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LACK OF AGE-DEPENDENT CHANGES IN RAT HEART MITOCHONDRIA

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SUMMARY

The effects of aging on the composition and function of cardiac mitochondria from rats exhibiting significant decreases in synaptic brain mitochondria composition and function have been studied. Cytochrome content and cytochrome absorbance wavelength maxima do not change in heart mitochondria. Respiratory activities, respiratory control ratios, ADP/O ratios, and H^+/O ratios do not change with increasing age. Unlike in brain synaptic tissue, energy output of the heart does not decrease with age.

Key words: Heart mitochondria; Cytochromes; Respiration; Aging; Phosphorylation; Proton translocation

INTRODUCTION

Age-related decreases in heart function have been reported in rats [1] and may be related to the production of energy by the mitochondria. Mitochondria are responsible for the oxidation of energy-rich substrates, oxygen consumption, generation of electrochemical gradients, and ATP synthesis. In addition, the mitochondrion is the seat of the tricarboxylic acid cycle and fatty acid oxidation and is involved in the generation of metabolic precursors by intermediary metabolism. Age-dependent alteration of mitochondria could significantly affect cardiac function.

This laboratory has previously reported substantial decreases in cytochrome content and respiratory activity in brain mitochondria of synaptic (neuronal) but not non-synaptic origin [2]. In this study the effects of age on cardiac mitochondrial composition, res-

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Abbreviations: NADH, reduced nicotinamide adenine dinucleotide; RCR, respiratory control ratio; TTFA, thenoyltrifluoroacetone.

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piration, and energy-linked functions have been investigated in animals in which we have shown changes in brain mitochondria.

MATERIALS AND METHODS

Virgin female Fisher 344 rats were obtained from the National Institute of Aging colony at Harlan-Sprague–Dawley, Inc., Indianapolis, IN. Rat heart mitochondria were isolated from four animals following decapitation by the procedure developed for pigeon heart by Chance and Hagihara [3]. Five milligrams proteinase (Sigma) per heart was used in the proteolytic digestion step.

Oxygen consumption was measured at 25°C using a glass water-jacketed chamber fitted with a Clark oxygen electrode. Heart mitochondria were suspended in medium containing 0.25 M sucrose and 40 mM Tris-SO_4 (pH 7.4). Succinate at 9 mM or 4.5 mM malate plus 4.5 mM glutamate was used as substrates. Succinate-driven and malate plus glutamate-driven activities are expressed as thenoyltrifluoracetone (TTFA) or rotenone sensitive activities, respectively. Respiration was inhibited by the addition of 45.1 μM TTFA or 9.5 μM rotenone. Respiratory control ratios (RCR) and ADP/O ratios were calculated from oxygen consumption recordings after the addition of approximately 250 μM (final concentration) Tris-ADP to the above reaction medium.

Proton translocation was determined at pH 6.5 at 25°C by the oxygen pulse procedure described by Hinkle and Horstman [4] except that mitochondria were used in place of submitochondrial particles. Changes if pH were measured with a Corning Model 12 pH meter and combination micro-electrode.

The cytochrome content of the mitochondria was calculated from reduced MINUS oxidized difference spectra recorded by a DBS-3 Johnson Research Foundation (University of Pennsylvania) scanning dual wavelength spectrophotometer using millimolar extinction coefficients (1 cm light path) of 28, 19.5, and 24 for cytochrome *b* (562 or 566 minus 575 nm), cytochromes $c + c_1$ (552 minus 538 nm), and cytochromes aa_3 (605 minus 630 nm), respectively. Cytochrome b_{562} with a midpotential of +30 mV [5] was calculated from the absorbance increase following the addition of 9.5 mM succinate and 122 μM NaCN (final concentrations). Total cytochrome *b* content was determined following dithionite addition to the mitochondrial suspension. Cytochrome b_{566} content was calculated from the absorbance at 566 nm in the computer-generated dithionite-reduced MINUS succinate-reduced spectra. The wavelengths of maximum absorbance for the cytochromes were determined from the reduced MINUS oxidized difference spectra.

Protein concentrations were determined by the mothod of Yonetani [6] using bovine serum albumin as standard.

As shown in Table I, the contents of the *a*-, *b*-, and *c*-type cytochromes do not decrease with increasing age. Furthermore, the observed wavelength maxima of each mitochondrial cytochrome does not change significantly with age (*cf.* Table II). Similar data

TABLE I

EFFECT OF AGE ON CYTOCHROME CONTENT OF HEART MITOCHONDRIA

Age (months)	*Cytochrome content (nmol/mg protein)*				
	$c + c_1$	b_{562}	b_{566}	b_{total}	aa_3
3	1.09 ± 0.07 (7)	0.34 ± 0.02 (7)	0.10 ± 0.01 (7)	0.39 ± 0.03 (7)	0.60 ± 0.04 (7)
12	1.01 ± 0.11 (7)	0.34 ± 0.06 (7)	0.10 ± 0.02 (6)	0.39 ± 0.05 (7)	0.50 ± 0.04 (7)
28	1.00 ± 0.08 (7)	0.31 ± 0.03 (7)	0.10 ± 0.01 (7)	0.37 ± 0.04 (7)	0.55 ± 0.01 (7)

Number in parentheses is n = number of observations, $P > 0.05$ for all values, values are means ± S.E.M.

obtained from the frozen hearts of animals used in the previous brain study [2] show similar findings except for a 25–33% decrease in all *b* cytochromes in senescent animals (data not shown).

The effects of aging on respiratory activity are shown in Table III. The state 3 (phosphorylating, respiration-limited) and state 4 (ADP-limited) activities for the first and second ADP addition in each trial do not show statistically significant or marked decreases in specific activity when either succinate or malate PLUS glutamate is used as substrate. Only 20% or less decreases in activity is observed at 28–30 months of age. The 25% decrease in state 4 respiration with succinate at 12 months is unexplained; state 3 respiration is not affected at this age.

The ratio of state 3/state 4 respiration is called the respiratory control ratio (RCR). Since marked decreases in respiration for each age group are not observed (Table III), differences in RCR are neither anticipated nor observed as shown in Table IV. Significant differences in RCR between the first and second ADP addition are not observed.

Determination of the RCR and respiratory activities also allows calculation of ADP/O ratios for each ADP addition. As shown in Table V, age-dependent differences in ADP/O ratios for either substrate are not observed. The ADP/O ratios for the second ADP addition when malate PLUS glutamate are substrates are approximately 10% higher than

TABLE II

EFFECT OF AGE ON CYTOCHROME WAVELENGTH MAXIMA

Age (months)	*Wavelength (nm)*				
	$c + c_1$	b_{562}	b_{566}	b_{total}	aa_3
3	551.4 ± 0.1	561.2 ± 0.6	566.3 ± 0.4	561.4 ± 0.6	605.9 ± 0.2
12	551.3 ± 0.1	561.7 ± 0.2	566.1 ± 0.3	562.1 ± 0.2	606.4 ± 0.1
28	551.2 ± 0.1	561.8 ± 0.2	565.0 ± 1.0	561.8 ± 0.5	605.9 ± 0.2

$n = 7$ for all values, $P > 0.05$ for all values, values are means ± S.E.M.

TABLE III

EFFECT OF AGE ON RESPIRATION IN HEART MITOCHONDRIA

Age (months	*Respiration (nmol 0/min per nmol heme a)*			
	1st ADP addition		*2nd addition*	
	State 3	*State 4*	*State 3*	*State 4*
Succinate				
3	838.8 ± 100.2 (6)	337.7 ± 26.0 (5)	684 ± 75.7 (5)	272 ± 28.4 (4)
12	949.0 ± 230.2 (6)	251.8 ± 40.7 (4)	659.8 ± 20.0 (5)	205.4 ± 36.6 (4)
28	719.2 ± 56.7 (7)	315.2 ± 23.4 (7)	564.7 ± 58.6 (5)	278.3 ± 14.4 (5)
Malate + glutamate				
3	490.5 ± 46.0 (5)	137.11 ± 23.3 (5)	394.8 ± 33.9 (5)	92.1 ± 6.2 (4)
12	473.1 ± 114.0 (6)	121.56 ± 28.7 (6)	382.9 ± 105.8 (6)	79.1 ± 13.9 (5)
28	403.8 ± 19.0 (7)	122.30 ± 9.5 (7)	306.6 ± 24.3 (5)	92.5 ± 7.9 (5)

Number in parentheses denotes n = number of observations, $P > 0.05$ for all values, values are means ± S.E.M.

for the first ADP addition; this difference is not observed with succinate as substrate. The observed values are very close to the expected values of 2 and 3 for succinate and malate PLUS glutamate, respectively.

As shown in Fig. 1, the calculated H^+/O ratios obtained following pulsing succinate-driven anaerobic mitochondria with oxygen are similar at all ages and very close to the value of 4.5 protons translocated from the mitochondria for every oxygen atom reduced during respiration. In agreement with the pattern of data in Tables III–V, no significant differences are observed between the first and second pulses (data not shown) of oxygen that stimulate respiration responsible for mitochondrial proton efflux. The results of RCR, ADP/O, and H^+/O ratio determinations indicate that the permeability of the mito-

TABLE IV

EFFECT OF AGE ON RESPIRATORY CONTROL RATIO (RCR) OF HEART MITOCHONDRIA

Age (months)	*Respiratory control ratio*			
	Succinate		*Malate + Glutamate*	
	1st ADP addition	*2nd ADP addition*	*1st ADP addition*	*2nd ADP addition*
3	2.25 ± 0.23 (6)	2.38 ± 0.12 (4)	3.66 ± 0.23 (6)	3.75 ± 0.39 (5)
12	2.28 ± 0.09 (6)	2.39 ± 0.14 (6)	3.90 ± 0.09 (6)	3.68 ± 0.15 (6)
28	2.32 ± 0.17 (7)	2.05 ± 0.21 (5)	3.39 ± 0.24 (7)	3.43 ± 0.38 (5)

Number in parentheses is n = the number of observations, $P > 0.05$ for all values, values are means ± S.E.M.

TABLE V

EFFECT OF AGE ON ADP/0 RATIO OF HEART MITOCHONDRIA

Age (months)	*ADP/0 ratio*			
	Succinate		*Malate + glutamate*	
	1st ADP addition	*2nd ADP addition*	*1st ADP addition*	*2nd ADP addition*
3	2.24 ± 0.07 (5)	2.22 ± 0.07 (4)	2.73 ± 0.1 (5)	3.03 ± 0.08 (5)
12	2.40 ± 0.12 (5)	2.35 ± 0.02 (5)	2.69 ± 0.09 (5)	3.13 ± 0.04 (5)
28	2.24 ± 0.06 (7)	2.26 ± 0.09 (4)	2.74 ± 0.14 (7)	3.07 ± 0.03 (4)

Number in parentheses is n = number of observations, $P > 0.05$, values are means ± S.E.M.

chondria to protons does not increase with age and that ATP synthesis and proton translocation do not become uncoupled from substrate oxidation. The mitochondria are equally capable of generating proton-motive force at all ages.

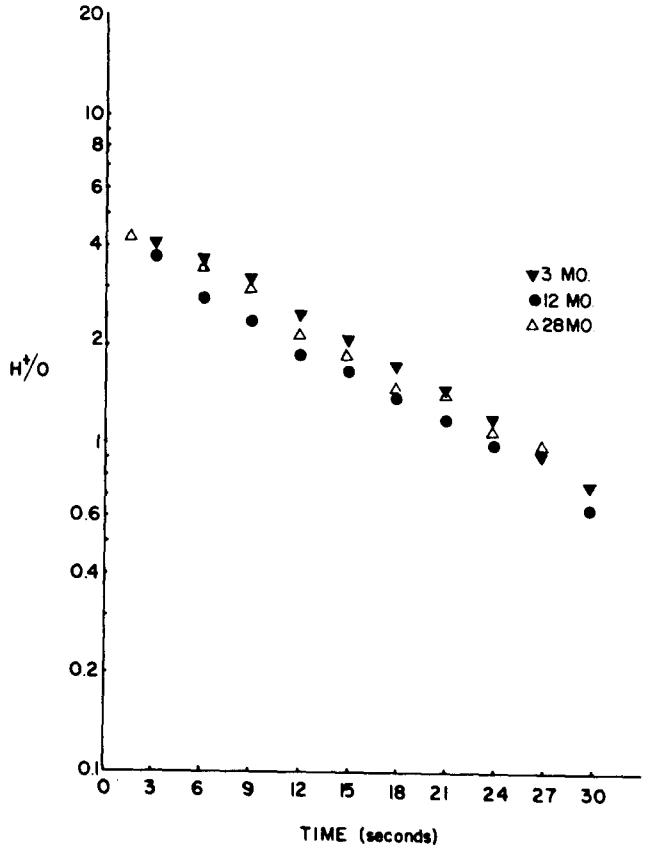

Fig. 1. H^+/O ratio *vs.* time in mitochondria from hearts of 3-, 12-, and 28-month-old rats.

DISCUSSION

Age-dependent changes in cardiac mitochondria have been investigated by others. Many conclusions are similar even though various sexes and strains of rats have been used.

Abu-Erreish and Sanadi [7] using male Sprague-Dawley rats found up to approximately 33% decrease in the content of all cytochromes yet no decrease in succinate oxidase activity when calculated on a per mg protein basis. The specific activity and turnover number of cytochrome oxidase was not affected since the activity was proportional to the amount of cytochrome aa_3 present. Beyer *et al.* [8] also using male Sprague–Dawley rats, showed decreases in coenzyme Q content in the heart. Since coenzyme Q is present in at least 3-fold greater quantity than other electron carriers [9], a 30–40% decrease in coenzyme Q may have little effect on mitochondrial respiration.

Using Wistar rats, Nohl [10] demonstrated changes in mitochondrial shape while Vitorica *et al.* [11] showed that the number and volume of mitochondria in cardiac tissue decreases less than 10% with age. Vitorica *et al.*, [11] showed that the activities of succinate dehydrogenase and NAD^+-linked malate dehydrogenase increase with age. Nohl [10] and Nohl and Kramer [12] demonstrated a 40% decrease in adenine nucleotide ADP/ATP exchange rates but no changes in "energy charge" (phosphate potential) or number of nucleotide binding sites. Nohl and Kramer [12] suggest that the activity decrease may be due to an age-dependent decrease in membrane fluidity; Lewin and Timiras [13] using male Long-Evans rats showed changes in phospholipid compositions in cardiac mitochondria that would result in decreased membrane fluidity.

Hansford and Castro [14] with male Wistar rats showed a decrease in Ca^{2+} uptake and Na^+-dependent Ca^{2+} release but concluded that these decreases are not caused by the altered rates of substrate oxidation shown in an earlier study [15]. This and other studies indicate that energy-linked respiratory activities in Wistar rats are not affected by age. Gold *et al.* [16] could not demonstrate decreases in ADP/O, RCR, B-OH-butyrate-driven respiration, or cytochrome oxidase content in heart mitochondria from 12- and 24-month-old rats. Lemeshko *et al.* [17] observed no decrease in ADP/O, RCR, or succinate or malate PLUS glutamate-driven respiration. They did note a 15% decrease in cytochomre aa_3 content, however. In contrast, age-dependent decreases in respiration driven by lipid-derived substrates (e.g. B-OH-butyrate) [15] or the ATPase/synthetase activities of rats fed purified lipid diets [18] have been reported.

Schmucker and Sachs [19] could not demonstrate an age-dependent change in the volume fraction of mitochondria in ventricular muscle in male Fisher 344 rats. In his review, Hansford [20] notes that changes in mitochondrial number and volume in heart tissues are not clearly evident.

Chiu and Richardson [21] with male Fisher 344 rats found no or only small (<6%) changes in ADP/O ratios or state 4 respiration with either succinate or malate PLUS glutamate as substrates. Age-related decreases in state 3 with succinate were not observed although an increase in state 3 activity at 12 months was noted. Malate PLUS glutamate-driven state 3 respiration increased at 6 and 12 months. Chen *et al.*, [22] were unable

to demonstrate changes in ADP/O, succinate oxidase, or cytochrome oxidase activities but observed decreases in state 3 respiration with glutamate + malate, glutamate + pyruvate, B-OH-butyrate, and palmitylcarnitine-driven respiration.

The observed lack of change in cytochrome content or cytochrome absorbance wavelengths in this report indicates that respiratory decrease due to electron carrier loss is not expected. Respiratory decreases in excess of 20% are not observed in agreement with the findings of others. In the brain, we observed marked age-dependent decreases in cytochromes *c* and aa_3 content, malate + glutamate-driven respiration, and changes in cytochrome *b* wavelength maximum. We observed no changes in the content or activity of heart mitochondria.

Roberts and Goldberg [1] noted species differences in age-dependent changes in cardiovascular activities in rats. The review of recent literature indicates few species differences in mitochondrial characteristics. Goldberg and Roberts [23] also note difficulties encountered in comparing published reports due to a lack of uniformity in reporting enzymatic activities or quantity of organelle. In this and previous reports [2,24] we have related all activities to the content of heme *a*. While species differences are not likely in heart, we do observe significant differences in tissue response to aging. The basis for differences in these two post-mitotic tissues is not known.

If the ATP synthesizing mechanism is unimpaired with age, then the energy-transducing ability of the heart tissue should not be impaired. The RCR, ADP/O, and H^+/O ratios are not affected, indicating that the ATP synthesizing and proton pumping mechanisms are not altered. Total energy production in heart is not decreased with age.

The findings from this and other studies suggest that age-related decreases in cardiac function are not due to a decrease in mitochondrial output or energy production but likely in some other aspect of heart function.

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