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Linoleic Acid Metabolism in Primary Cultures of Adult Rat Cardiomyocytes Is Impaired by Aging

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Many of the changes that occur in the rat cardiac muscle with advancing age are related to modifications in membrane fatty acid composition, polyunsaturated fatty acids decreasing and saturated increasing as the animal develops. In the present study, using cultured adult cardiomyocytes isolated from the hearts of rats of a broad (1-24 months) age range, we demonstrated that the modifications in the fatty acid pattern of cardiomyocytes have to be related to alterations in the mechanism of desaturation/elongation of essential fatty acids. In fact, independent of the age of the animal, heart cells in culture were capable of rapidly metabolizing radiolabeled linoleic acid taken up from the surrounding medium, but to a different extent. The ability of heart cells to metabolize linoleic acid to higher and more unsaturated metabolites decreased with the animal's age. As the age of the animal increased, the pattern of fatty acids of the cultured cardiomyocytes showed a gradual but significant shift, similar to those reported in the whole heart. Data here reported confirm that the basic aging-related process in the cellular model system may also be relevant to aging in the whole animal.

A characteristic profile of biochemical and contractile changes in cardiac muscle of the rats occurs with adult aging: the transmembrane action potential is markedly prolonged, the myoplasmic Ca^{2+} transient following excitation is prolonged, and the myosine isoenzyme distribution shifts to an isoform that hydrolyses ATP at a slower rate (1,2). Many of these changes that occur with advancing age are related to modifications in membrane lipid composition. Awad and Clay (3) reported age-related lipid compositional changes in heart sarcolemma and, in particular, alterations in fatty acid composition, polyunsaturated fatty acids (PUFAs) decreasing and saturated increasing as the animal develops.

The abundance of more saturated fatty acids may imply that the desaturation mechanism is altered; we previously demonstrated that the aging process greatly affects delta-6-desaturase activity in rat heart microsomes (4).

The aim of the present study is to examine the effect of age on the desaturation and elongation of linoleic acid (18:2 n-6, LA) in rat heart cells and its consequence on the fatty acid composition using cardiac myocytes isolated from rats of different ages.

Freshly isolated cardiac myocytes from the adult heart have been successfully used in a variety of biochemical and electrophysiological studies on myocardial mechanisms at the cellular and molecular level. These studies include investigations on contractile activity, regulation of myocyte energy balance, synthesis of polynucleotides and proteins, and metabolism of fatty acids (5,6). Primary cultured adult cardiomyocytes represent an ideal system to approach the study of aging influence on essential fatty acid metabolism in the heart. In fact, they give the advantage of the availability of a homogeneous population in each age group so that ambiguities in the interpretation of biochemical data arising from uncertainties as to the cell type where a particular constituent is located or a given reaction is taking place may be avoided.

Data here reported demonstrate a decrease in LA conversion into PUFAs during aging and are to be unambiguously considered as referred only to pure cardiac cells.

MATERIALS AND METHODS

Materials. [1-^{14}C]18:2 (53 mCi/mmol) of 99% radiochemical purity was purchased from NEN (Boston, MA). Medium 199, fetal calf serum (FCS), cytosine-β-D-arabino-furanoside (Ara-C), insulin, and bovine albumin were obtained from Sigma Chem. (St. Louis, MO).

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Collagenase B (0.59 U/mg) was from Boehringer (Mannheim, Germany). All chemicals and solvents were of analytical grade.

Methods. Single myocytes were prepared by a modification of the technique described by Capogrossi et al. (7). Adult (1-,3-,6-,9-,12- and 24-month-old) male Wistar rats were killed by decapitation. The heart was quickly removed and placed in a beaker containing EBSS salt solution (5.4 mM KCl, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 0.8 mM $MgCl_2$, 5 mM D glucose). The aorta was then cannulated and perfused with the same solution (37°C) non recirculating, in order to wash out the blood from myocardium. Perfusion was then switched to a recirculating EBSS solution supplemented with 0.2 U/ml collagenase and 25μM $CaCl_2$. Perfusate was continuously gassed with 95% O_2 and 5% CO_2; the perfusion was terminated when the heart became flaccid, which occurred after 15-25 minutes. The ventricles were then isolated, placed in a beaker with the same perfusate, minced and pipetted several times. The suspension was then filtered into a conical tube. After about 5 minutes at 37 °C, a pellet was formed by gravity and was resuspended three times in EBSS buffer with scalar $CaCl_2$ concentrations (100, 250 and 500 μM). The pellet was finally resuspended in medium 199, 4% FCS and seeded in pretreated Petri dishes (medium 199 and 4% FCS, 37°C, 5% CO_2, 95% humidity for 6-12 h) at 2×10^5/ml concentration. After 4 h, medium was replaced with medium 199, 4% FCS supplemented with 0.07 μg insulin/ml, 2 mg/ml albumin and 2.4 μg/ml AraC. This procedure allows the attachment of only viable myocytes to the dish. After 24 h incubation, some dishes were radiolabeled with 0.1 μCi [1-^{14}C]LA/ml medium for additional 24 h. Then cells were washed three times with 0.9% NaCl and scraped off in ice-cold methanol.

Lipids were extracted according to Folch et al. (8) and methyl esterified according to Stoffel et al. (9).

In the radiolabeled cardiomyocytes the desaturating/elongating activities for LA were evaluated by separating fatty acid methyl esters on thin layer chromatography plates coated with silica gel G, impregnated with 10% (w/v) $AgNO_3$, and identifying them by comparison with authentic standards. Plates were developed in hexane/diethyl ether (8:2 v/v), and spots made visible under ultraviolet light by spraying with 2′,7′-dichlorofluorescein (0.2%, w/v in ethanol). The spots were scraped off into scintillation vials and immediately counted in 10 ml of liquid scintillation mixture (Instagel, Packard) using a 1900 TR Packard liquid scintillation spectrometer.

The fatty acid composition of cardiomyocyte total lipids was determined by gas chromatography (Carlo Erba mod. 4160, Italy) using a capillary column (SP 2340, 0.10-0.15 μm i.d.) at a programmed temperature (160-210°C, with an 8°C/min gradient), as previously reported (10).

Data are means ± S.D. of 5 different cultures. Statistical differences were evaluated using the analysis of variance.

RESULTS

In order to examine the ability of adult rat cardiomyocytes to desaturate/elongate LA, and to evaluate the influence of aging on these activities, we incubated the cells with the radioactive substrate for 24 h and measured the per cent distribution of radioactivity in the different fatty acid classes.

Independent of the animal age, cultured cardiomyocytes were able to desaturate/elongate LA, and since cultures were pure cultures of cardiomyocytes, these desaturating/elongating activities were localized in myocytes and not in other heart cells such as fibroblasts.

In figure 1 the desaturating/elongating activities for LA of cultured cardiomyocytes derived from rats of different ages are reported. These activities were evaluated as the conversion of LA to longer and more unsaturated fatty acids, and are expressed as per cent radioactivity in the highly unsaturated fatty acid (HUFA) fractions related to total radioactivity recovered.

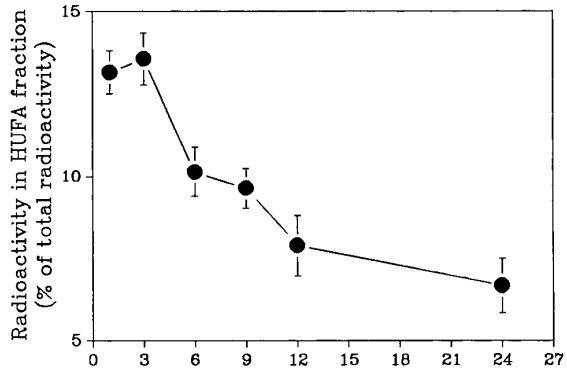

FIG. 1. Desaturating/elongating activities for linoleic acid of cultured adult cardiomyocytes derived from rats of different age. Cultured cardiomyocytes were radiolabeled with [1-^{14}C] linoleic acid (0.1 μCi/ml medium) for 24 h, and the desaturating/elongating activities were evaluated as the conversion of LA to longer and more unsaturated fatty acids (HUFAs). Results are expressed as per cent radioactivity recovered in the HUFA fraction/total radioactivity recovered. Data are means ± S.D. of 5 different cell cultures. Statistical analysis was by the one way analysis of variance: $p < 0.001$.

In cardiomyocytes from young animals (1- and 3-month-old rats) about 13% of the radioactivity was recovered in the HUFA fraction. Aging caused a significant reduction in the conversion of LA into HUFAs ($p < 0.001$). About a 30% decrease in LA conversion was observed in cardiomyocytes from 6- and 9-month-old rats in comparison to young animals; the decrease of LA conversion in cardiomyocytes from 12- and 24-month-old animals was about 42% and more than 50% respectively in comparison to young animals.

The fatty acid composition of total lipids of cultured cardiomyocytes obtained from rats of different ages is reported in table 1. The fatty acid composition of cultured cardiomyocytes was modified by the process of aging; in comparison to cells obtained from young animals, cells from old rats (more than 6-month-old) appeared less unsaturated, due to an age-dependent decrease in the relative molar content of the main PUFAs, particularly arachidonic (AA) and docosahexaenoic acid (DHA), which are the main products of the desaturation/elongation process of LA and α-linoleic acid. The AA/LA ratio significantly decreased with the animal age; this is in agreement with the reported decrease in desaturating/elongating activities for LA.

DISCUSSION

Recent advances in techniques to isolate adult cardiac myocytes have allowed the isolation of individual

TABLE 1

Fatty Acid Composition (mol/100 mol) of Cultured Cardiomyocytes from Rats of Different Ages

Fatty acid	1-month-old (n=5)	3-month-old (n=5)	6-month-old (n=5)	9-month-old (n=5)	12-month-old (n=5)	24-month-old (n=5)
14:0	1.60 ± 0.21	1.28 ± 0.42	1.66 ± 0.20	1.34 ± 0.79	1.61 ± 0.84	2.55 ± 1.43
15:0*	0.39 ± 0.18	0.90 ± 0.28	1.12 ± 0.18	1.20 ± 0.55	1.79 ± 0.85	2.19 ± 0.82
16:0	18.76 ± 0.58	18.59 ± 2.70	19.67 ± 1.10	19.61 ± 3.11	20.20 ± 2.14	20.65 ± 2.42
16:1°	2.16 ± 0.32	2.03 ± 0.67	3.33 ± 0.59	3.48 ± 1.46	2.99 ± 1.11	3.81 ± 1.03
17:0*	0.83 ± 0.05	0.69 ± 0.13	0.85 ± 0.03	1.00 ± 0.39	1.13 ± 0.31	1.90 ± 0.10
18:0	20.93 ± 1.21	22.39 ± 0.48	22.41 ± 0.61	22.70 ± 1.42	21.53 ± 0.95	21.74 ± 2.16
18:1*	7.70 ± 0.30	7.13 ± 0.96	7.70 ± 0.30	10.43 ± 0.71	8.88 ± 1.33	9.61 ± 0.83
18:2n-6*	14.08 ± 0.45	14.60 ± 1.69	13.06 ± 0.76	11.75 ± 1.87	11.71 ± 1.68	9.61 ± 0.80
18:3n-6*	0.73 ± 0.04	0.80 ± 0.24	0.97 ± 0.10	1.62 ± 0.39	1.36 ± 0.42	1.73 ± 0.42
18:3n-3°	0.63 ± 0.41	0.31 ± 0.21	0.98 ± 0.18	0.50 ± 0.41	0.89 ± 0.27	0.49 ± 0.27
20:3n-6*	tr	tr	tr	0.61 ± 0.47	1.21 ± 0.45	1.46 ± 0.52
20:4n-6*	17.33 ± 0.37	17.08 ± 1.85	14.72 ± 0.92	12.41 ± 2.40	11.95 ± 2.53	9.78 ± 1.81
20:5n-3*	tr	tr	0.55 ± 0.17	0.48 ± 0.42	0.97 ± 0.26	1.33 ± 0.51
22:4n-6*	1.88 ± 0.1	0.62 ± 0.41	1.18 ± 0.30	1.28 ± 0.83	2.33 ± 0.72	3.05 ± 1.27
22:5n-6*	0.14 ± 0.03	0.20 ± 0.09	0.28 ± 0.04	0.30 ± 0.25	0.72 ± 0.32	0.56 ± 0.13
22:5n-3	1.39 ± 0.21	1.90 ± 0.61	1.75 ± 0.58	1.58 ± 0.15	1.73 ± 0.36	1.70 ± 0.60
22:6n-3*	11.36 ± 0.61	11.24 ± 1.85	10.21 ± 0.12	9.74 ± 1.02	8.25 ± 0.71	6.80 ± 0.93
20:4/18:2°	1.23 ± 0.10	1.17 ± 0.12	1.13 ± 0.11	1.05 ± 0.09	1.02 ± 0.10	1.01 ± 0.10
U.I.*	194.75 ± 3.42	190.43 ± 3.17	180.76 ± 2.59	170.60 ± 3.77	169.39 ± 1.85	158.94 ± 2.29

The fatty acid composition analysis (as methyl esters) was performed as reported in Methods. Data are means ± S.D. of 5 different cell cultures. Statistical analysis was by the one-way analysis of variance: °$p<0.05$, *$p<0.001$. U.I., unsaturation index.

myocytes that remain viable in physiological saline solutions (5). It has been demonstrated that cultured adult myocytes isolated from intact heart can retain the morphological properties and exhibit characteristics similar to those observed in the intact muscle (7,11). In the present study we sought to determine more closely the metabolism of LA in cultured myocytes isolated from hearts of rats of a broad (1-24 months) age range, and to compare fatty acid composition of young cells to values for old cells.

Independent of the age of the animal, heart cells in culture are capable of rapidly metabolizing radiolabeled LA taken up from the surrounding medium, but to a different extent. The ability of heart cells to metabolize LA to higher and more unsaturated metabolites decreased with the animal age.

As the age of the animal increased, the pattern of fatty acids of the cultured cardiomyocytes showed a gradual but significant shift. In cells from young (1- and 3-month-old) rats the AA/LA ratio was similar to that reported by Rogers for intact adult rat hearts this ratio decreased with the animal age.

The modification of the total lipid fatty acid composition and the decrease of delta-6-desaturase (D6D) activity were previously demonstrated in heart microsomes from old rats (4); data here reported confirm that the basic aging-related process in the cellular model system may also be relevant to aging in the whole animal.

It is possible that the process of aging changes the structural physico-chemical properties of the membrane which then modulate the desaturating/elongating activities. Alternatively, desaturating/elongating activities may respond first to aging, and may alter lipid composition. Whether age-related changes in physico-chemical properties are a consequence or a cause of changes in desaturating/elongating activities remains to be determined. Anyway, the effects of the lipid derived physical properties of membrane on its biological functions are well recognized, and the modification of the fatty acid residues may have profound effects on membrane functions.

The biophysical and biochemical mechanisms that govern cardiac muscle function change with age, resulting in characteristic alterations in muscle functions (2); these deficits of the senescent muscle may be related in part to the diminished calcium pumping rate by sarcoplasmic reticulum (SR) in senescent muscle and to age differences in the conditions required for spontaneous SR Ca^{2+} release (2). Many studies (13-15) document the importance of lipids in maintaining calcium homeostasis and suggest a role for lipid alterations in the mechanism of damage during the aging process by altering intracellular calcium concentration.

In agreement to data reported by Awad and Clay (3) in the whole heart, the age related fatty acid pattern modifications in cultured cardiomyocytes mainly involved a decrease in PUFAs, especially LA, AA and DHA, and well correlated with the reported decrease in desaturating/elongating activities. Similar modifications in fatty acid composition have been reported even in cardiomyopathic hamsters (16) and turkeys

(17), possibly implying that the desaturation mechanism is altered also in the diseased animals and that these changes are integral to the aetiological mechanisms of cardiomyopathy.

Despite obvious differences which exist between the cell in culture and the tissue from which it is derived, it is apparent from this investigation that cultured heart cells can contribute significantly to our understanding of lipid metabolism within the myocardium in both physiological and pathological conditions.

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