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COUPLED AND NONCOUPLED RESPIRATION IN RAT CARDIOCYTES AND MITOCHONDRIA

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ABSTRACT
The respiration of rat cardiocyties while oxidizing different substrates was studied polarographically to quantify rates of coupled and noncoupled respiration at 37^0C in incubation medium. Cardiocyte respiration rates were higher in state-4 with succinate than with NAD-linked substrates especially being used high substrate concentration. The rate of the oxygen consumption in intact cardiocytes did not change after addition of NADH and ADP. In the presence of digitonin causing increase of cytoplasmic membrane permeable. ADP increased cardiocyte respiration rate approximately three-fold with pyruvate (+ malate) as substrate and less than two-fold with succinate as substrate. In presence of digitonin (30 ng/ml and cytochrome *c* (2 mg/ml), the cells oxidized exogenous NADH (1 mM) at a high rate that did not change after addition of ADP. Rotenone blocked the oxidation of exogenous NADH. In the presence of digitonin and cytochrome *c* (2 mg/ml), ascorbate (20 mM) was also oxidized at a high rate which did not change after ADP addition. Oligomycin, which blocks the ATP synthase, did not decrease the rate of succinate oxidation in state-4 and NADH or ascorbate oxidation. Mitochondria isolated from collagenase treated hearts responded similarly to the different substrates and inhibitors as did the cardiocytes. Based on these findings, it appears that cardiocytes are able to oxidize substrates by two pathways, one coupled to the synthesis of ATP (coupled respiration) and one not inhibited by oligomycin and thus not connected with synthesis of ATP (noncoupled respiration).

KEYWORDS: Cardiocytes, mitochondria, NAD-linked substrates, succinate, NADH, ascorbate, cytochrome *c*, coupled and noncoupled respiration.

1. INTRODUCTION
It has been reported many times that mitochondria isolated from muscle tissues oxidize exogenous NADH and that rotenone blocks this oxidation.[1,2,4,6,34,35,29] The oxidation occurs without synthesis of ATP, so adding ADP does not stimulate respiration and adding oligomycin does not reduce respiration rate. Some researchers consider that this oxidation is a native property of mitochondria with functional importance for tissues.[41,3,4,6, 34,35, 29,30] In addition, it is well known that isolated mitochondria oxidize succinate[15,41, 25,26,38] and ascorbate in the presence of cytochrome *c*[39] at a high rate in the absence of ADP (state 4 respiration).

Based on studies of the oxidation of these and other substrates by mitochondria isolated from different tissues, we have proposed that there are two subpopulations of mitochondria, those that are coupled (ATP synthesis is coupled to respiration) and those that are noncoupled.[3,5,6] The noncoupled mitochondria have a high membrane permeability to substrates and high rate of proton leakage and which would account for the observed noncoupled respiration by isolated mitochondria. However, it is possible that noncoupled mitochondria result from damage of phosphorylating mitochondria during isolation. On the other hand, noncoupled respiration may be a native property of mitochondria, as high rates of noncoupled respiration have been measured even in mitochondria isolated using the mildest conditions in the presence of factors that stabilize membranes.[3,5,6] In addition, high rates of noncoupled respiration during succinate oxidation have been measured in cells isolated from mammalian brown adipose tissue[31], liver[17,28] and myocardium.[23, 24,33, 8,21]

Currently it was shown the presence of specific uncoupling proteins in the membranes of mitochondria of different tissues.[32,36] In these researches, it is believed that these proteins induce the activation of proton leakage involved in tissue thermogenesis. In evaluating the intensity of proton leakage succinate is used generally as oxidation substrate.[37, 22,18] The contribution

of some substrates in this process is not considered. Definite interest perform such substrates as NADH or ascorbate (+ cytochrome c), and study of oxidation would deepen understanding of the mechanisms of cellular respiration and proton leakage. In particular, it was shown previously that exogenous NADH is oxidized rapidly in intact mitochondria of muscle tissue[9,34,35] without ATP formation. However, evaluation of role of this oxidation, and its participation in the proton leak is not carried out until now. In our opinion, the most appropriate object is cell preparation for analyzing of these problems.

The objective of the present study was to determine if noncoupled respiration could be measured in intact rat cardiocyte cells when oxidizing substrates that deliver reducing equivalents at different steps of the main respiratory chain. Cardiocyte respiration rate was quantified in the absence and presence of ADP while oxidizing exogenous NADH (substrate of complex I or UQ-oxidoreductase), succinate (substrate of complex II or succinate dehydrogenase), and ascorbate with cytochrome *c* (substrate of complex IV or cytochrome *c* oxidase).

2. MATERIALS AND METHODS

Wistar Rats (Rattus nigericus) were used in experiments which weighed 200-250 g and were sacrificed by Guillotine.

Cardiocyte preparation. Cardiocytes were separated from rat myocardial muscle by methods described previously[8,21] using Tyrode's solution (120 mM NaCl, 5.8 mM KCl, 4.3 mM $NaHCO_3$, 1.4 mM KH_2P0_4, 1.5 mM $MgS0_4$, , 14.1 mM glucose, 20 mM HEPES buffer, pH 7.2). Rat heart was immediately removed and perfused with Tyrode's solution saturated with oxygen in mixture with carbon dioxide (95 % O_2 and 5 % CO_2). The heart was perfused for 5 min to remove calcium and erythrocytes. Then 20 ml Tyrode's solution with 25 mg collogenase have been used for perfusion in 30 min. Further 7 mg collogenase, 10 mg hyaluronidase, 10 mg albumin (BSA, 5-fraction) and 300 μM of $CaCl_2$ was added to Tyrode's solution (second Tyrode's solution) for increase the cellular calcium tolerance. The total amount of perfusion medium (second Tyrode's solution) was 20 ml and additional perfusion time was 30 min (37 ^oC), after which the heart became very friable. The heart was then removed from the perfusion vehicle and placed in this Tyrode's solution. Then the heart was cut into small-sized slices and these slices placed in the second Tyrode's solution solution again, and this mixture was slowly stirred on a magnetic stirrer for 30 min (+25 ^oC). The cardiocytes, separated from the friable tissue, were drained into a separate tube. This procedure was repeated three times. The cells were washed 3-4 times in second Tyrode's solution (without calcium only) to remove collogenase, $CaCl_2$ and destroyed cells, and every time centrifuged at 200 x g for 1-2 min (+25 ^oC). The washed cells were also suspended in second Tyrode's solution (30-40 mg cellular protein ml^{-1}), stored at 25^0C. Total time for cell isolation was approximately 90 min. Fresh cell preparation was used in polarographic experiments that were completed within 30-50 min after preparation.

Testing intactness of cardiocytes. At first estimation intactness of cardiocytes made visually with the help of a light microscope. At the amount of the square (intact) more than 85 % and rounded (injured) cells less than 15% within cell preparation it is considered as intact for usage in experiments.

Intactness of cardiocytes was determined also by the biochemical tests using polarographic method. Isolated cells was incubated in second Tyrode's solution and them intactness was checked up by adding in incubation medium of low succinate (0.2 mM) concentration. Small stimulation (20-30 %) of initial cell respiration by succinate indicates them intactness.[28,19,42]

In an alternative variant testing cardiocytes were incubated in second Tyrode's solution and introduced ADP to medium (without digitonin). Lack of ADP stimulating effect on of the succinate (5 mM) or pyruvate (5 mM) with malate (2 mM) oxidizing rate is considered as intactness of cellular preparation.

Mitochondria isolation. Beside cells the study was also curried out with mitochondrial preparations. Preliminary the heart was perfused in first and second Tyrode's solution containing collagenase for 30 min as described during cellular preparation. Then soften heart tissue was used for isolation of mitochondria in medium containing 300 mM sucrose, 10 mM HEPES buffer, 2 mM EGTA, 0.2% BSA pH 7.5 at 0-2 ^{o} C, Tissue was minced by Teflon homogenizer and twice was centrifuged for isolation of mitochondria as described.[20,15,3,4] Mitochondrial sediment was suspended in solution containing 300 mM sucrose, 10 mM HEPES, pH 7,5 and it was stored at 0-2^o C.

Protein of cellular and mitochondrial preparations was determined by Lowry[27] and that were contained 30-40 mg protein ml^{-1}.

Measurement of coupled and noncoupled substrate oxidation. Cellular and mitochondrial preparations were used in polarografic studies of the respiration parameters[14] (at 37 ^{o}C for cells, at 25 ^{o}C for mitochondria) by adding approximately 2 mg protein in 1 ml medium of polarographic cell.

Incubation medium for cells was the second Tyrode's solution and for mitochondria that was medium containing 120 mM KCL, 10 mM HEPES, pH 7.5, 5 mM KH_2PO_4 and oxidizing substrate.

In all experiments it was used two types of substrates: a) 5mM pyruvate with 2 mM malate and 5 mM succinate

were used for evaluating of phosphorilating (coupled) respiration, b) 1 mM NADH with cytochrome *c* (2 mg ml^{-1} for cells, 200μg ml^{-1} for mitochondria) and 20 mM ascorbate with cytochrome *c* (2 mg ml^{-1}) to estimate noncoupled respiration in all preparations.

For an estimation of phosphorylating (coupled) cardiocyte respiration the second Tyrode's solution was introduced in a polarographic cell (1 ml of volume) then a oxidizing substrate and cellular suspension (approximately 2 mg protein) were put in polarographfic cell. For induction of a cytoplasmic membrane, permeability to ADP a digitonin (30 ng ml^{-1}) was introduced in medium. The subsequent ADP (500μM) produced a stimulation of phosphorylating respiration called as a state-3. Oligomicin (2μg ml-1) was applied for blocking of phosphorylating respiration. At strong block of oxidative phosphorylation by oligomycin shows a good coupled respiration, whereas at gentle oligomycin effect shows a small coupled respiration. Usually, during a pyruvate with malate oxidation the maximum degree of coupled respiration emerges in cellular preparation that was served as test for an estimation of a phosphorylating respiration intensity.

Cardiocyte respiration rates were expressed in μmol O/min on mg of cells. Respiration in state-3 was initiated by the addition of ADP (500μM or 1 mM) into incubated cellular suspension and after 1-2 min it was added 2 μg ml^{-1} oligomycin for receiving respiration in a state-4. The respiratory control ratio (RCR) was determined as the ratio of cardiocyte respiration rate in a state-3 to state-4. Parameters of oxidative phosphorylation in isolated mitochondria were determined according Chance and Williams.[14] The RCR is determined as the ratio of ADP-stimulated respiration rate (state 3) to respiration rate in state-4.RCRs were measured in the presence of 200μM ADP with succinate (5 mM) and pyruvate (5 mM)+malate (2 mM) as oxidative substrates and in absence of oligomycin.

For an estimation of not phosphorylating (noncoupled) respiration the cardiocytes were also incubated in second Tyrode's solution. For this purpose basically a substrate NADH with cytochrome *c* have used. For induction of permeability through a cytoplasmic membrane the digitonin (30 ng ml^{-1}) was introduced in the incubating cellular suspension and determined a degree of a stimulation of respiration by NADH. In order to confirm of noncoupling type of oxidation of NADH was affected by oligomycin which usually blocks the phosphorylating respiration but not non-phosphorylating (noncoupled) one. Therefore this approach served as a test for determine of noncoupled type of oxidation. For establish that NADH oxidation occurs in a 1-complex of a main respiratory chain, a specific inhibitor rotenone (1 μg ml^{-1}) have been used. The blocking by it of NADH oxidation testified to participation of a 1-complex of respiratory chain in noncoupled respiration.

For an clarifying of noncoupled oxidation of the second complex of a respiratory chain a substrate a succinate with oligomycin (2 μg ml^{-1}) was used in experiments. Oligomycin blocks a phosphorylating part of succinate oxidation only and does not depress noncoupled part of oxidation. If cardiocytes release a high intensity of respiration in this condition the succinate oxidizes in the noncoupled respiration pathway.

Noncoupled oxidation in the third segment of a respiratory chain was clarified with ascorbate (20 mM) as substrate combination with cytochrome *c* (2 mg ml^{-1}). To an incubation cellular suspension was introduced this substrate and a intensity of its oxidation in presence of oligomycin was judged participation of 3-segment of the main respiratory chain in noncoupled respiration.

Statistical analyses

Data are expressed as means ± S.E.M., with the statistical significance of results being analyzed using Student's test.

3. RESULTS AND DISCUSSION

Cardiocyte nonphosphorylating respiration with succinate and NAD-linked substrates. As indicated in table 1, the respiration rate of intact cardiocytes with 5 mM succinate as substrate is greater than that with the NAD-linked substrate (pyruvate + malate), with or without digitonin, which increases the permeability of the cytoplasmic membrane. Therefore, the differences in oxidation of succinate and NAD-linked substrates may be explained by properties of the mitochondrial respiratory enzymes, not by properties of the cytoplasmic membrane.

Experimental conditions are described in section "Methods". Concentration of ADP, uncoupler (2,4-dinitrophenol) are consequently 500μM, 50μM. In inverted commas are number of experiments. Uncoupler increases the oxidation of succinate and pyruvate + malate without effect on NADH oxidation. Differences between data with and without ADP are > 0.05.

In the absence of digitonin, the addition of ADP does not simulate respiration for any of the substrates studied (Fig. 1).

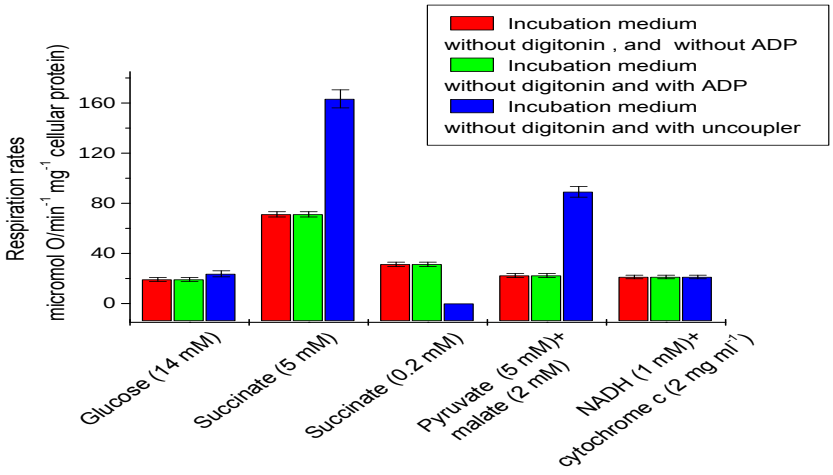

Fig 1: Rates of cardiocyte respiration (μmol O/min^{-1} mg^{-1} cellular protein) of different substrate oxidation without digitonin in medium und during adding of ADP or the uncoupler 2, 4-dinitrophenol (n = 5-12).

When digitonin is present and ADP is absent, the only substrate studied that increases respiration rate significantly is NADH + cytochrome *c*, and that rate is unaffected by the addition of the uncoupler 2,4-dinitrophenol. Dinitrophenol does cause an increase in the respiration rate of cardiocytes oxidizing the substrates succinate and pyruvate + malate. Hence, access of substrates into mitochondria (without digitonin) and the mitochondrial electron-transport system of the isolated cardiocytes appears to be normal.

Some authors[28,19,42] have used a low rate of succinate oxidation as an index of the intactness of isolated cells. However, low rates of succinate oxidation occur only when the succinate concentration is low. Similarly, in our experiments, the low succinate concentration (0.2 mM) did not cause a pronounced increase in cell respiration (Fig. 1).

In experiments, glucose possessed a weak rate of oxidation as on this substrate cardiocytes did not react to uncoupler (Fig. 1) or ADP (Fig. 2). Probably during cell preparation a soluble glycolitic enzymes leave intercellular space that result in lowering glycolitic activity.[19]

Cardiocyte respiration and synthesis of ATP with succinate and NAD-linked substrates. It is well known that ATP synthesis by mitochondria is blocked by oligomycin. We studied this process in cells treated with digitonin. ADP-stimulated respiration is reduced by oligomycin approximately three-fold with pyruvate + malate as substrate and only 45 % with succinate as substrate (Fig. 2). In addition, this table shows that respiration is stimulated by ADP and then oligomycin returns it to control values (before ADP) - i.e., oligomycin blocks only the ADP-stimulated respiration, as expected.

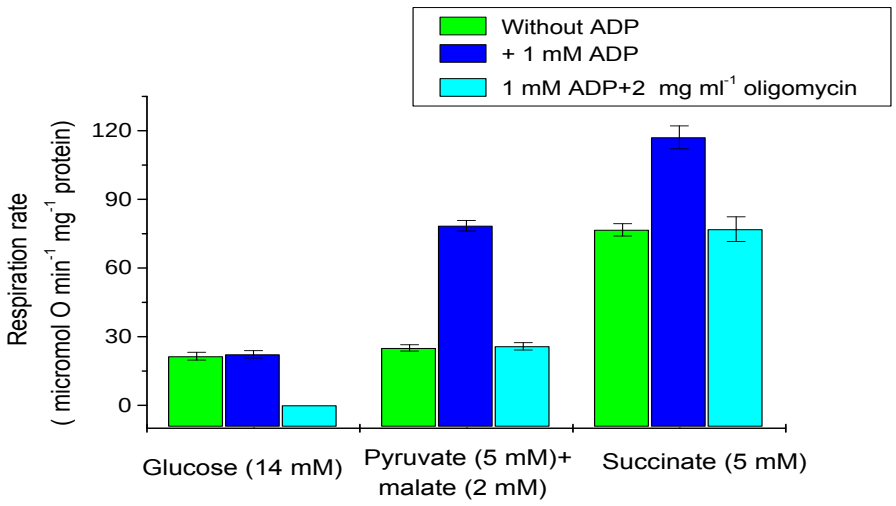

Fig 2: Rates of coupled (ATP-synthesizing) respiration of cardiocytes using different substrates in the presence of digitonin (n = 9-21).

30 ng ml^{-1} digitonin presents in incubation medium. Other conditions are as described in methods. Means with ADP differ significantly from those without ADP on pyruvate+ malate or succinate as substrates ($P < 0,05$).

The initially high rate of succinate oxidation (without ADP) in intact cells is maintained in the presence of oligomycin. Therefore, we conclude that the cardiocytes utilize succinate in two ways: by mechanisms that are coupled and are not coupled with ATP synthesis.

*Cardiocyte respiration in the presence of exogenous NADH and ascorbate***.** As an oxidizing substrate we also have used NADH together with cytochrome *c* (Fig. 3).

It is known, that the oxidation exogenic NADH by isolated mitochondria is esteemed as result of mitochondrial membrane damaging.[26] However this conclusion is drawn up at liver mitochondria research[26,19] whereas the oxidation exogenic NADH is esteemed as native property of muscle mitochondria.[41, 3,5,6,34,36,30]

It is necessary to note that earlier this question was not studied on isolated cellular preparations. Using exogenic NADH on cardiocytes we found out that its oxidation was intensively in presence of cytochrome *c* if digitonin was added in medium. In particularly rate of NADH oxidation was 69.1±5.6 μmol O min^{-1} mg^{-1} protein that was closely to succunate oxidation one when experiment was performed without ADP or uncoupler (compare Fig. 2 and 3).

However NADH oxidation rate was essentially higher than that pyruvate + malate oxidation in cells when ADP or uncoupler were absent in medium. Difference in rate oxidation between these two substrates was more than three fold (compare Fig. 2 and 3).

Oligomycin or ADP did not produce change in an NADH oxidizing rate in our experimental conditions (Fig. 3). Therefore, this oxidation completely is not coupled with phosphorylation process. Rotenone (blocker of 1- complex of the main respiratory chain) occurred depressing of NADH oxidation approximately 3 fold. Therefore, it can be said that observed NADH oxidation proceeds on the main respiratory chain of noncoupling pathway.

Also we used ascorbate as a substrate of oxidation which delivers reduction equivalents in the last part of the respiratory chain (complex IV), activates the consumption of oxygen by cardiocytes in the presence of cytochrome *c* (Fig. 3). The addition of oligomycin had no significant effect on the cardiocyte respiration rate, indicating that ascorbate oxidation is poorly coupled or is not coupled with ATP synthesis. Earlier[3,6] we demonstrated that isolated heart mitochondria utilized ascorbate in high rate too, that was extremely blocked by sodium cyanide – inhibitor of cytochromoxidase enzyme of main respiratory chain.

Therefore, it is possible to believe that the intensive oxidation NADH and other substrates in absence of ADP occurs in the main respiratory chain and is not coupled with ATP synthesis.

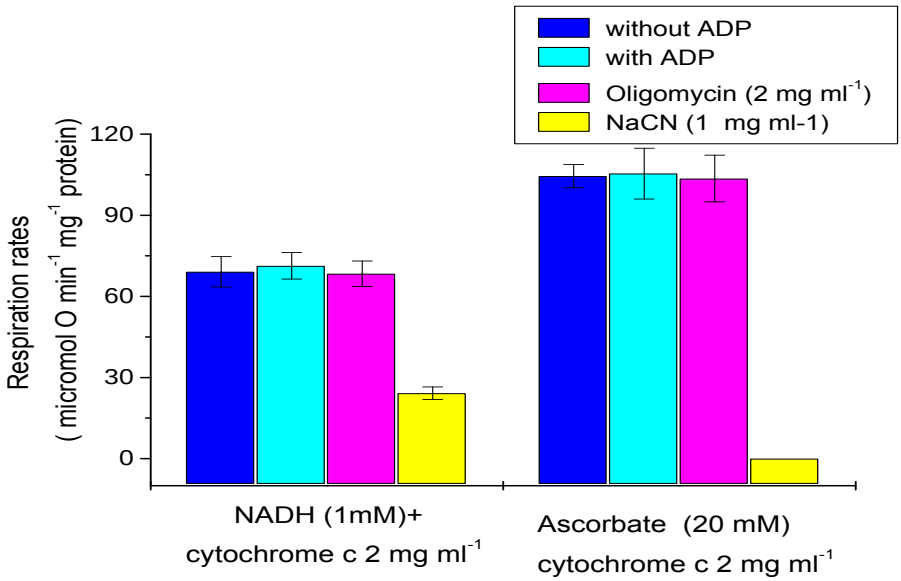

Fig 3: Rates of oxidation of NADH and ascorbate in the presence of cytochrome *c* by cardiocytes treated with digitonin (n = 5-20).

Experimental conditions are as in table 1. Differences between data with and without ADP are not sufficiently ($P > 0,05$).

Effects of collagenase and digitonin on isolated mitochondrial respiration. We also studied respiration of isolated heart mitochondria. (Fig**.** 4). In this case, heart

was perfused previously about 30 mines at temperature 37 ^{o}C with Tyrode's solution containing collagenase and hyaluronidase enzymes. However these procedures have not resulted in infringement of mitochondrial function as RCR of our mitochondrial preparations have reached approximately to six and the ADP/O ratio was been nearer to three at pyruvate with malate as substrate oxidation . According to Almatov (1990), incubation of a mitochondria at 37^{o}C result in complete damage of the mitochondrial coupling system for 10-15 minutes. Hence, the cells are capable to preserve mitochondria from temperature damage. This fact is one of the important moments for research of mitochondrial damage mechanisms in vitro conditions.

We have tested digitonin influence on mitochondria, which increases a permeability of cytoplasmic membrane usually. As it can be seen from table 4, this reagent does not offend against the couple system of the isolated mitochondria on pyruvate with malate as substrates. Even in these, condition the coupled respiration is supervised which little increased because of increase of a state 3-respiration rate. These results indicate that digitonin does not influence on mitochondrial membranes sufficiently so their function is saved as in the cellular preparation.

On the isolated mitochondria, we also tested the digitonin effect on oxidation of succinate and NADH. RCR at the succinate oxidation reached approximately to two and mitochondria had high respiration rates in states 2, 3 and 4 that corresponded to other author data obtained without digitonin.[34,35,30,29] The application digitonin did not result in decrease or increase of a respiration rates in states 2 or 4. Respiration in these states were not altered also by oligomycin blocking mitochondrial respiration in a state-3 in our experiments (Fig. 4).

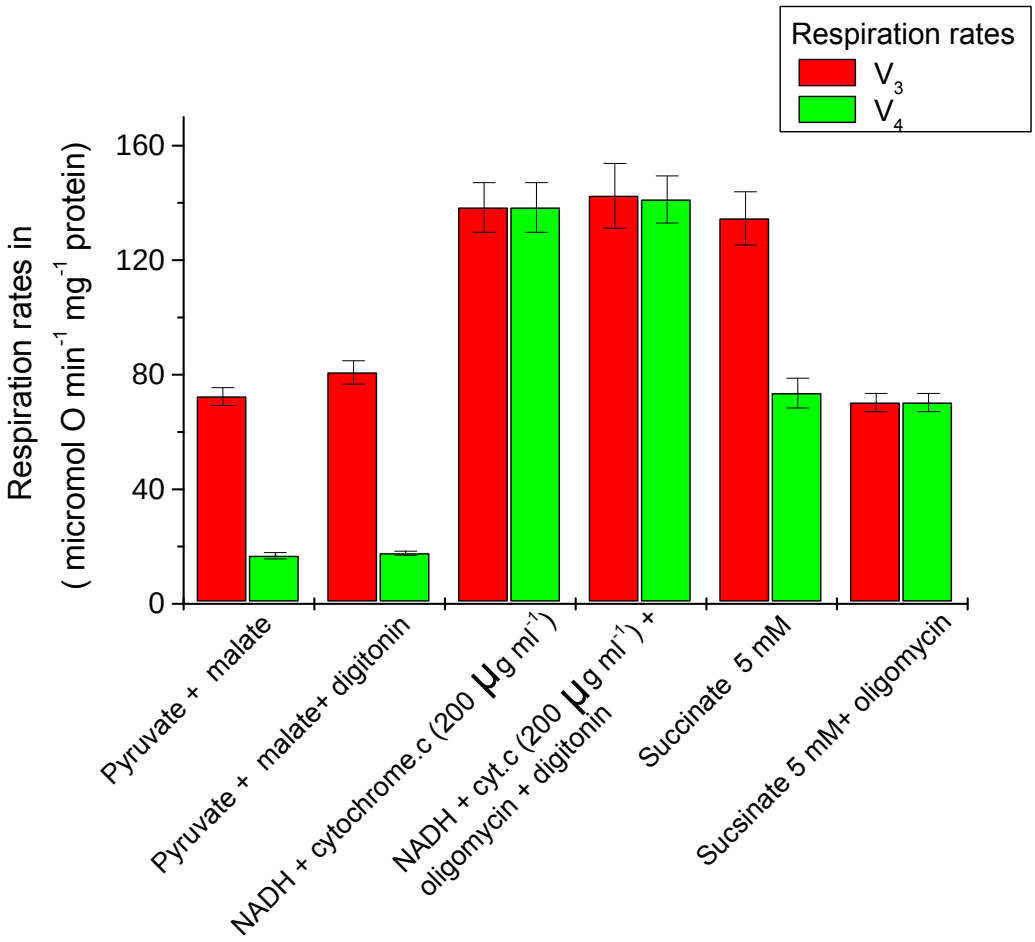

Fig 4: Effect of digitonin on respiration of isolated mitochondrial (n = 5-12)

Digitonin (*60* n*g/ml*) is present in experiments 2 and 4, oligomycin was used 2 µg ml^{-1} (in 4 and 6 experiments). Metabolic parameters of mitochondria were determined according to Chance and Williams (Chance, Williams, 1955). V_3 is respiration rate during 200µM ADP utilization by mitochondria, V_4 is respiration rate after

exhaustion ADP in medium. Concentrations of substrates and inhibitors are same as in above tables. Mean of digitonin effects on V_4 respiration does not sufficiently ($P > 0,05$)

At use NADH as substrate we found a high rate of its oxidation in the isolated mitochondria (Fig. 4). Unlike other substrates the NADH oxidation continues with high rate at oligomycin using that indicates the phosphorlative oxidation lacking in mitochondria in this condition. Besides we also shown that digitonin is not produces the appreciable change of NADH oxidation rate in mitochondria.

Therefore the respiration of isolated mitochondria during digitonin application correspond to the cellular respiration so degree of their coupled and noncoupled parameters are similar to each other.

4. CONCLUSION

In the literature, many works are devoted to the study of respiration coupled with ATP synthesis in mitochondria, as while it is generally accepted that the basic energetic function of mitochondria is the synthesis of ATP. However, noncoupled mitochondrial oxidation of exogenous NADH in muscle mitochondria[41,34,35,30] and of other substrates[3,4,6] has been repeatedly reported. There has been some controversy over whether noncoupled respiration is a consequence of damage of mitochondria during isolation, or if it a native property of mitochondria. In present experimental work, we have shown that, in rat cardiocytes, as well as in isolated heart mitochondria, the oxidation of different substrates is coupled to different extents with ATP synthesis and there is noncouped part of oxidation process. Thus according our data the noncoupled respiration is characteristic of the intact cardiac cells, supporting the idea that noncoupling respiration is native property of cells.

In cardiocytes and isolated heart mitochondria, the substrates that produced the highest coupling respiration with ATP synthesis occurred during pyruvate (+malate) oxidation (Fig. 2 and 4). As show earlier, many NAD-linked substrates[3,4,6] and fatty acids[6] possess a high level of coupling with ATP synthesis in heart mitochondria.

During succinate oxidation, part of respiration is coupled with oxidative phosphorylation but approximately half of this oxidation (respiration in a state-4) is not coupled in cardiocytes and heart mitochondria isolated with collagenase and incubated in presence of digitonin. Cardiocytes and isolated mitochondria remain a high rate of respiration in a state-4 at presence of oligomycin that is the important indicator on noncoupling of this form respiration with ATP-synthesis. The similar results was shown by us[3,5,6] and by other authors[15, 41, 25,26,38] earlier in the heart mitochondria isolated without treatment by collagenase enzyme or digitonin. use Hence, last factors (collagenase or digitonin) do not appreciable effect on respiratory function of mitochondria in various substrate oxidation including succinate and NADH (table 4). Therefore, noncoupled respiration measured in cardiocytes and we consider isolated mitochondria as native property of heart mitochondria.

Primarily the pathway that is not coupled with phosphorylation (in an ADP-independent pathway) as added ADP oxidizes exogenous NADH and ascorbate and oligomycin does not influence noticeable on high rate oxidation of these substrates in either cardiocytes or isolated cardiac mitochondria. We consider that their noncoupled oxidation is native property of mitochondria because we now have shown that it occurs in intact cardiac cells of heart. It is important to note that oxidation of these substrates occurs in the main respiratory chain of mitochondria because oxidation NADH in cardicytes is suppressed by rotenone which inhibits complex 1 of mitochondrial respiratory chain, and noncoupled oxidation of ascorbate in isolated heart mitochondria is inhibited by cyanide sodium, which inhibits cytochrome oxidase.[3,5,6]

The unequal degree of coupling of respiration during oxidation of different substrates in cardiocytes and isolated mitochondria is proposed to be due to the function of the two subpopulations of mitochondria[1,4,5,6] which possess two independence respiratory chains. Inner membrane of noncoupled ones could contain uncoupling proteins (porin or thermogenin like protein) that occurs high membrane permeability. We have shown that heart mitochondrial membranes contain analogical proteins.[7] Addition to intact cardiac mitochondria (besides ATPase synthesing ATP) content magnesium stimulated ATPase that hydrolyses ATP and blocks by oligomycin also.[16,2] It is possible that these ATPases may be located in various mitochondrial subpopulations and this question would be investigated in cellular preparation.

Also it is possible that the earlier described exo-NADH-dehydrogenase enzyme[34,35,29,13,22] is a component the noncoupled respiratory pathway studied here.

Noncoupled substrate oxidation could also result from high rates of proton leak across the inner mitochondrial membrane, as described by Brand and others.[10,11,12,13] It may be possible that, in isolated mitochondrial suspensions, a proportion of the mitochondria possess high membrane permeability to ATP, ADP, NAD, NADH, cytochrome *c*, H^+ and others. We think that described here noncoupled mitochondrial system is connected by proton leak process. However, conditions for study of noncoupled respiration and proton leak are essential distinctions. First process was studied with different oxidative substrates including NADH and ascorbate (separately each substrate) and added cytochrome *c* in medium. The noncoupled mitochondrial subpopulation can lose a lot of cytochrome *c* during its

isolation and washing procedures because of high permeability of their membranes. Therefore studying of noncoupled respiration will be necessary to carry out in presence cytochrome *c* otherwise, its activity may be low.[6] As the proton leak is studied without added cytochrome *c* and at presence of a substrate oxidation blocker.[10] So, for inhibition of succinate oxidation is used malonate as blocker succinate dehydrogenase enzyme that inhibits either coupled or noncoupled respirations simultaneously. For an establishment of interaction of noncoupled respiration with proton leak it is necessary to take into account the above-stated factors in experiments. Our experimental data show that the two subpopulations of mitochondria coexist in cells. One – little permeable to the proton and carries couple respiration, and the other - has a high membrane permeability to the proton (and other substances) and carries out a non-coupled respiration.

Because noncoupled respiration transfers chemical energy directly into heat and displays at high activity in mitochondrial and cellular preparations, it may participate in thermogenesis in endothermic animals. Previous work has demonstrated that the rate of noncoupled respiration is higher in mitochondria isolated from endothermic animals than in those from ectothermic ones.[3,5,6,7] Undoubtedly, questions, raised in this work require further investigation.

5. ACKNOWLEDGEMENTS

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