fig. 1. Kinetic parameters of the permeabilized hepatocytes respiration in control group rats upon the succinate oxidation in the mediums containing 0.1 and 1 μM Ca^{2+}: *A* and *C* – the concentration dependence of succinate influence within semilogarithmic coordinates at rotenone absence (*A*) and presence (*C*); *B* and *D* – linearization within modified Eadie–Hofstee coordinates, at rotenone absence (*B*) and presence (D), the designations are the same as at a graph *A*, during calculations of the kinetic parameters of oxygen consumption velocity dependence on succinate concentration, the respiration rate index after ADP adding was subtracted; [rotenone] = 10 μM, [succinate] = 0.1–5 mM, n=4

Рис. 1. Кінетичні параметри дихання пермеабілізованих гепатоцитів контрольної групи щурів за окиснення сукцинату в середовищах з 0,1 і 1 мкМ Ca^{2+}: *A* і *C* – концентраційна залежність впливу сукцинату в напівлогарифмічних координатах без ротенону (*A*) та з ротеноном (*C*); *B* і *D* – лінеаризація у змінених координатах Іді–Хофсті , без ротенону (*B*) та за наявності ротенону (*D*), позначення, як на графіку *A*; під час розрахунків кінетичних параметрів залежності швидкості споживання кисню від концентрації сукцинату віднімали показник швидкості дихання, який був після додавання АДФ; [ротенон] = 10 мкМ, [сукцинат] = 0,1–5 мМ, n = 4

Finally V_{max} index of hepatocytes succinate-stimulated respiration is two times higher than this one, registered in pancreas cells upon the same experimental conditions [13]. It is taking into consideration the increased oxidative ability of the liver tissue.

Pyruvate-stimulated respiration rate dependence on calcium concentration in medium. Cell energy supply processes have a critical significance in adaptation of various living tissues to the different loads. Oxidation substrates with Ca^{2+}-regulated use, belong to the factors, providing maximal adaptation accordingly to the demands. Ponsot and coauthors [19] have carried out studies using various rat muscles (gastrocnemius, soleus and heart muscle) with estimation of ADP-stimulated respiration upon escalating pyruvate, pyruvate with malate, glutamate with malate, palmitoyl-carnitine concentrations. They made a conclusion about qualitative differences of mitochondria metabolic pathways dependently on the cells functions. In particular, mitochondria of glycolytic muscle fibers are adapted to the maintenance of the necessary oxidation-reduction state of these cells, and heart mitochondria have developed an important ability to use the fatty acids. The key factor of the mitochondrial energy supply fitting to the intact neurons demand is also cytosolic Ca^{2+} [8]. Ca^{2+} accumulation by mitochondria proceeds at its high concentrations (> 600 nM) and it increases the level of the intramitochondrial dehydrogenases activity. Ca^{2+}-induced escalation of oxygen consumption intensity in neurons mitochondria at state S_3 diminishes, dependently on oxidation substrate, in a such sequence: glutamate > α-ketoglutarate > α-glycerophosphate > pyruvate [8].

Adding of the exogenous pyruvate on the malate background (0.01–5 mM) leads to the dose dependent increasing of oxygen consumption velocity as at 0.1, so at 1 μM Ca^{2+} in the medium. $K_{0.5}$ is much less, than for succinate, in both cases. It is no wonder, because not only the endogenous pyruvate, but also that one, which arrives as lactate with the blood from glycolytic tissues, proceeds oxidation in liver [10]. Simultaneously, the V_{max} amount of pyruvate-stimulated respiration is significantly less.

There is a significant difference between the kinetic parameters of pyruvate oxidation processes at various Ca^{2+} concentrations. Thus, $K_{0.5}$ is 0.16 ± 0.01 mM at 0.1 μM Ca^{2+} and 0.015 ± 0.001 mM at 1 μM Ca^{2+}, i.e. difference amounts 10.7 times. And V_{max} is 1.5 times larger at the higher Ca^{2+} content, in comparison with Ca^{2+} concentration of 0.1 μM (0.4 ± 0.008 and 0.27 ± 0.01 nmol O_2 / (s × million cells) respectively, n = 4). It indicates the greater system affinity to pyruvate and more intensive its oxidation at higher Ca^{2+} concentration in permeabilized hepatocytes. It is harmonized with a fact that, in particular, pyruvate dehydrogenase is one of Ca^{2+}-sensitive mitochondrial enzymes, established using only isolated organelles. Similar variations of $K_{0.5}$ and V_{max} of pyruvate-stimulated respiration, though at some different range of Ca^{2+} concentration (0.1 and 0.5 μM), are inherent for pancreacytes also [13].

It was cleared up that the intensity increasing of oxygen consumption by hepatocytes at Ca^{2+} concentration 1 μM proceeds upon only in the range from 0.01 to 0.2 mM pyruvate application. The respiration rate indices begin to diminish at the later rise of substrate content (0.35–5 mM), i.e. substrate inhibition develops. This fact can be explained by the existence of homeostatic mechanisms of the reverse negative impact which decrease oxygen consumption upon reaching the mitochondria maximal oxidative activity (that proceeds at saturating substrate concentrations), and, thus, initiate the inhibition process of the later membrane potential increasing in these organelles. Hill

coefficient partly evidence of the previous hypothesis, which is less is than 1 at both Ca^{2+} concentration in medium. It indicates the existence of the negative feedbacks within a system.

Such well expressed substrate inhibition of pyruvate-stimulated respiration (and succinate-stimulated as well) is not inherent for pancreacytes in the investigated substrate concentrations diapason [13]. It is due, quite possibly, the greater oxidative ability of liver tissue and, thus, the presence of specific protection systems.
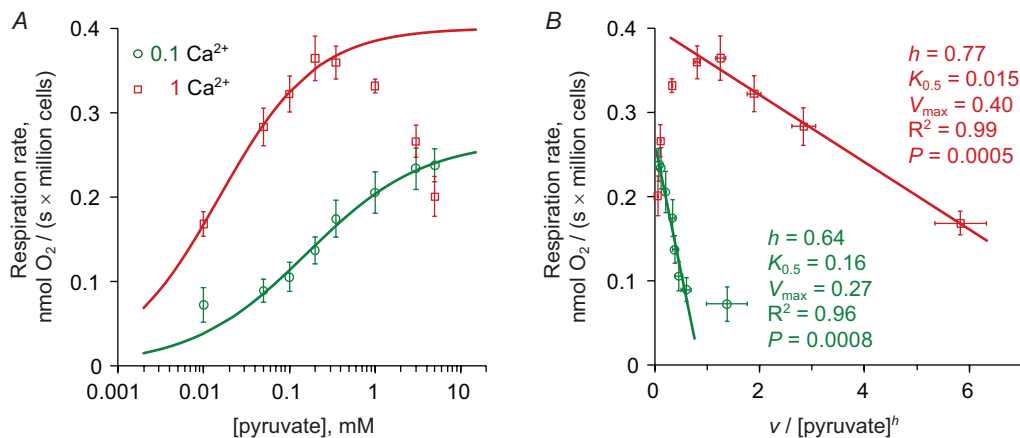

Fig. 2. Kinetic dependence of the respiration rate of permeabilized liver cells in control group animals on pyruvate concentration upon malate presence within the media containing 0.1 and 1 μM Ca^{2+}: *A* – the dependence graph of oxygen consumption velocity on pyruvate concentration, built within semilogarithmic coordinates; *B* – linearization within modified Eadie–Hofstee coordinates, designations are the same as at a graph *A*; the indexes of ADP-stimulated oxygen consumption velocity were subtracted; [malate] = 1 mM, [pyruvate] = 0.01–5 mM, n = 4

Рис. 2. Кінетична залежність швидкості дихання пермеабілізованих клітин печінки контрольної групи тварин від концентрації пірувату за наявності малату в середовищах з [Ca^{2+}] 0,1 і 1 мкМ: *A* – графік залежності швидкості споживання кисню від концентрації пірувату, побудований у напівлогарифмічних координатах; *B* – лінеаризація у змінених координатах Іді–Хофсті, позначення, як на графіку *A*; в обох випадках показники швидкості споживання кисню, стимульованого АДФ, віднімали; [малат] = 1 мМ, [піруват] = 0,01–5 мМ, n = 4

Respiration rate dependence on calcium concentration hin the medium in the permeabilized hepatocytes of rats injected with taurine. Endogenous metabolites, such as taurine, are also involved in energy and Ca^{2+}-metabolism regulation. Taurine *in vitro* is known to enhance Ca^{2+}-accumulation by the isolated mitochondria of rat liver [17]. It is accompanied with the mitochondrial respiration rate increasing upon succinate oxidation [16].

We have investigated the taurine *in vivo* influence on oxygen consumption by isolated hepatocytes dependently on Ca^{2+} and oxidation substrates concentration. Prolonged taurine injection to the rats does not modify the endogenous respiration intensity in permeabilized hepatocytes of these animals. Oxygen consumption velocity in liver cells decreases as consequence of rotenone adding to the polarographic chamber upon the endogenous substrates oxidation. Such reduction occurs in the medium containing either 0.1 or 1 μM Ca^{2+} – for 47 ± 8 % (n = 4, P ≤ 0.05) and 26 ± 7 % (n = 4, P ≤ 0.01) respectively. Since rotenone does not modify the endogenous respiration rate at 0.1 μM

Ca^{2+} in control group, taurine, nevertheless, enhances inflow of NAD-dependent endogenous substrates. Taurine effect is smoothed over at higher Ca^{2+} concentration. It corroborates the assumption that its impact is realized due to the intensification of Ca^{2+} accumulation within mitochondria.

At rotenone presence V_{max} index of succcinate-stimulated respiration increases upon taurine influence, as well as in control animals, with Ca^{2+} concentration rise (from 0.83 ± 0.03 (at 0.1 μM Ca^{2+}) to 0.91 ± 0.06 (at 1 μM Ca^{2+}) nmol O_2 / (s × million cells). Whereas $K_{0.5}$ conversely diminishes upon these conditions (from 0.52 to 0.41 mM), unlike the control. This reduction is nonessential and can be neglected. At the same time, the Hill coefficient h stays unchangeable in control rats also. The V_{max} amounts themselves after prolonged taurine injection are lower, in comparison with control, at both Ca^{2+} concentrations. Development of "substrate inhibition" is also observed at the highest succinate concentration (5 mM) in animals receiving taurine (Fig. 3). No significant changes in hepatocytes respiration kinetics upon succinate oxidation after prolonged taurine injection were registered, as well as a dependence of this kinetics on Ca^{2+} concentration.
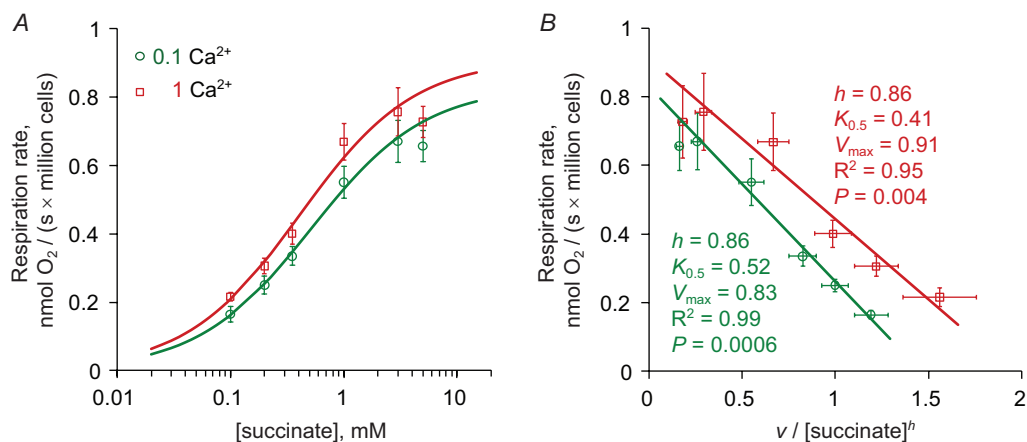

Fig. 3. Kinetic characteristics of oxygen consumption velocity in the permeabilized hepatocytes of rats receiving taurine, upon succinate oxidation at rotenone background, in media containing 0.1 and 1 μM Ca^{2+}: *A* – concentration dependence of succinate influence within semilogarithmic coordinates; *B* – linearization within modified Eadie–Hofstee coordinates, designations are the same as at a graph *A*; the indexes of ADP-stimulated respiration velocity were subtracted; [rotenone] = 10 μM, [succinate] = 0.1–5 mM, n = 4

Рис. 3. Кінетичні характеристики швидкості споживання кисню пермеабілізованими гепатоцитами щурів, яким вводили таурин, за окиснення сукцинату на тлі ротенону в середовищах з [Ca^{2+}] 0,1 і 1 мкМ: *А* – концентраційна залежність впливу сукцинату в напівлогарифмічних координатах; *В* – лінеаризація у змінених координатах Іді–Хофсті, позначення, як на графіку *А*; показники швидкості дихання, стимульованого АДФ, віднімали; [ротенон] = 10 мкМ, [сукцинат] = 0,1–5 мМ, n = 4

V_{max} index of pyruvate-stimulated respiration in rats, receiving taurine for a long time, 1.6 times increases with Ca^{2+} concentration rise in the medium from 0.1 to 1 μM (Fig. 4), i.e. practically the same as in control animals (see Fig. 2). $K_{0.5}$, conversely, diminishes 6 times – essentially less than in control – first of all due to its reduction already at 0.1 μM Ca^{2+} in the medium under taurine effect. Virtual Hill coefficient is significantly

higher at 0.1 μM Ca^{2+} in experimental animals in comparison with control ones, and has a value of 1.12. That is negative cooperativity, inherent to the respiration rate dependence on pyruvate concentration in control, disappears or even is replaced with the positive cooperativity upon a prolonged taurine effect. Ca^{2+} concentration increase within mitochondria matrix underlies such transformation, because this transformation is eliminated at 1 μM Ca^{2+} in the medium. Generally, the appearance of the respiration rate positive cooperativity with pyruvate concentration and some decrease of $K_{0.5}$ at 0.1 μM Ca^{2+} in the medium have to be interpreted as an evidence of pyruvate oxidation intensification upon prolonged taurine effect. It occurs despite the calculated V_{max} amounts are lower in experimental animals at both Ca^{2+} concentrations in the medium.
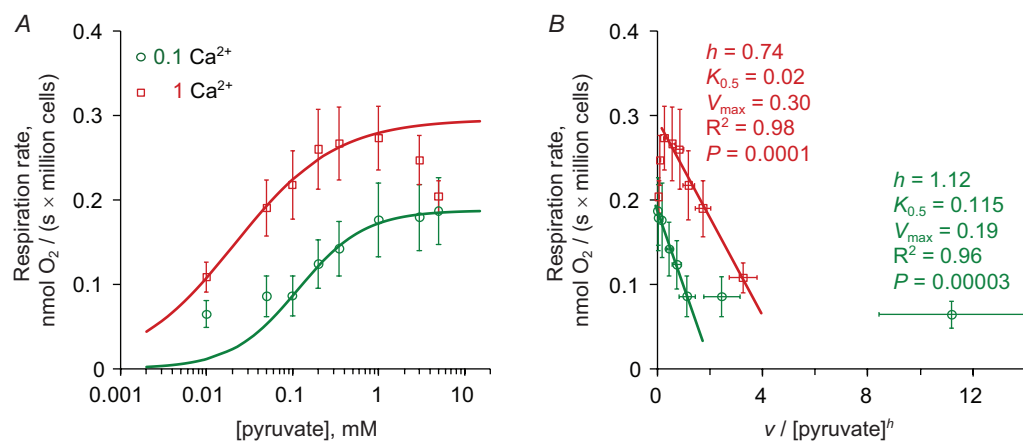

Fig. 4. Kinetic parameters of oxygen consumption velocity in the permeabilized hepatocytes of rats receiving taurine, upon pyruvate oxidation and malate presence within the mediums containing 0.1 and 1 μM Ca^{2+}: *A* – concentration dependence of pyruvate influence, represented within semilogarithmic coordinates; *B* – linearization within modified Eadie–Hofstee coordinates, designations are the same as at a graph *A*; the indexes of ADP-stimulated oxygen consumption velocity were subtracted; [malate] = 1 mM, [pyruvate] = 0.01–5 mM, n = 4

Рис. 4. Кінетичні параметри швидкості споживання кисню пермеабілізованими гепатоцитами за окиснення пірувату й наявності малату в середовищах з [Ca^{2+}] 0,1 і 1 мкМ у щурів, яким вводили таурин: *A* – концентраційна залежність впливу пірувату, представлена у напівлогарифмічних координатах; *B* – лінеаризація у змінених координатах Іді–Хофсті, позначення, як на графіку *A*; показники швидкості споживання кисню, стимульованого АДФ, віднімали; [малат] = 1 мМ, [піру-ват] = 0,01–5 мМ, n = 4

Besides, substrate inhibition inherent to the respiration rate dependence on pyruvate concentration at 1 μM Ca^{2+} in the medium, starts to develop at one fold higher than in control, this substrate concentration (3 mM in comparison with 0.35 mM), under a prolonged taurine effect. Such elimination of substrate inhibition by taurine is an evidence of pyruvate oxidation intensification.

It has to be noted, that dispersion (deviations range) of pyruvate-stimulated respiration rate (not succinate-stimulated) is larger upon taurine influence, in comparison with control rats. Two experimental points, characterizing the respiration rate upon the lowest pyruvate concentration (0.01 and 0.05 mM) at Ca^{2+} concentration 0.1 μM, are not situated on a curve computed with use of Hill equation. There is only one such point in control – 0.01 mM pyruvate. This "basic" pyruvate concentration dislocation at 0.1 μM Ca^{2+}

to the right caused by prolonged injection of taurine, is similar to substrate inhibition displacement at 1 μM Ca^{2+}. The curves reflecting pyruvate oxidation kinetics upon taurine effect come closer to each other at both Ca^{2+} concentrations (it is well observed within semilogarithmic coordinates). It also indicates indirectly a change of Ca^{2+}-dependent component of pyruvate-stimulated oxygen consumption in hepatocytes.

CONCLUSION

A prolonged *in vivo* effect of taurine, does not influence the succinate-stimulated rotenone-insensitive respiration and intensifies the processes of pyruvate-stimulated oxygen consumption in rat hepatocytes. A change in the parameters of Ca^{2+}-regulation of mitochondrial respiration underlies such intensification. It causes a reduction in $K_{0.5}$ and appearance of positive cooperativity in pyruvate oxidation at 0.1 μM Ca^{2+} in the medium and substrate inhibition decrease at 1 μM Ca^{2+}.

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КІНЕТИЧНІ ПАРАМЕТРИ ДИХАННЯ ПЕРМЕАБІЛІЗОВАНИХ ГЕПАТОЦИТІВ ЩУРІВ ЗА РІЗНОЇ КОНЦЕНТРАЦІЇ Ca²⁺ У СЕРЕДОВИЩІ ТА ТРИВАЛОГО ВПЛИВУ ТАУРИНУ

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Досліджено залежність швидкості дихання пермеабілізованих гепатоцитів щурів від концентрації субстратів окиснення за 0,1 і 1 мкМ Ca^{2+} у середовищі, а також вплив аміносульфокислоти таурину на цю залежність. Дослідним тваринам упродовж 28 днів вводили таурин у дозі 40 мг / кг маси, а контрольним – воду. Клітини печінки пермеабілізували дигітоніном (20 мкг на 1 млн клітин). Швидкість дихання визначали полярографічно з використанням електрода Кларка за окиснення сукцинату або пірувату за наявності малату. Параметри рівняння Хілла розраховували, використовуючи координати {v; v/[S]^h}. У середовищах з досліджуваними [Ca^{2+}] кінетична залежність дихання за окиснення сукцинату і пірувату добре описується рівнянням Хілла як у контролі, так і за дії таурину. Коефіцієнт Хілла h і константа напівактивації $K_{0,5}$ для сукцинату за наявності ротенону у контрольних тварин не змінюються зі збільшенням [Ca^{2+}] від 0,1 до 1 мкМ, а максимальна швидкість V_{max} трохи зростає. Унаслідок тривалого введення таурину кінетичні параметри дихання за окиснення сукцинату і обох [Ca^{2+}] суттєво не змінюються. Між кінетичними

показниками піруватстимульованого дихання за різних [Ca²⁺] є значно суттєвіша різниця. Так, у контролі за 0,1 мкМ Ca²⁺ V_{max} є в 1,5 разу меншою, а $K_{0,5}$ – у 10,7 разу більшою, ніж за 1 мкМ Ca²⁺. Коефіцієнт Хілла за обох [Ca²⁺] є меншим 1. Унаслідок введення тваринам таурину V_{max} за низької концентрації Ca²⁺ є меншою, ніж за високої в 1,6 разу, $K_{0,5}$ – більшим, але тільки у 6 разів. За 1 мкМ Ca²⁺ коефіцієнт Хілла h цього процесу внаслідок впливу таурину збільшується і становить 1,12. Субстратне інгібування, яке притаманне залежності швидкості дихання від концентрації пірувату за 1 мкМ Ca²⁺ у середовищі внаслідок тривалої дії таурину починає розвиватися за вищої, ніж у контролі, концентрації цього субстрату (3 мМ порівняно з 0,35 мМ). Отже, підвищення концентрації Ca²⁺ від 0,1 до 1 мкМ стимулює дихання мітохондрій гепатоцитів щурів за окиснення екзогенного пірувату, але не сукцинату. Таурин за тривалої дії *in vivo* практично не впливає на сукцинатстимульоване ротеноннечутливе дихання й інтенсифікує процеси піруватстимульованого дихання гепатоцитів.

Ключові слова: гепатоцити, мітохондрії, дихання, кальцій, таурин.

КИНЕТИЧЕСКИЕ ПАРАМЕТРЫ ДЫХАНИЯ ПЕРМЕАБИЛИЗИРОВАННЫХ ГЕПАТОЦИТОВ КРЫС ПРИ РАЗНОЙ КОНЦЕНТРАЦИИ Ca²⁺ В СРЕДЕ И ДЛИТЕЛЬНОМ ВЛИЯНИИ ТАУРИНА

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Исследована зависимость скорости дыхания пермеабилизированных гепатоцитов крыс от концентрации субстратов окисления при 0,1 и 1 мкМ Ca²⁺ в среде, а также влияние аминосульфокислоты таурина на эту зависимость. Подопытным животным в течение 28 дней вводили таурин в дозе 40 мг / кг массы, а контрольным – воду. Клетки печени пермеабилизовали дигитонином (20 мкг на 1 млн клеток). Скорость дыхания определяли полярографически с использованием электрода Кларка при окислении сукцината или пирувата и при наличии малата. Параметры уравнения Хилла рассчитывали, используя координаты {v; v/[S]^h}. В средах с исследуемыми концентрациями Ca²⁺ кинетическая зависимость при окислении сукцината и пирувата хорошо описывается уравнением Хилла как в контроле, так и при воздействии таурина. Коэффициент Хилла h и константа полуактивации $K_{0,5}$ для сукцината при наличии ротенона у контрольных животных не изменяются с увеличением концентрации Ca²⁺ от 0,1 до 1 мкМ, а максимальная скорость V_{max} несколько возрастает. Вследствие продолжительного введения таурина кинетические параметры дыхания при окислении сукцината и обеих концентраций Ca²⁺ существенно не изменяются. Между кинетическими показателями пируватстимулированного дыхания при разных концентрациях Ca²⁺ существует более значительная разница. Так, в контроле при 0,1 мкМ Ca²⁺ V_{max} в 1,5 раза меньше, а $K_{0,5}$ – в 10,7 раза больше, чем при 1 мкМ Ca²⁺. Коэффициент Хилла при обеих концентрациях

Ca^{2+}меньше 1. Вследствие введения животным таурина V_{max} при низкой концентрации Ca^{2+} меньше, чем при высокой, в 1,6 раза, $K_{0,5}$ – больше, но лишь в 6 раз. При 1 мкМ Ca^{2+} коэффициент Хилла h этого процесса вследствие влияния таурина возрастает и составляет 1,12. Субстратное ингибирование, присущее зависимости скорости дыхания от концентрации пирувата при 1мкМ Ca^{2+} в среде вследствие длительного действия таурина начинает развиваться при более высокой, чем в контроле, концентрации этого субстрата (3 мМ по сравнению с 0,35 мМ). Следовательно, повышение концентрации Ca^{2+} от 0,1 до 1 мкМ стимулирует дыхание митохондрий гепатоцитов крыс при окислении экзогенного пирувата, но не сукцината. Таурин при продолжительном воздействии *in vivo* практически не влияет на сукцинатстимулированное ротенончувствительное дыхание и интенсифицирует процессы пируватстимулированного потребления кислорода гепатоцитами.