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AGE-RELATED CHANGES OF ENERGY METABOLISM OF RAT PANCREAS

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ABSTRACT

In this paper, it has been studied the changes in body weight, pancreatic weight and respiration, oxidative phosphorylation of mitochondria of the pancreas in growing, adult and old rats. It is found that the pancreas and body weight of animal increased by age, but mitochondrial respiration of pancreas, in contrast, is reduced. It should be noted that the decrease of mitochondrial respiration function in aged rat markedly suppressed especially by succinate.

KEYWORDS: rat, pancreas, postnatal ontogenesis, mitochondrial respiration, oxidative phosphorylation.

1. INTRODUCTION

Energy metabolism includes the processes of complex organic molecules oxidation and transforms energy into a form suitable for further use by an organism. Natural changes in indicators of energy metabolism in animals and humans go with them throughout life and are found at many levels and systems of an organism. But still there is no objective possibility to determine definitively the cause of aging or a result of they are. However, we know that the direction and pace of these processes (energy and aging changes) is largely correlated, if the body does not have a place of some pathology or is not withdrawn outside the physiological norm.

Currently, there are three hypotheses to explain the molecular and genetic mechanisms of aging. One of them is the fact that with age, in the process of enzymes biosynthesis increases the number of errors that occur in the stages of transcription and translation and reparation of the genome of the system cannot cope with them. As a result, there are defective enzymes and normal functioning of cells is disrupted. The essence of the second hypothesis is as follows: throughout its life, cell constantly uses only 0.4% of the information contained in the DNA of the cell nucleus.

At the same time, many genes are repeated in the DNA molecule. Usually, repeated sequences are repressed for what they, incidentally, are called "silent" genes. Such a provision of genetic information, apparently, is a kind of protection against the random molecular defects: In case of significant damage of the active gene is replaced by a reserved "silent" gene. And so it continues as long as the entire reserve is exhausted. In short, the more DNA, the longer life-span. In the third hypothesis, age-related changes are seen as a natural extension of the genetic processes regulating the development from the moment of its conception to puberty.

It is possible that possibility of "aging genes" existence of that are able to gradually close the various biochemical pathways, thereby reducing the functional capabilities of living cells and cause predictable age-related changes. All three hypotheses are quite logical and even have the indirect experimental confirmation. But best of all, age-related changes are explained by mitochondrial theory of aging.[1-3] There are many cell organelles of living organisms with individual functions that support the cellular activity. Among these organelles mitochondria is the hemi of cell that produces energy necessary for the functioning of cells.[4-6] Mitochondria are often referred as power plants of cells. After all, this is where the biological oxidation of proteins, fats and carbohydrates occur and at the same time released energy consumed in the synthesis of ATP - the connection in which the stored energy required for the biosynthesis of proteins, active transport of molecules, of division and movement processes of cells. Briefly, the normal life of any cell in the body depends entirely on mitochondria. Perhaps that is why many scientists believe that the cause of cells aging (as well as the whole body) can become an energy crisis, arising at damaging of these organelles. In spite of tens of thousands of studies on mitochondrial functions, the role of mitochondria in cell activity and their involvement in the regulation of intracellular processes still remain unclear.
Change of tissues energetic during ontogenesis can be a criterion for characterizing the conversion functionality

of the body on the borders, separating the individual periods of development.[7] Therefore, sequential study of age-related changes in respiratory and energy metabolism must begin with physiological systems require for their operation of large energy reserves and greater energy consumption. We have previously shown that the respiratory activity of mitochondria and energy-producing activity of pancreas developed much weaker compared to the activity of the system in the mitochondria of other organs.[8,9] It is well known that the delivery of oxygen and metabolites to the tissues of organs correlated with their level of functional activity. It means that the metabolism in pancreatic tissue compared with the other organs of the gastrointestinal tract of humans and animals occurs with a low rate.

In recent years, more and more attention of researchers attracted to the study of changing features of respiration and oxidative phosphorylation of mitochondria in age-related aspect, especially in aging. We act on the premise, that the increase in life-span and especially its healthy period is absolutely beneficial to humans. However, the existing concepts and approaches have not led to a significant extension of their maximum life-span. Due to the improvement of living conditions, health and hygiene measures only managed to increase the average life-span that is to reduce deaths from accidental causes, some pathologies and infections. Due to the above-mentioned, considerable interest is also the study of changes in the respiratory and energy-producing function of mitochondria of the pancreas in a postnatal ontogenesis and identification of features of the process at aging.

The objective of this study was to evaluate the respiratory phosphorylation and mitochondrial function of the pancreas in postnatal ontogenesis.

2. MATERIALS AND METHODS

Albino rats were used in experiments. Animals were kept in a well-ventilated room at air temperature 18-26°C and a relative humidity of 40-60%. They were fed with standard diet of the vivarium with unlimited access to food and water. The composition of the diet of rats was: white bread, cow's milk, cotton oil, liver sausages, salt, wheat, oats, yeast, carrots.

In this work, we have identified some energy datum in rats of three age groups: growing (1-1.5 mos.), adult (3-4 mos.) and old (24-26 mos.). 45 rats in all were used in the experiment. Rats were sacrificed by decapitation.

Pancreas of several (3-4) decapitated rats were carefully freed from fatty tissue and placed in a chilled medium containing 0.25 M sucrose, 50 μM Tris-HC1 buffer (pH 7.5), 5 μM of ethylene diaminetetraacetate (EDTA) and bovine serum albumin (3g / g tissue) at 15 ml of buffer. Pancreatic tissue minced with scissors, homogenized in a glass homogenizer with Teflon pestle for 40-50 seconds, followed by an equal solution of the same buffer without bovine serum albumin.[8]

At 0-2°Cat 650 x g for 10 minutes whole unbroken cells and nuclei separated by centrifugation in refrigerator centrifuge CLR -1. The precipitate was removed and the supernatant of mitochondria were pelleted by centrifugation at 5000xg for 15-20 minutes. The resulting mitochondrial pellet was resuspended in the selection medium of the above composition without bovine serum albumin and stored until the end of experiments at 0-2°C.

The rate of mitochondrial respiration in pancreas in various metabolic conditions (V2 - prior to the addition of ADP, V3 - in the presence of ADP, V4 - after exhaustion of ADP) recorded by polarographic method using a rotating electrode under standard conditions in a cell volume of 1 ml at 20°C. Measuring medium used: sucrose - 0.25 M Tris-HC1 buffer - 5 mM (pH 7.5), KH_2PO_4 - 5 mM. Oxidation substrates: succinate and α-ketoglutarateon 4 μM (pH 7.5).

The reaction was started by addition of the mitochondrial suspension with concentration of ADP - 100 μM.[8,10] Respiratory coupling with phosphorylation was assessed in terms of the coefficient of ADP/O and respiratory control values of Chance (V3:V4).[11] Mitochondrial protein was determined by Lowry et al.[12]

3. RESULTS AND DISCUSSION

Our studies have shown (Table 1) that the weight in growing rats was 85.3 ± 5.9 g, pancreas - 0.35 ± 0.02 g. Weights of animals and pancreas increased by age. Thus if weight of adult rats compared to growing increased by 2.44 times, while the old by 4.50 times, the mass of the pancreas of adult rats increased by only 1.66 and compares old rats -on 2.34 times. One can compare that the ratio of pancreas mass to body weight in growing rats is 0,410, adults - 0.278, old - 0.213. This means that the mass of animal pancreas decreases markedly with age.

Table 1: Body and pancreas weight alteration in rats of postnatal ontogenesis (M ± m; n = 5-6)

Animals age	Indicators				
	Body weight, g	%	Pancreas mass, g	%	The ratio of body weight / to mass of pancreas
Growing	85,3±5,9	100	0,35±0,02	100	0,410
Mature	208,2±14,5****	244,1	0,58±0,03****	165,7	0,278
Old	384,3±30,7****	450,5	0,82±0,04****	234,3	0,213

All analyses are the mean of triplicate measurements ± standard deviation. Means not sharing a common letter were significantly different at $P \leq 0.05$.

Thus, body and pancreas weight increases by age. However, increase in pancreas weight compared to age increase of the animals significantly was backward. Due to the above substantial interest is the identification of the role of mitochondrial respiration and oxidative-phosphorylation function of pancreas in postnatal ontogenesis.

Alteration of the rate of □-ketoglutarate and succinate oxidation in various metabolic states of pancreatic mitochondria in rats of different age groups are given in Table 2 and 3. Mitochondrial respiration of pancreatic α-ketoglutarate in growing rats equals 10.90 ± 0.74 ng oxygen / min mg of protein, with succinate - 17,75 ± 2,24. With age, mitochondrial respiration decreased in this state. Thus, if respiration of mitochondria in adult rats compared to growing animals decreased to 16.7% in the old rats to 44.2%. Now, it is obvious that functioning of mitochondria in *in vivo* conditions at rest bulk of mitochondria is in a state 2 (V_2). This condition is characterized by a good maintenance of oxygen and mitochondrial substrates. Thus, mitochondrial respiration of pancreas of growing rats is very active. Mitochondrial respiration gradually decreases with age. In our opinion, this is due to stabilization of membranes, with the result that substrates to the active site of dehydrogenases and oxidases enzymes have difficulty in access. Therefore, with age, the rate of electron transfer along the respiratory chain to molecular oxygen slows.

Table 2: Alteration of the rate of □-ketoglutarate in pancreatic mitochondria of rats in postnatal ontogenesis (M ± m; n = 12-15)

Readings	Rate of substrate oxidation, ng of atom oxygen/min mg of protein		
	Animals age		
	Growing	Pubescent	Old
State 2 (V_2)	10,90±0,74	9,08±0,65	7,17±0,92****
%	100	83,3	65,8
State 3 (V_3)	26,72±1,35	21,14±1,23*	17,99±2,06****
%	100	79,2	67,3
State 4 (V_4)	7,91±1,03	5,98±1,04	4,97±1,20****
%	100	75,7	62,9
RC_{Ch}	3,38±0,10	3,53±0,11	3,62±0,11**
%	100	104,4	107,1
ADP/O	2,57±0,09	2,68±0,08	2,84±0,07*
%	100	114,3	110,5

Table 3: Alterations of succinate oxidation rate in pancreatic mitochondria of rats in postnatal ontogenesis (M±м; n = 12-15)

Readings	Rate of substrate oxidation, ng of atom oxygen/min mg of protein		
	Animals age		
	Growing	Pubescent	Old
State 2 (V_2)	17,75±1,24	15,14±1,50	9,64±1,89****
%	100	85,3	54,3
State 3 (V_3)	38,23±2,12	33,79±2,14	24,35±2,26****
%	100	88,4	63,7
State 4 (V_4)	12,90±1,40	10,81±1,61	7,48±1,98****
%	100	83,8	58,0
RC_{Ch}	2,96±0,10	3,12±0,09	3,25±0,08**
%	100	105,4	108,4
ADP/O	1,72±0,08	1,81±0,07	1,95±0,06**
%	100	105,2	113,4

However, respiratory activity is suppressed due to the fact that it is associated with phosphorylation processes and in a quiescent tissue core adenine nucleotides exchange fund used for intracellular transport of energy is in ATP form. Lack of appropriate acceptor of phosphate is the main brake of cellular respiration. Increasing the cell activity leads to energy expenditure and hydrolysis of ATP. Appearance of phosphate acceptor in ADP form leads to activation of respiratory activity of mitochondria (respiratory control) and this will continue as long as the cell will waste of high-energy phosphate compounds and deliver ADP to mitochondria. In the experiment, conducted by the polarographic method, which allows a relatively short period of time to record respiratory activity of mitochondria. Activation of respiratory activity of mitochondria is achieved additives to the incubation medium of certain amounts of ADP. Respiratory activity of mitochondria is dramatically activated meanwhile. Time is taken into account spent by mitochondrial suspension to phosphorylation of each additive. Phosphate acceptor is depleted and mitochondria pass

from active third state to the fourth - inhibition of respiration. Mitochondrial respiration of pancreas with α-ketoglutarate is 26.72 ± 2,35ng oxygen/ min mg of protein, succinate - 38,23 ± 3,12. With age, rate of phosphorylated oxidation is also reduced. At the same time, if in adult and old rats V_3 and α-ketoglutarate decreased respectively to 20.8 and 42.9%, with succinate to 14.7 and 45.7%. With age, similar character of changes observed in the state 4. In this case, the highest rate of respiration occurs in growing animals, low - in old rats. This means that compared to growing rats, mitochondrial respiration rate of pancreas in V_3 and V_4 also decreases.

The ratio of the 3rd state rates (V_3) to the respiration rate in the 4th state (V_4) is one of the parameters that characterize mitochondria and is referred as respiratory control of Chance ($RC = V_3/V_3$). Other indicators characterizing the activity of mitochondria in the 3rd condition is ADP/O ratio (ADP/O = K: $V_3 \bullet \Delta t$, where Δt – time of phosphorylation of ADP standard addition, K - value of standard ADP additives). If value of the respiration control reflects degree of relations of energy transformation and accumulation processes by mitochondria with energy processes inside the cell, value of ADP/O describes the functional organization of mechanisms, underlying the process of ADP phosphorylation in mitochondrial membrane and their connection with activity of the terminal respiratory chain.

In our work, rate of respiratory control and ADP/O ratio gradually rises with age. So if in adult rats respiratory control rate at oxidation by α-ketoglutarate and succinate increased to 4.4 and 5.4% respectively, in old animals - 8.4 and 8.8%. In this case, ratio of ADP/O in adult rats by oxidation with α-ketoglutarate and succinate increased respectively 14.3 and 5.2%, in old animals - 10.5 and 13.4%. This means, mitochondria economically consume oxygen and oxidation cell substrates with age. The larger ADP/O, the less oxygen is expended on phosphorylation, the correspondingly higher efficiency in terms of the mitochondrial energy storage for subsequent intracellular metabolic processes. Thus, with age, mitochondria try to provide more ATP for the cell.

Currently, there is no a single point of view with respect to regulation of mitochondrial respiration and regulation of energy metabolism at the cellular level. The most widely discussed in the literature are two hypotheses: hypothesis of "equilibrium" model of oxidative phosphorylation, which received its name because of the alleged dynamic equilibrium between the redox state of the transporters and phosphate potential of cytosol[13] and translocase hypothesis.[14] According to the first rate, mitochondrial respiration is controlled by four factors: 1) concentration of carriers of the respiratory chain, 2) concentration of molecular oxygen, 3) inner mitochondrial concentration of substrates and intramitochondrial ratio of NAD +/NAD.N, 4) utilization rate of ATP in the cytosol and phosphate potential of ATP/ADP.Pn in cytosol.

Second, translocase hypothesis postulates that the exchange of adenine nucleotides (ATF^{4-}/ADP^{3-}) between mitochondrial matrix and the cytosol, carried out by a special transport system - translocase, determines the gross rate of respiration. Authors of this hypothesis suggest that translocase - narrow link in oxidative phosphorylation system, and operation of this system depends on translocase activity. Since this carrier has a central role in the respiratory system, a large number of works devoted to the study of its structure and functional properties. Translocase isolated in non-denatured form, it has determined its molecular weight and amino acid sequence, carried out biosynthesis and characterized two conformational states. Experiments with highly specific inhibitors – atractilate and bongcrecate allowed reproducing the molecular mechanism of translocase functioning on a "portal pores" principle.[15] With the help of fluorescent probes it was shown coexistence in membrane both non-mobile carriers (fixed portal pore) and mobile, that carrying out rotational and lateral diffusion in the plane of the membrane.[16] The most essential feature of translocase is its electrogenic. This means in energized mitochondria transport of nucleotides is being realized in the same direction: ADP from cytosol to mitochondria, ATP - from mitochondria into cytosol, where K_M for exogenous ATP for more than 100 times higher than for exogenous ADP; ratio of cytosolic ATP/ADP: mitochondrial ATP/ADP is in direct linear dependence on the size of the membrane potential.[14] Translocase of adenine nucleotides operating synchronously with H+ -ATP-synthetase system and oxidative enzymes.[17,18] is controlled by intramitochondrial pool of adenine nucleotides and linearly dependent on size of this pool.[19] This means that intramitochondrial concentration of adenine nucleotides (more specifically - intramitochondrial concentration of ATP) is a regulator that controls translocase activity and H+-ATP synthase, thus predetermining respiration rate and generation of ATP.[20] At reducing of total intracellular ATP levels to 15-20%, intensity of the energy-dependent cell function falls to 75-80% of the initial value, which leads to the development of multisystem pathologies dysfunction of the central nervous system, heart function, synthetic processes in the liver, kidney and etc. In close dependence on intracellular ATP is the cell's ability to maintain its specific energy dependent function (electrogenic, neurotransmitter, receptor, contractile and ions transport of transmembrane potentials, synthetic processes and so forth.[21]

Thus, from obtained data it should be noted that the functional activity of mitochondria of rat pancreas has age-related features, while in growing rats there is a high rate of oxygen consumption in various metabolic conditions. This indicates a greater degree of coupling processes of oxidative phosphorylation in growing

animals. With increasing of animals age readings characterizing the functional activity of mitochondria, stabilize. Meanwhile mitochondrial respiration gradually reduced, but the efficiency of coupling parameters - ADP/O ratio and respiratory control values by Chance, by contrast, increased slightly. In old age oxygen consumption and ATP synthesis was markedly suppressed, especially succinate, despite the increase of ADP/O coefficient and respiratory control values.

Previously, it was shown by V.P. Skulachev[22] that oxidation rate and degree of respiration coupling with phosphorylation associated by reverse correlation. This is the regulatory mechanism by which ATP synthesis rate adapts to the tissues needs. Thus, succinate pathway of mitochondrial oxidation of pancreas noticeable disrupted at aging. It is found that succinate is not only the substrate in the Krebs cycle in mitochondria, but also functions as a regulator of physiological and biochemical processes. Signal effects of succinic acid appear in the activation of a variety of physiological functions.[23] Recently it has been shown that succinate normalizes physiological state and a number of indicators acid-base balance during acidosis caused by physical overload or other factors. The effect achieved with this metabolite of tricarboxylic acid cycle, is quite stable. Most likely, in this case the mechanism of action of succinic acid is associated with its energizing effect on mitochondria and the corresponding change of hydrogen ions outside of mitochondria.[6]

Analyzing the results, it can be concluded that by age of animal pancreatic mass is increased, but mitochondrial respiration, in contrast, is reduced. Respiratory and energy function of mitochondria of rat pancreas was highest in growing animals and the lowest - in the old ones. It should be noted that the decrease of mitochondrial respiratory function in old rats markedly suppressed by succinate. In our opinion this is due to the fact that in growing rats dehydrogenase and oxidase activity of respiratory chain enhancement of enzymes biosynthesis and in old - on the contraryweakening of enzymes biosynthesis.

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